SUPREME COURT OF ARIZONA

STATE OF ARIZONA, Appellee, v. RODNEY CHRISTOPHER JONES, Appellant.	Arizona Supreme Court No. CR-18-0370-PR Court of Appeals Division One No. 1 CA-CR 16-0703 Yavapai County Superior Court No. P1300CR201400328

AMICUS CURIAE BRIEF OF ARIZONA DISPENSARIES ASSOCIATION IN SUPPORT OF APPELLANT

(Filed with consent of all parties)

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INTRODUCTION

This is a case about whether the Arizona Medical Marijuana Act (AMMA) permits the medicinal use of the marijuana plant's extracted oil, whether in the form of hashish or other concentrate-based products (including edibles).

The Opinion warrants review because this case impacts far more than just one defendant. It affects tens of thousands of medical marijuana patients and more than 100 dispensaries throughout Arizona. The Opinion directly jeopardizes $200 million of sales annually in Arizona and upends settled expectations of an entire industry. Moreover, the majority fundamentally misunderstood what hashish and other concentrates are and how they're made and used.

This brief explains the significance of the Opinion, puts the regulatory environment in context, provides a primer on hashish and other concentrates, and demonstrates why the Opinion leads to absurd results that contradict AMMA's text.

INTEREST OF AMICUS CURIAE

The Arizona Dispensaries Association (ADA) is the voice of Arizona's cannabis industry. Its membership includes licensed dispensary owners and

those actively engaged in business in Arizona's medical marijuana industry. The organization is dedicated to advancing the Arizona cannabis industry through political advocacy, public education, and professionalization.

The ADA's membership consists of 58 medical marijuana license-holders, accounting for about 60% of the retail dispensaries in Arizona and about 80% of the major cultivators in Arizona. Its members come from every county in Arizona except Apache.

All of the ADA's member dispensaries manufactured or dispensed concentrates before the Opinion was issued. The ADA and its members thus have a strong interest in ensuring that concentrates continue to be legal to dispense under a proper interpretation of AMMA.

REASONS TO GRANT REVIEW

I. The Opinion warrants review because it will have an enormous impact across Arizona.

Medical marijuana has become a major industry in Arizona after Arizona voters passed Proposition 203 in 2010. Today, more than 100 licensed dispensaries operate throughout Arizona, dispensing about $387 million in marijuana-based products last year. *See* Ryan Randazzo, *How Arizona's 'Non-Profit' Medical Marijuana Industry Makes Millions*, Arizona

Republic, https://www.azcentral.com/story/money/business/consumers/2018/01/26/how-arizonas-non-profit-medical-marijuana-industry-makes-millions/907082001 (Jan. 26, 2018). The vast majority of Arizona dispensaries dispense concentrates. The ADA estimates that products affected by the Opinion make up more than 50% of total medical marijuana revenues in the State.

The Opinion effectively outlaws entire categories of products that are dispensed widely throughout Arizona, affecting thousands of patients and over 100 dispensaries in the State. Resolving the legal status of concentrates (including edibles) thus is an "important issue[]" of statewide importance that warrants this Court's review. Ariz. R. Crim. P. 31.21(d)(1)(C).

II. The Opinion upends the industry's settled expectations.

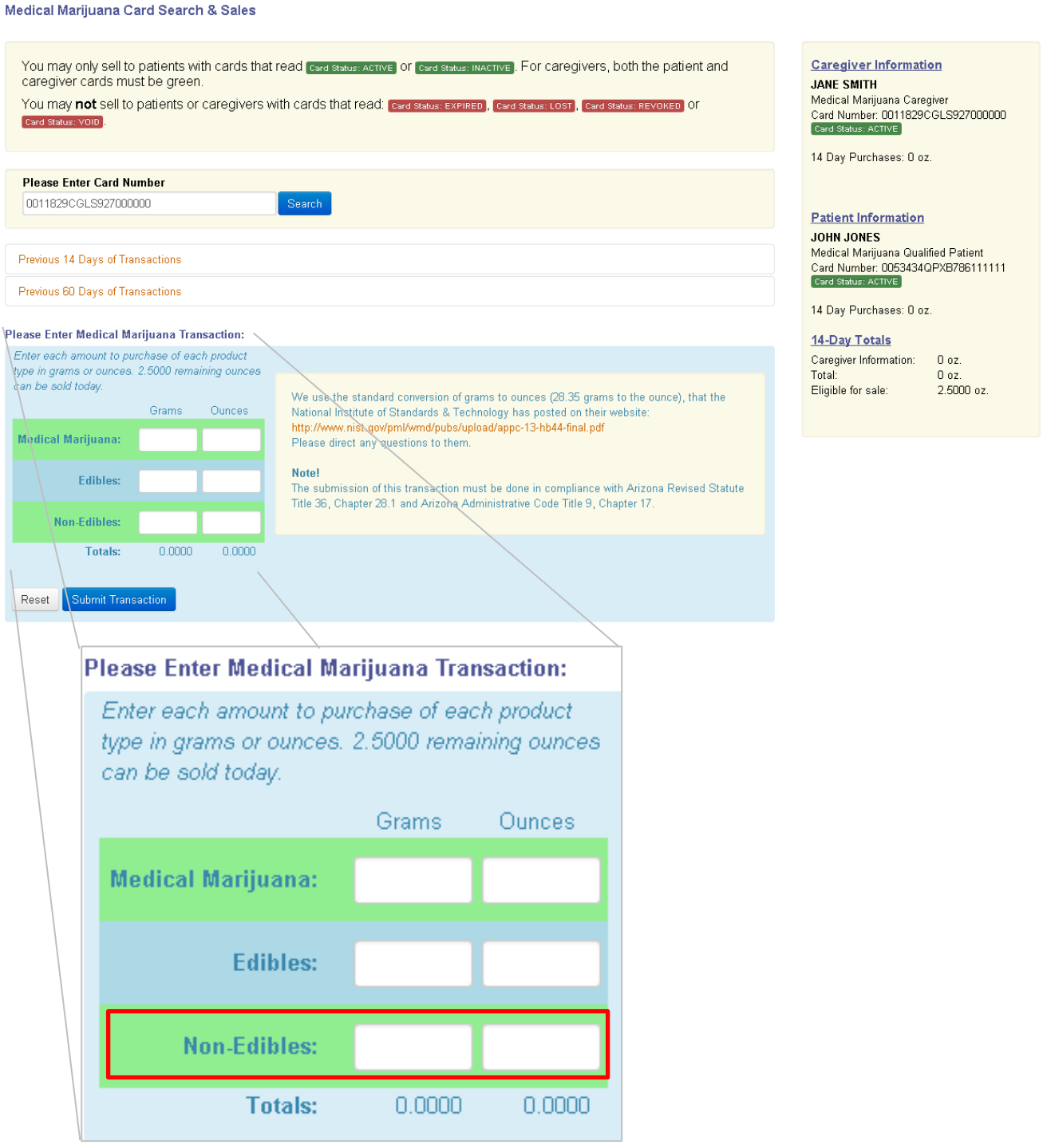
Dispensaries operate in an extremely regulated environment. They must register with the Arizona Department of Health Services (ADHS), including disclosing the identities and personal information of directors and officers (who cannot have been "convicted of an excluded felony offense"). A.R.S. § 36-2804(A)-(B). Dispensaries must be not-for-profit entities, with detailed restrictions on operations and sources of marijuana. *See* A.R.S. § 36-2806. Dispensaries may dispense marijuana only to registered patients and

caregivers and must submit to the government details about each transaction (including "how much marijuana is being dispensed to [each] registered qualifying patient"). A.R.S. § 36-2806.02(B)(1). Every employee gets fingerprinted and background-checked. *See* A.R.S. § 36-2819.

Dispensaries are also subject to detailed regulations promulgated by the ADHS. *See* A.A.C. R9-17-101 to R9-17-323. The regulations are so detailed that, for example, they not only require video cameras, but actually specify the resolution (704x480 pixels) and placement of the required cameras. *See* A.A.C. R9-17-318(G)(1)(c)(iii).

ADHS unquestionably contemplated that dispensaries could and would manufacture and dispense concentrates and concentrate-based products. ADHS regulations required each dispensary to specify whether it intends to "[p]repare, sell, or dispense marijuana-infused" products, both "edible" and "non-edible." A.A.C. R9-17-304(C)(8)(b)(v)-(vi). ADHS's electronic Medical Marijuana Verification System ("ADHS System") specifically directs a dispensary to report the weight of marijuana being dispensed as "dried flower," "[e]dibles," and "[n]on-edibles." ADHS, *Medical Marijuana Verification System: Dispensary Handbook* 11 (June 8, 2017 ed.) ("ADHS Handbook") [APP028]. The ADHS Handbook defines "[n]on-

edibles" as "any non-edible items, *such as concentrates*, sold that contain medical marijuana." *Id.* [APP028] (emphasis added). An annotated screenshot of the ADHS System appears below:



Id. at 12 [APP029].

Based on AMMA's text as well as the administrative regulations, guidance, and systems, Arizona dispensaries reasonably concluded that AMMA permitted them to manufacture and dispense concentrates. (The Petition and other amici explain why AMMA's text supports this view.) This conclusion became an established understanding in the industry—all or nearly all Arizona dispensaries made or dispensed concentrates before the Opinion.

The dispensaries would not have done so without a firm and settled understanding about concentrates' legality. The risks of getting things wrong are simply too high to deviate an inch from the law. If a dispensary does not comply with the various laws and regulations, its registration is "immediately revoke[d]" and its officers and directors are debarred from serving any other dispensary. A.R.S. § 36-2815(B). And that says nothing of the criminal penalties under A.R.S. Title 13. Dispensaries have no hope of flying under the radar, either. As explained above, they self-report each sale of concentrate products into the ADHS System and are subject to announced and unannounced ADHS inspections. *See* ADHS Handbook at 11-12 [APP028-29] (reporting); A.A.C. R9-17-309 (inspections). No reasonable

dispensary or its officers, directors, or employees would risk exposure to the severe civil and criminal consequences by manufacturing and dispensing illegal products. After all, if concentrates were illegal, then all or nearly all Arizona dispensaries have literally been *self-reporting their own crimes* by entering transaction information into the ADHS System every single day.

The Opinion disturbs the reasonable settled expectations of an entire private industry and literally makes criminals out of dispensary owners and operators who have been complying with the law as explained by ADHS. The Opinion thus deserves this Court's review.

III. Dispensaries manufacture concentrates using well-known, long-established processes that yield products that satisfy a wide range of patient requirements and preferences.

A. Concentrates can be made using extremely simple processes.

The fundamentals of making concentrates are simple. In essence, the desirable medical properties of marijuana come from the cannabinoids in the *oil* (cannabis oil) of the marijuana plant's flowers or "buds." When dried, the flowers contain both the desirable oil and inert plant material, so the goal is to extract the oil from the plant's resin glands and discard the remaining plant matter. The extracted oil is more versatile and can be used as an

ingredient in a wide variety of products. This concept is the same as extracting corn oil from corn, or even orange juice from oranges.

For centuries, people have rubbed marijuana flowers together between their fingers and palms and then scraped the resulting residue from their hands. This residue is a concentrate known as hashish (at issue in this case). Hashish can be made in many other ways, such as by compressing dried flowers that have been ground and sifted. But it is nothing more than the residue from rolled or squeezed marijuana flowers. Ed Rosenthal, *Beyond Buds: Next Generation* 174, 198 (2018) [APP056-57].

Other simple processes work, too. A patient may grind up dried marijuana flowers and then sift them through a fine sieve. Because of the physical properties of the flowers' resin glands, the powder that falls through the sieve contains a higher ratio of oil than the material blocked by the sieve. This simple process results in a rudimentary concentrate known as kief. *Id.* at 169-171 [APP053-55].

Another type of concentrate can be made simply by applying pressure to marijuana flowers, literally squeezing the oil out of the plant matter. Patients can do this with an ordinary bench vice from Home Depot or Lowe's. Applying heat makes the process more efficient, so some patients

use an ordinary hair straightener from Target or Wal-Mart to simultaneously heat and squeeze the oil out. This simple process yields a concentrate known as rosin. *Id.* at 207-210 [APP058-61].

The oil can also be separated from the rest of the plant matter by dissolving it. Oil won't dissolve in water because water is polar (i.e., has an uneven electron density) whereas oils are nonpolar (i.e., have a symmetrical electron density). But it will easily dissolve in a nonpolar solvent such as fat. So a patient may mix ground-up dried flowers in melted butter and then strain off the plant matter, leaving cannabis-infused butter behind—a concentrate known as cannabutter. (Edibles often use cannabutter, as explained in § IV.B below.) Other oils like olive oil also work as solvents to extract cannabis oil. *Id.* at 245 [APP066].

B. More advanced manufacturing methods are identical to standard processes in food production.

The simple processes described above are inefficient. They leave too much desirable oil in the discarded material and leave too much non-usable plant matter in the oil.

To improve efficiency and reduce waste, the industry borrowed standard processes from agricultural food production. Common oils like

corn oil, canola oil, and soybean oil can be made using the above processes: by grinding up corn, rapeseed, or soybeans and physically squeezing out the oil from the remaining plant matter. But scientists discovered that oils will easily dissolve in nonpolar solvents such as hexane, propane, butane, ethanol (alcohol), or carbon dioxide, giving better yields. Although any of these solvents will work, "[t]he most widely used solvent to extract edible oils from plant sources is hexane" because of cost, boiling point, and other factors. Haizhou Li et al., *High Intensity Ultrasound-Assisted Extraction of Oil from Soybeans*, 37 Food Res. Int'l 731, 731 (2004) [APP030].

Corn oil, canola oil, and soybean oil are typically manufactured using this *solvent extraction* method. After a first pass grinding or pressing seeds, a liquid solvent such as hexane is poured through the ground-up matter. Egon Stahl, et al., *Extraction of Seed Oils with Liquid and Supercritical Carbon Dioxide*, 28 J. Agric. Food Chem. 1153, 1153 (1980) [APP076] ("[P]ressing . . . is often followed by extracting" with solvents.). The oil dissolves in the solvent. The non-usable plant matter gets strained off, leaving a solution of oil dissolved in the liquid solvent. After that, the solution gets heated. Because hexane and the other solvents have a higher boiling temperature than oil, the solvent evaporates first, leaving just the oil behind. *See id.*

[APP076]. (This process is called distillation, and is the same process used to make distilled water or liquor.)[1]

The process for manufacturing marijuana concentrates is identical. A solvent is poured through ground marijuana flowers, and then the solution gets distilled to leave only the oil behind. Rosenthal at 89 [APP047], 141-42 [APP051-52]. (The cannabutter process described above (§ III.A) is a simple solvent extraction process. It omits the distillation step because the solvent (butter) is edible and therefore does not need to be removed.)

At that point, the marijuana is almost ready, but it still must be decarboxylated. The medicinal properties of marijuana come from tetrahydrocannabinol (THC, or Δ^9-THC) and other cannabinoids not relevant here. Marijuana flowers contain essentially no THC. Instead, they have tetrahydrocannabinol acid (THCA). THCA must be converted to THC through "decarboxylation," which "is a rather common chemical reaction in which a carboxyl group splits off from a compound as carbon dioxide." Helene Perrotin-Brunel et al., *Decarboxylation of Δ^9-Tetrahydrocannabinol:*

[1] At various stages the solution can be filtered through paper filters (like coffee filters), activated carbon (like a Brita filter), or diatomaceous earth (like a swimming pool filter) to remove additional impurities.

Kinetics and Molecular Modeling, 987 J. Molecular Structure 67, 68 (2011) [APP039]. Although it sounds complicated, the process merely requires heat and time (like cooking a steak), and it releases harmless carbon dioxide. The highest yield is around "110 °C and 110 min" (i.e., 230 °F for close to two hours). *Id.* [APP039]. Higher temperatures require less time. When smoking marijuana, decarboxylation occurs naturally through the flame at extremely high temperatures. But when used in other products, the concentrate must be decarboxylated first by applying heat. Rosenthal at 113 [APP050].

C. Oil serves as the foundation for other products.

Isolating the oil from the rest of the plant matter increases versatility and allows manufacturers to manufacture a wide range of products for a wide range of applications. The oil can be distilled to be nearly odorless and flavorless, which further improves its versatility as an ingredient in other products.

The oil can be packaged in gelcaps to make pills like any other medicine. The oil can also be used in a vaporizer pen, which avoids having to inhale the smoke from burned plant matter. It can be made into a tincture (a liquid to drop under the tongue), lotion or transdermal patch (for topical application), or metered-dose inhaler. It can also be made into wax, a butter-

like substance (known as budder), or a glassy material (known as shatter) similar to peanut brittle. The oil can also be combined with other ingredients to manufacture edible products. Brownies are the most famous example, but teas, cookies, gummies, sodas, chocolate bars, and more can be made simply by adding the extracted oil to a recipe. Rosenthal at 45 [APP046], 109-10 [APP048-49], 234 [APP064], 256, 267, 282 [APP067-69].

These products have obvious benefits. Most obviously, some patients cannot or should not smoke. Surely every mother with an epileptic child would want the child to take an oral solution or eat a gummy chewable rather than smoke a joint. Likewise for patients with lung problems. And some patients simply have different preferences—some patients may want to avoid the social stigma or preconceived notions of smoking marijuana.

The various products also allow patients to control how the active ingredient (THC) gets absorbed, and how quickly. Edibles get processed relatively slowly and pass through the digestive system and liver. Inhaled products get processed more quickly and get absorbed through the lungs. Products administered under the tongue get absorbed sublingually through tissue. Lotions and patches get absorbed into the skin.

These various delivery mechanisms mirror traditional pharmaceuticals. The same antibiotic may come in a pill, in a powder, as a cream or gel, in an oral solution, or through an IV drip. Products for children are frequently altered to be more age-appropriate (e.g., gummies or cherry-flavored liquids). Like with medical marijuana, some of the different forms for traditional pharmaceuticals exist for medical reasons—topical antibiotics and pills treat different conditions. Some of the differences, however, account for patient preferences or ease of administration. As just one example, many asthma patients strongly prefer to receive medication through a diskus of inhaled powder versus the identical medication administered via metered-dose aerosol inhaler. *See generally* Ketan Sheth et al., *Patient Perceptions of an Inhaled Asthma Medication Administered as an Inhalation Powder via the Diskus or as an Inhalation Aerosol via a Metered-Dose Inhaler*, 91:1 Annals Allergy, Asthma & Immunology 55 (2003) [APP070].

Or consider how people consume caffeine. Some people drink coffee; others reach for tea, soda, energy drinks, concentrated energy shots, caffeine pills, or even caffeinated gum. For social reasons, the same person might drink coffee in the morning, soda with lunch, and an energy drink before an important presentation—all for the same pick-me-up purpose.

Medical marijuana is no different. Concentrates enable a wide variety of products and administration methods that can be used to fulfill a wide variety of patient needs and preferences. AMMA even contemplates alternate methods of administration: it permits nursing homes, hospice, and other entities to require that their patients consume marijuana "by a method other than smoking." A.R.S. § 36-2805(A)(3).

IV. The Opinion leads to absurd results.

A. The Opinion criminalizes this entire range of products and processes.

The Opinion criminalizes this entire range of extraction methods (from simple to complex) and the entire range of products (from pills to edibles), with whole dried flowers as the only exception. The Opinion therefore necessarily restricts medical marijuana patients to only one method of administration—smoking—regardless of whether other methods of administration are more appropriate or even medically necessary.

Under the rationale of the Opinion, if a cardholding patient legitimately buys whole dried flowers from a licensed dispensary and rubs them between her hands, she could go to prison merely for possessing the resulting substance. A.R.S. § 13-3408.

That's not an exaggeration. Here, Jones went to prison for possessing hashish and a glass jar. A licensed dispensary gave him the hashish, but he could have made the same hashish by purchasing dried flowers from the same dispensary and rubbing them between his hands, following the centuries-old traditional way of making hashish.

The Opinion criminalizes all of the do-it-yourself methods that patients can do in their own homes. It means patients can't grind up the flowers (like they would black peppercorns in a pepper mill). They can't sift them (like they would unsifted wheat flour). They can't use a press to squeeze the flowers (like they would use a garlic press). And because the raw ingredients of black peppercorns, wheat flour, and garlic are legal, no one would ever dream that it would be illegal to use any of those processes familiar to any home cook. Likewise with prescription medication. No parent would think twice about the legality of grinding up an antibiotic tablet to mix into applesauce for a child. By criminalizing the same simple processes for making medical marijuana products, the Opinion thus leads to absurd results.

Likewise for the products that require more advanced extraction methods (e.g., gelcaps, vaporizing oil, tinctures, wax, and edibles). Under

the Opinion, asthmatics will have to smoke marijuana rather than take a pill, and children will have to smoke rather than eat a medicated gummy bear. Nothing in Proposition 203's history suggests that Arizona voters intended to legalize only *smoking joints* for medical purposes, while continuing to treat gelcaps, gummies, and tinctures as criminal, even though those are much more common forms of delivery for traditional pharmaceuticals. Without such history, this result is absurd.

B. The Opinion's reasoning would bar any effective edibles or drinks, contrary to AMMA's text.

Edibles provide perhaps the most powerful display of the Opinion's absurd results. AMMA contemplated that marijuana would be "prepared for consumption as food or drink." A.R.S. § 36-2801(15). The Opinion recognizes that "'consumables' such as brownies and the like" are permitted. Op. ¶ 12. By simultaneously (1) recognizing that AMMA permits brownies and edibles while (2) holding that AMMA extends only to whole dried flowers and not concentrates, the majority implicitly assumes that edibles are made from dried flowers and not concentrates. To the contrary, brownies and other edibles and drinks cannot be made using unprocessed dried flowers.

Dumping dried flowers into brownie batter would yield mostly inert, inedible, and pointless brownies. The internal temperature of brownies typically doesn't exceed 170-210 °F, even when baked at 350 °F. *See How to Bake Perfect Brownies Every Time*, https://www.preparedpantry.com/blog/how-to-bake-perfect-brownies-every-time. But to decarboxylate and "activate" the active ingredient in marijuana, the resin typically must be held at 220 °F for half an hour. (Ideal conditions are 230 °F for close to two hours. *See* Perrotin-Brunel at 68 [APP039].) Dried-flower brownies would have limited medicinal effects. And they would be gag-inducing, too. Even homemade brownies are made using concentrates such as oil, cannabutter, or budder. Rosenthal at 113 [APP050] ("to be used in edibles, it must first be decarboxylated"). Thus, although even the majority assumes that AMMA immunizes marijuana "brownies and the like" (Op. ¶ 12), the majority's reasoning actually prohibits making edible and effective brownies.

The same holds true for "drink[s]," which AMMA also expressly contemplates. A.R.S. § 36-2801(15). As explained above (§ III.A), cannabis resin is not water-soluble, so adding dried flowers to any water-based beverage would not work—the active ingredient would not dissolve. And without decarboxylation, it would have no medicinal properties. Stirring

dried flowers into soft drinks, for example, would yield an inert (and disgusting) drink. Cannabis beverages can be made only from decarboxylated concentrates.

AMMA allows for edibles and drinks, but the rationale of the majority would prohibit such products. These absurd results show that the majority misconstrued the statutes.

V. Under any measurement standard, Jones's hashish fell far below the statutory allowable amount of 2.5 ounces.

Jones had only 0.05 ounce of hashish—*50 times* less than the 2.5-ounce "allowable amount of marijuana" that patients are permitted to have. A.R.S. §§ 36-2801(1)(a)(i); 36-2811 (A)(1)(b). Even assuming that the "allowable amount" means the equivalent potency one could achieve from 2.5 ounces of dried flowers *before processing*, Jones's amount unquestionably fell below that limit.

Hashish is among the most rudimentary, and therefore least potent, concentrates. Even if his hashish were somehow 100% pure THC (which is literally impossible, or else it would be oil), it would take dried flowers of less than 2% potency to yield Jones's 0.05 ounces of hashish using the

2.5-ounce maximum of dried flowers—far less than the typical 15-20% potency of typical commercial medical marijuana.

CONCLUSION

The Court should grant the Petition.

RESPECTFULLY SUBMITTED this 31st day of October, 2018.

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APPENDIX
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ARIZONA DEPARTMENT OF HEALTH SERVICES

Medical Marijuana Verification System

Dispensary Handbook

Published: 06/08/2017 12:53:00 PM
Source: http://sharepoint.hs.azdhs.gov/ITS/MM/Shared Documents/Dispensary Handbook/2016-10/Dispensary Handbook.docx

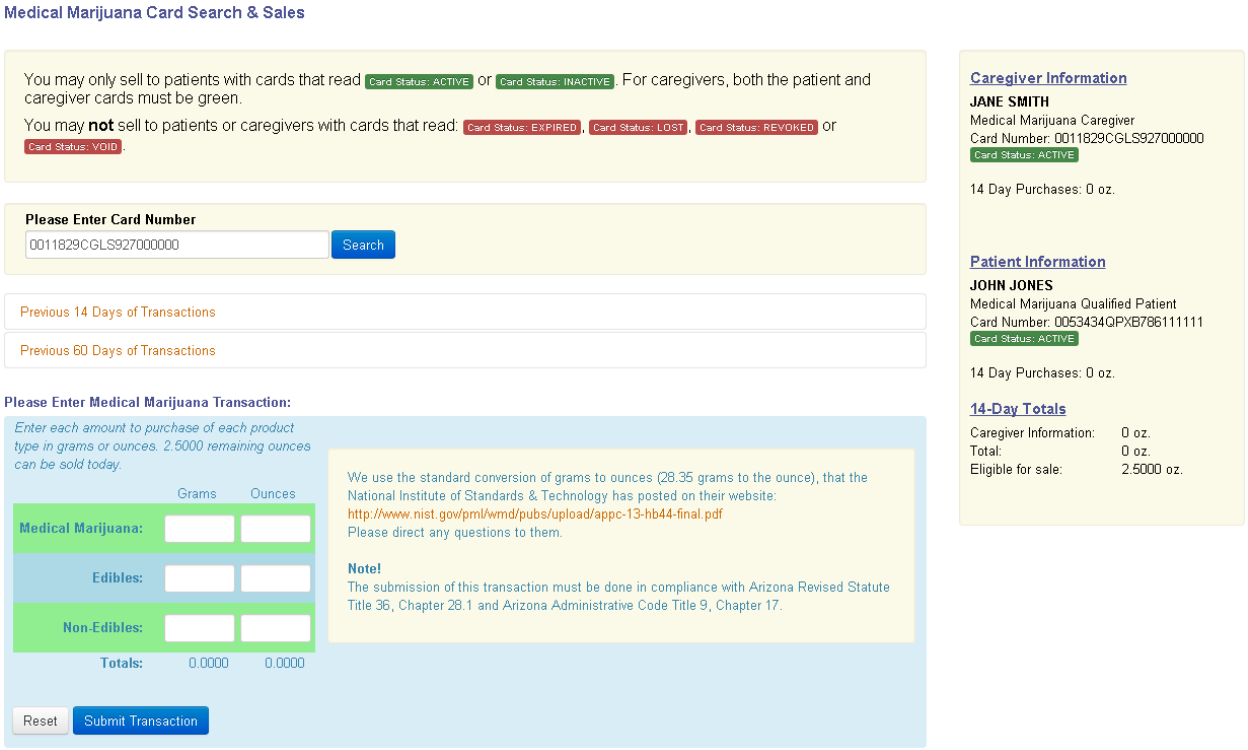
- On the right side of the page, information about the card will be shown including:
  - The customer's name and card number
  - Whether the customer is a caregiver, patient, or minor
  - The status of the card
  - The amount of product the customer has purchased within the last 14 days
  - If the customer is a caregiver, then information about the patient will also be shown below it
  - The 14 days total at the bottom and the remaining amount that is eligible for sale
- Below the "Search" button you will see a section of two header items named:
  - The Previous 14 Days of Transactions
  - The Previous 60 Days of Transactions
- You will need to click on these header items to expand the details if you would like to review the transaction history.
- If the customer's card is valid, then below you will see a green entry form where you can enter the grams or ounces of:
  - **Medical Marijuana** is the dried flower of the marijuana plant.
  - **Edibles** are any items sold for consumption that contain medical marijuana. The amount of medical marijuana in the edible must be labeled and entered into the system during a transaction.
  - **Non-edibles** are any non-edible items, such as concentrates, sold that contain medical marijuana. The amount of medical marijuana in the non-edible must be labeled and entered into the system during a transaction.
- The form will guide you through the rules and warnings (if any) pertaining to your sale.

The following rules apply to all transactions:

- You may only sell to cards for caregivers or adult patients
  - You may not sell to cards for minor patients (patients under 18 years old). Minor patients can only receive their medication through their designated caregiver. There is a specific case for cardholders who turn 18 years of age while still holding a card for a minor patient. The system will evaluate the age of the patient and if it is 18 or more years – it will permit the sale.
  - You may not sell to cards for dispensary agents or members
- You may only sell to valid cards; i.e. ACTIVE or INACTIVE
  - You may not sell to cards that are REVOKED, LOST, REPORTLOST, EXPIRED, or VOID
  - If the customer is a caregiver, both the caregiver and related patient cards must be valid
- You may not create a single transaction that exceeds 2.5 ounces in total
- You should only sell to customers that have not purchased more than 2.5 ounces in the last 14 days for the patient's card
  - You should not sell to a caregiver that has a patient that has purchased more than 2.5 ounces in the last 14 days.

- NOTE: The system will allow you to record a transaction for less than 2.5 ounces[1] that will exceeded the patient's 2.5 ounce limit for a 14 day period, but you will be warned that a violation will be recorded.
- You will be given the opportunity to cancel the transaction.

Figure 11 - Card Search and Sales



Dispensary Members

Initially, when the dispensary was created and approved for operation, a list of initial dispensary members were recorded on the certificate application. This meant that your dispensary had at least one dispensary member who had all the information needed to request the Dispensary Member user role.

This section assumes you are a board member or principal officer of a dispensary. As a dispensary member, you will be able to perform all operations that a Dispensary Agent can perform plus additional functions.

Register

If you are not a dispensary member, you can register to become one by following the instructions in Choose a Role.

[1] Dispensing amount may be reported in ounces or grams. The MMV system uses the standard conversion of grams to ounces (28.35 grams to the ounce), that the National Institute of Standards & Technology has posted on their website: http://www.nist.gov/pml/wmd/pubs/upload/appc-13-hb44-final.pdf.

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High intensity ultrasound-assisted extraction of oil from soybeans

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Abstract

The application of 20 kHz high-intensity ultrasound during extraction of oil from two varieties of soybeans (TN 96-58 and N 98-4573) using hexane, isopropanol and a 3:2 hexane–isopropanol mixture was evaluated. In a simplified extraction procedure, ground soybeans were added to solvents and ultrasonicated between 0 and 3 h at ultrasonic intensity levels ranging from 16.4 to 47.6 W/cm^2. Oil was recovered after distillation and yield and composition determined. Using hexane as a solvent, yield generally increased as both application time and intensity of ultrasound increased. Solvent type influenced the efficiency of the extraction, i.e., the highest yield was obtained using ultrasound in combination with the mixed solvent. Gas chromatography analysis of ultrasonicated soybean oil did not show significant changes in fatty acid composition. Results were attributed to mechanical effects due to ultrasonically induced cavitation increasing permeability of plant tissues. A comparison of scanning electron microscopy images of raw and ultrasonicated soybeans indicated development of microfractures and disruption of cell walls in ground soybean flakes. Our study suggests that high-intensity ultrasound may reduce time required to extract edible oils from plant sources and hence improve throughput in commercial oil production processes.

Keywords: High-intensity ultrasound; Extraction; Oil; Soy; Solvent

1. Introduction

Plant-based lipophilic compounds such as edible oils, phytochemicals, flavors, fragrances and colors are valuable products in the food, pharmaceutical and chemical industry. Extraction is one of the key processing steps in recovering and purifying lipophilic ingredients contained in plant-based materials (Liu, 1999). Classical extraction technologies are based on the use of an appropriate solvent to remove lipophilic compounds from the interior of plant tissues. The choice of a suitable solvent in combination with sufficient mechanical agitation influences mass transport processes and subsequently efficiency of the extraction. The most widely used solvent to extract edible oils from plant sources is hexane. Hexane is available at low cost and is efficient in terms of oil and solvent recovery (Mustakas, 1980; Serrato, 1981). More recently, the use of alternative solvents such as alcohols (isopropanol or ethanol) and supercritical carbon dioxide has increased due to environmental, health and safety concerns (Dunnuck, 1991). Alternative solvents are often less efficient due to a decreased molecular affinity between solvent and solute and costs for solvent and process equipment can be higher (Baker & Sullivan, 1983; Freidrich & Pryde, 1984; Karnofsky, 1981).

A potential new technology that may improve extraction of lipophilic compounds from plants is high-intensity ultrasound. High-intensity ultrasonication can accelerate heat and mass transport in a variety of food process operations and has been successfully used to improve drying, mixing, homogenization and extraction (Fairbanks, 2001; Mason, 1992; Mason, Paniwnyka, & Lorimera, 1996; Povey, 1998). Ultrasonication is the application of high-intensity, high-frequency sound waves and their interaction with materials (Luque-García & Luque de Castro, 2003). The propagation and

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interaction of sound waves alters the physical and chemical properties of materials that are subjected to ultrasound (Mason & Lorimer, 1988). In the case of raw plant tissues, ultrasound has been suggested to disrupt plant cell walls thereby facilitating the release of extractable compounds and enhance mass transport of solvent from the continuous phase into plant cells (Vinatoru, 2001).

Hui, Etsuzo, and Masao (1994) utilized ultrasound to extract saponin from ginseng and observed that yield of total extraction increased by 15% and yield of saponin by 30%. Romdhane and Gourdon (2002) investigated extraction of pyrethrines from pyrethrum flowers and oil from woad seeds. In both cases, acceleration of extraction kinetics and increase in yield was observed, however less so in the case of woad seeds. Vinatoru et al. (1997) showed improved yields of lipophilic compounds extracted from herbs such as coriander and fennel.

Based on these studies, we hypothesize that application of high-intensity ultrasound may improve extraction of oil from soybeans. The objective of this study was to test this hypothesis by determining the influence of sonication time and intensity in combination with different solvents on the efficiency of oil extraction from soybeans.

2. Materials and methods

2.1. Materials

Two soybean varieties, TN 96-58, a popular Tennessee variety, and N 98-4573, a North Carolina specialty variety, were obtained from the Crops Laboratory at The University of Tennessee. Compositional analysis of the two soybean varieties indicated a total lipid content of 19.6% for TN 96-58 and 19.1% for N 98-4573, a protein content of 42.2% for TN 96-58 and 42.7% for N 98-4573 and an ash content of 5.43% for TN 96-58 and 5.34% for N 98-4573 (Stassi, 2003). AOCS Mix No. 3, a fatty acid standard for GC analysis, was purchased from Alltech Corporation (Deerfield, IL, USA) and kept in a refrigerator at 4 °C until analysis. Hexane and isopropanol (99.8% purity) were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

2.2. Methods

2.2.1. Soybean flake preparation

Raw soybeans were cleaned using a grading procedure established by the Federal Grain Inspection Service (FGIS, 1997) to remove any foreign material such as small stones, sand and plant leaves that may be present after harvesting, drying, transportation and storage. Soybeans (125 g) were sieved and soybeans larger than 3.18 cm ($\frac{8}{64}$ in.) were collected. The cleaned, raw soybeans (moisture content approx. 8% w.b.) were stored in a environmental chamber containing potassium iodide solution (69.9% relative humidity at 22 °C) to adjust their moisture content to the optimal value suitable for subsequent grinding and extraction (Liu, 1999). Moisture content of soybeans was recorded every two hours using a single kernel moisture tester (CRT-160E, Shizuoka Seiki, Japan) until a final moisture content of 11% was reached. Cleaned and conditioned soybeans were ground using a hammer mill (Standard Model No. 3, Arthur Thomas Co., Philadelphia, PA, USA) running at 478 RPM. A stainless steel screen with a mesh size of 4 mm was used to obtain a consistent particle size distribution of soybean flakes (Fig. 1). Ground soybean flakes were then packaged in air-tight plastic bags until used.

2.2.2. Sonication and extraction procedure

Ground soybean flakes (100 g) were mixed with 150 ml solvent in a 600 ml plastic beaker. The soybean–solvent suspension was ultrasonicated for 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 h using a 20 kHz ultrasonic generator (S3000, Misonix Incorporated, Farmingdale, NY, USA) with a 1.27 cm probe that was submerged in the suspension. Ultrasonic wave intensities were determined calorimetrically (Eq. (3)) and ranged from 16.4 to 47.6 W/cm^2. Suspensions were kept in a waterbath at 25 °C during sonication and extraction. Suspensions were continuously stirred at a constant stirring rate using a magnetic stirrer to prevent heating of suspensions under the influence of high-intensity ultrasound. Controls included soybean flakes that were extracted using the same solvent without applying ultrasound. After extraction, oil was separated from the solvent–soybean suspension using a countercurrent distillation set-up with the heat source set to 110 ± 5 °C and water as the coolant (Li, 1999).

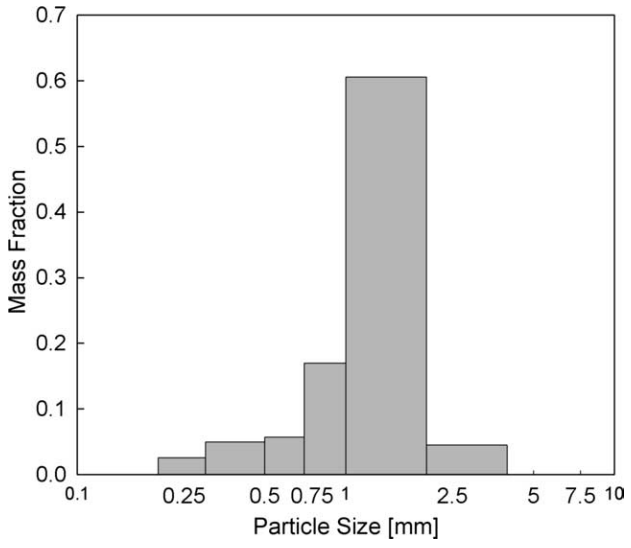

Fig. 1. Particle size distribution of ground soybeans.

2.2.3. Yield determination

Extraction yield was determined gravimetrically as

$$Y = \frac{m_e/m_t}{m_l/m_t} = \frac{x_{el}}{x_{tl}}, \quad (1)$$

where m_e is the mass of extracted lipids (g), m_t the ground soybean weight (g), m_l the total lipid mass of the soybean flakes (g), x_{el} the extracted lipid fraction and x_{tl} the total lipid fraction of soybeans (19.6% and 19.1% for TN 96-58 and N 98-4573, respectively).

2.2.4. Calorimetric determination of ultrasonic wave intensities

The intensity of the generated ultrasonic wave was determined using a calorimetric method (Mason et al., 1996). For each suspension, the temperature T was recorded with a thermocouple as a function of time under adiabatic conditions. From temperature versus time data, the initial temperature rise dT/dt was determined by polynomial curve fitting. The absolute ultrasonic power P was calculated as

$$P = mc_p\left(\frac{dT}{dt}\right), \quad (2)$$

where m is the total mass and c_p is the heat capacity of the solvent. The intensity of ultrasonic power dissipated from a probe tip with radius r is given by

$$I = \frac{P}{\pi r^2}. \quad (3)$$

For input power levels of 90, 120 and 180 W, the calculated intensities were 16.4, 20.9 and 47.6 W/cm^2, respectively.

2.2.5. Fatty acid profile determination by GC

Fatty acid (FA) profiles were determined according to the AOCS official methods that describe preparation of FAME (Ce 2-66) and GC analysis (Ce 1-62) (AOCS, 1998). FA profile determination included extraction of lipid samples with organic solvents, followed by transformation of the isolated lipid to fatty acid methyl esters (FAME) and quantification of FAME by gas chromatography. FA profiles were analyzed using a Hewlett–Packard 6890 gas chromatograph with cold on-column injection in a capillary column (HP-2980 (30 m × 0.25 mm × 0.1 μm)) and by flame ionization detection. Injection temperature was set at 130 °C, rising at 3 °C/min to 210 °C with a 10 min holding time and a detector temperature of 250 °C. Helium carrier-gas column flow rate was 1.8 ml/min with a make-up gas flow rate of 30 ml/min. The flow rate of hydrogen and air was 40 ml/min and 400 ml/min, respectively. Prepared FAME (2 μl) was introduced into the GC with a split ratio of 1:10. The ratio of unsaturated fatty acid to saturated fatty acid content was used as an indicator for soybean oil compositional changes.

2.2.6. Electron microscopy

An in-lens field emission scanning electron microscope (S-3500N, Hitachi SEM) was used at an operating voltage of 20 kV at a vacuum of 15 Pa. High resolution topographic images at low (100×), medium (1000×) and high (4000×) magnifications were digitally recorded with short dwell times to prevent beam induced damage. Samples were deposited on a silicon wafer and coated with a conductive material (gold) to ensure sufficient electron refraction.

2.2.7. Statistical analysis

Duplicate samples were used. All measurements were conducted in triplicates. Least square means were analyzed using the general linear model of the Statistical Analysis System (SAS Institute, Cary, NC).

3. Results and discussion

3.1. Solvent extraction in the absence of high-intensity ultrasound

The oil extraction capabilities of three different solvents (hexane, isopropanol, and hexane:isopropanol mixture, 60:40%, v/v) at extraction times ranging from 30 min to 3 h are shown in Fig. 2. When the extraction time increased from 30 min to 3 h oil yield of TN 96-58 increased by 4.5%, 5.8% and 8.8% using isopropanol, hexane, and the mixed solvent. In general, oil yield increased with treatment time irrespective of the type of solvent used, but the mixed solvent was superior in terms of oil yield increase (approx. 9%) when compared

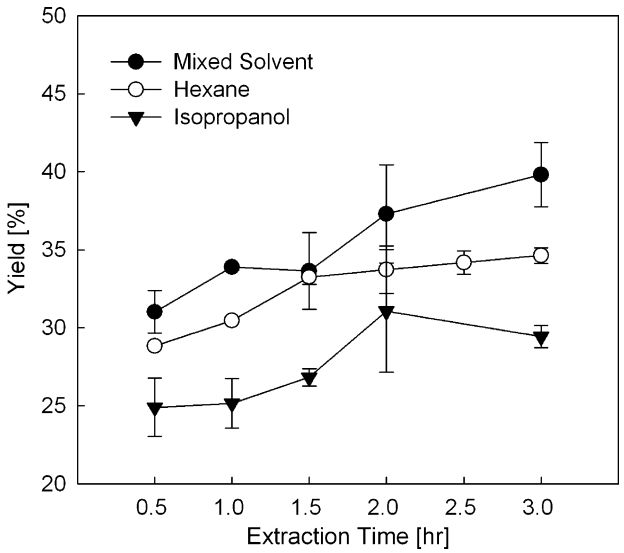

Fig. 2. Oil yield as a function of extraction time for soybean variety TN 96-58 using hexane, isopropanol and hexane:isopropanol as solvents at 25 °C.

to the efficacy of hexane or isopropanol. After 30 min using the mixed solvent, oil yield was 3.9% higher than that of hexane, which in turn was 2.2% higher than that of isopropanol. When the treatment time was increased to 3 h, the oil yield using the mixed solvent was 5.2% higher than that of hexane, which was 5.2% higher than that of isopropanol.

Our results indicate that the efficiency of the extraction process is a function of the molecular affinity between solvent and solute in agreement with earlier studies (Meniai & Newsham, 1992). The higher efficiency of the isopropanol:hexane mixture has previously been reported by Hara and Radin (1978) in a lipid extraction experiment using rat and mouse tissue and more recently by Schäfer (1998) who extracted cereal lipids using a 2:3 isopropanol:hexane mixed solvent. It should be noted that the overall extraction efficiency of our simplified extraction method after 3 h was low (absolute oil yields: 34.6% for hexane, 20.4% for isopropanol and 39.8% for hexane:isopropanol). This may be attributed to the fact that: (a) hulls were not removed in our simplified extraction procedure as is often practiced commercially, (b) the asymptotic final yield may only be obtained after significantly longer extraction times and (c) the use of a hammer mill instead of a flaking roll may yield non-optimal particle sizes. Thus higher yields may be obtained in a commercial process.

3.2. Influence of ultrasonic wave intensity on oil yield

The influence of different ultrasound intensity levels (16.4, 20.9, and 47.6 W/cm^2) on oil yield is shown in Fig. 3. Oil yield increased with increasing ultrasonic intensity. After 3 h at an ultrasound intensity of 47.6 W/cm^2, the increase in oil yield was 2.4% higher than at an ultrasonic intensity of 20.9 W/cm^2 and 9% higher than at 16.4 W/cm^2 (Fig. 3). Compared to the nonsonicated control, the oil yield after 3 h at 16.4, 20.9 and 47.6 W/cm^2 increased by 2.2%, 10.1% and 11.2% respectively. Thus, after three hours, the relative oil yield increase at 47.6 W/cm^2 was approximately five times higher than at 16.4 W/cm^2.

Improved soybean oils yields may be explained in terms of cavitational effects caused by the application of high-intensity ultrasound. As large amplitude ultrasound waves travel through a mass medium, they cause compression and shearing of solvent molecules resulting in localized changes in density and elastic modulus (Price, White, & Clifton, 1995). As a consequence, the initially sinusoidal compression and shear waves will at a finite distance from the ultrasonic transducer be distorted into shock waves. The abrupt decrease in pressure at the edge of the saw tooth shaped ultrasonic wave in the negative pressure cycle generates small bubbles. These bubbles collapse in the positive pressure cycle and produce turbulent flow conditions associated with high

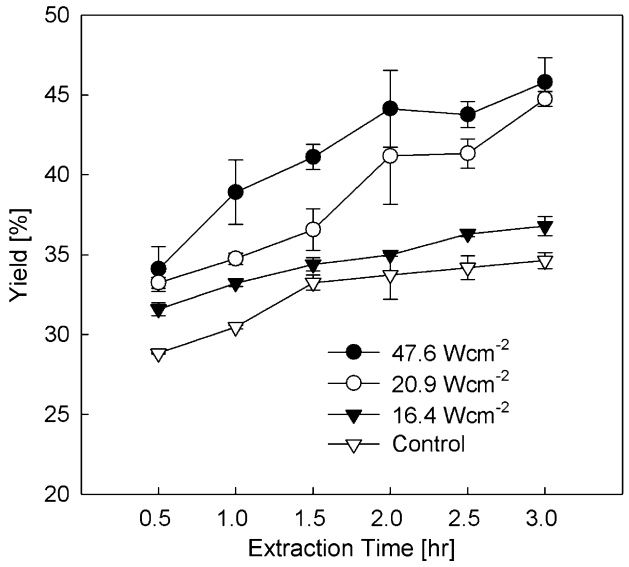

Fig. 3. Oil yield as a function of extraction time for soybean variety TN 96-58 using high-intensity ultrasound at ultrasonic intensities of 0, 16.4, 20.9 and 47.6 W/cm^2 using hexane as a solvent at 25 °C.

pressures and temperatures (Mason, 1997; Mason & Cordmas, 1996; Mason, 1992; Price, 1990, 1993). Since formation and collapse of bubbles occurs over very short periods of time, typically a few microseconds (Hardcastle et al., 2000), heat transfer from cavitational bubbles to the medium is small causing only gradual temperature increases in the medium. Therefore, decreases in solvent viscosity are small and are most likely not the principal cause of the yield increases. Rather, at increasing amplitudes, cavitational bubble collapse is more violent since the resonant bubble size is proportional to the amplitude of the ultrasonic wave (Suslick, Casadonte, Green, & Thompson, 1987; Suslick & Price, 1999). Bubble collapse in the vicinity of plant membranes may cause strong shear forces to be exerted that can cause microfractures to be formed in plant tissues (Vinatoru, 2001; Vinatoru et al., 1997).

Fig. 6 shows a set of SEM images of TN 96-58 soybean flakes at a magnification factor of 1000× (a) after 3 h of conventional hexane extraction, (b) 1 h of ultrasound-assisted hexane extraction and (d) 3 h of hexane assisted extraction. Microfractures appeared in the soybean flakes after application of ultrasound for 1 h (Fig. 6(b)) and the surface morphology of soybean flakes visibly changed after 2 h of sonication (Fig. 6(c)) that is the soybean flake surfaces became more porous.

3.3. Influence of soybean varieties on ultrasound-assisted extraction of soybean oil

The oil yield of both varieties of soybeans increased with application of ultrasound (Fig. 4) but the relative increase in oil yield of the two soybean varieties with extraction time differed. For the TN 96-58 variety, the yield

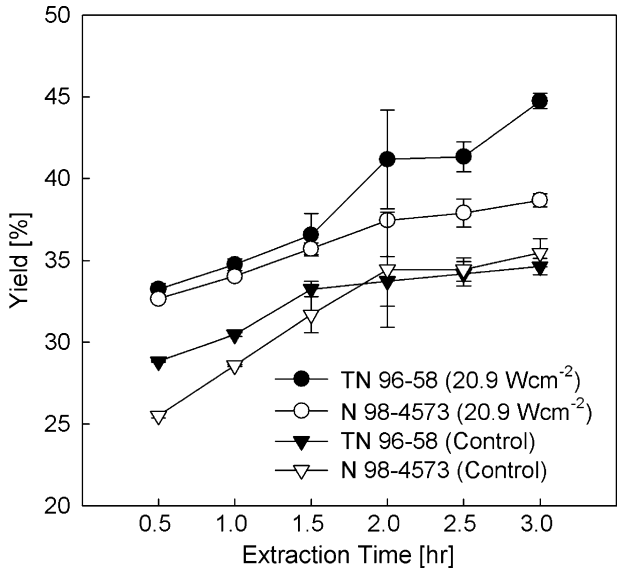

Fig. 4. Oil yield as a function of extraction time for soybean varieties TN 96-58 and N 98-4563 treated with ultrasound at an intensity of 20.9 W/cm^2 using hexane as a solvent at 25 °C.

increased by 4.4% between the control group and the 20.9 W/cm^2 ultrasound-assisted group at a reaction time of 30 min to reach a yield difference of 9.4% after 3 h. For this variety, ultrasound had a more pronounced effect on the yield in the latter stage of the extraction. In contrast, for N 98-4573, the oil yield difference between the control and the ultrasound-assisted group was 7.1% after extraction/sonication for 30 min and increased only by another 3.2% after 3 h. For this variety, ultrasound enhanced oil yield particularly in the early stage of the extraction process.

Results shown in Fig. 4 may be related to difference in soybean structure (Romdhane & Gourdon, 2002). As noted by Romdhane and Gourdon (2002), the rheological nature of the seed structure (hardness, compactness) may have a direct impact on the capability of ultrasound to improve extraction of lipid compounds from plant cells. While a compositional analysis of the two soybean varieties showed little difference between the two varieties in protein content (42.7% for N 98-4573 and 42.2% for TN 96-56), ash content (N 98-4573: 5.34%; TN 96-56: 5.43%) and total lipid content (N 98-4573: 19.1%; TN 96-56: 19.6%), a more in-depth analysis of the cell wall structure may help explain the exact nature of the observed differences between the two plant varieties.

3.4. Influence of molecular properties of solvents on ultrasound-assisted extraction of soybean oil

The difference between oil yield obtained with hexane and isopropanol as solvents after 30 min using the classical extraction process was 3.9% (Fig. 5). When the reaction time was increased to 3 h, the difference in yield increased slightly to 5.2%. Comparison of the relationship between yield and extraction time for the classical extraction using different solvents illustrates that the selection of solvent influences oil yield. In the case of ultrasound enhanced extraction using pure hexane and isopropanol, the difference between yields was less pronounced. After 30 min, the oil yield using hexane was 2.4% higher than with isopropanol. When the reaction time was increased to 3 h, the oil yield obtained with isopropanol was 1.1% higher than with hexane as a solvent. The difference between the ultrasound-assisted and the control group after 30 min of extraction using hexane was 4.4% while the difference between ultrasonicated and untreated soybeans using isopropanol was 5.9%. At a reaction time of 3 h, the difference increased to 10.1% and 16.4%, respectively. It is apparent in Fig. 5 that in the ultrasound-assisted extraction operation there was a greater increase in oil yield when isopropanol was used as a solvent than when hexane was used.

A solvent mixture was prepared by mixing hexane and isopropanol at a ratio of 60:40% (v/v). Oil yields obtained with all three solvents (hexane, isopropanol and the solvent mixture) both with and without ultrasound assistance are shown in Fig. 5. The mixed solvent clearly had a much better extraction performance than any of the other solvents. At an extraction time of 30 min, the oil yield using the mixed solvent was 2.2% higher than with hexane and 6.1% higher than with isopropanol. When the reaction time was increased to 3 h, the oil yield using the mixed solvent group increased by 5.2% and 10.4% when compared to hexane and isopropanol, respectively. The extraction capability of the mixed solvent was further

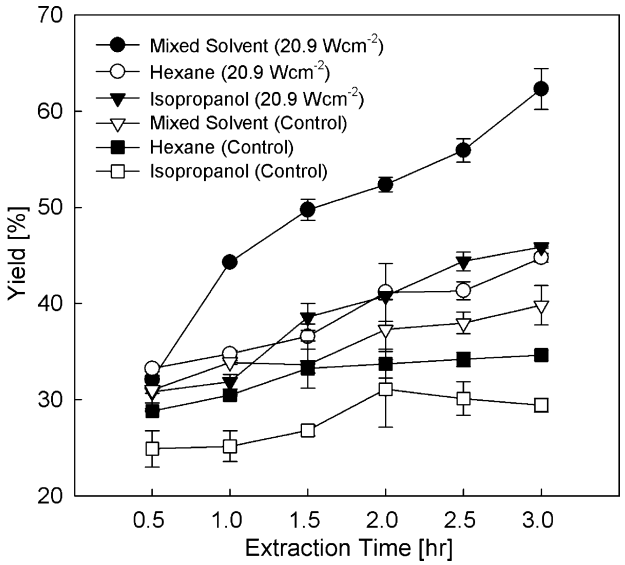

Fig. 5. Oil yield increase of soybean variety TN 96-58 as a function of extraction time using hexane:isopropanol mixture, hexane and isopropanol and treated with (20.9 W/cm^2) and without ultrasound at 25 °C.

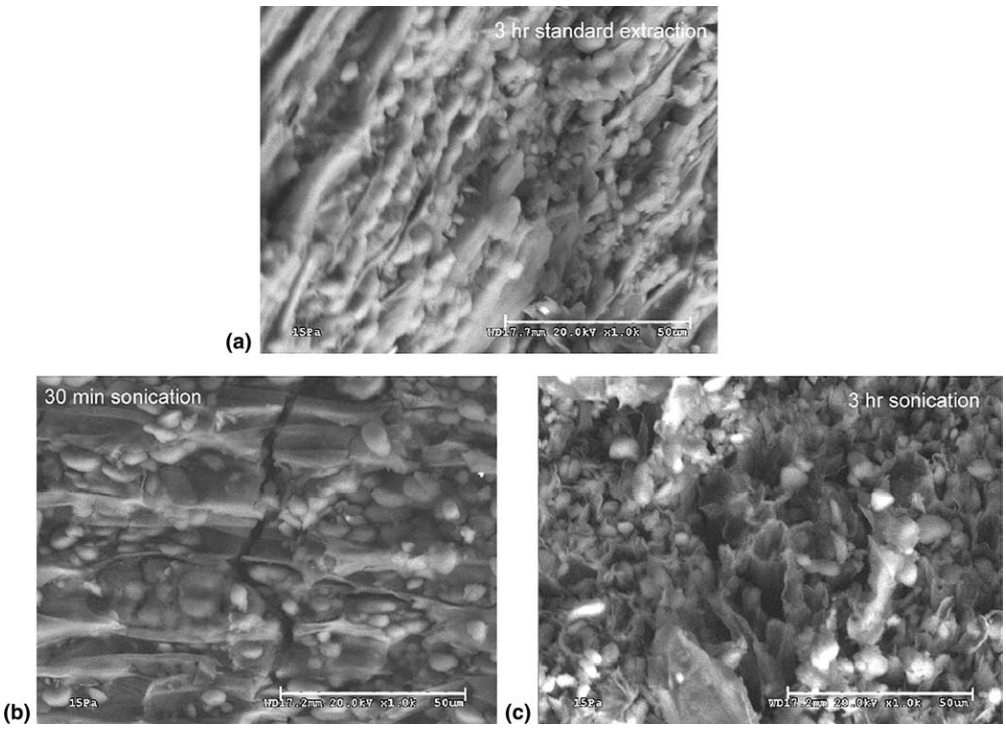

Fig. 6. Scanning electron microscopy images of soybean flakes after (a) 3 h classical hexane extraction, (b) 30 min ultrasound-assisted extraction and (c) 3 h ultrasound-assisted extraction.

enhanced by the application of ultrasound. The absolute oil yield was 32.0% after 30 min and increased almost twofold to 62.3% after 3 h when used in combination with ultrasound. The difference between the ultrasound-assisted group and the control group was only 1.0% at the beginning of the extraction. However, when a reaction time of 3 h was used, the difference increased to 22.5%. These results indicate that for the mixed solvent, reaction time is an important processing parameter affecting the oil yield.

Cavitation in a liquid continuous phase is impacted by the physical properties of the solvent. At 25°C, isopropanol has a vapor pressure of 43 mbar, a viscosity of 2.27 mPas, a density of 0.785 g/cm^3 and a surface tension of 21.7 mN/m while hexane has vapor pressure of 266 mbar, a viscosity of 0.31 mPa, a density of 0.664 g/cm^3 and a surface tension of 18.4 mN/m. Chivate and Pandit (1995) demonstrated for binary mixtures of ethanol and water that vapor pressure and surface tension are the two key factors that impact the cavitation intensity at a specific distance from the horn generator, i.e. cavitation intensity decreases as vapor pressure and surface tension increases. While the surface tension of the two solvents does not differ significantly, the vapor pressure of hexane is approximately five times higher than that of isopropanol. As previously stated, solvent affinity between oil and the mixed solvent is higher than for hexane or isopropanol (Hara & Radin, 1978; Schäfer, 1998). Results may thus be attributed to solvent–solute affinity and cavitational phenomena. Nevertheless additional studies will be required to quantify the contribution of the individual effects of high-intensity ultrasound and solvent on oil yield and to gain a better understanding of the mechanism of ultrasonication.

3.5. FA analysis of ultrasonically extracted soybean oil

Results of the GC analysis of sonicated and untreated soybean oil show a small decrease in the relative percentage of unsaturated fatty acids and an increase in the percentage of saturated fatty acids when ultrasound-assisted extraction was used (Table 1). This ratio is used as an indicator of the extent of fat deterioration because unsaturated fatty acids are more susceptible to oxidation, whereas saturated fatty acids are more stable to oxidation. In the control group, the C18:1/C16:0 ratio was 1.54 while in ultrasound-assisted extraction group it decreased to 1.49. The oxidation percentage was 3.4%. The ratio of C18:2/C16:0 was 5.08 and 5.05 in the control and ultrasound-assisted group, respectively. A difference of 0.52% in the linoleic acid content was observed. Results would indicate that oxidation of soybean oil does occur upon application of ultrasound, however the difference in the GC analysis between the ultrasonicated and the control group was small suggesting that ultrasonication did not noticeably influence composition of the extracted oil.

Table 1
Comparison of ratio of unsaturated to saturated fatty acid (C18:2/C16:0, C18:1/C16:0) of oil extracted for 3 h from TN 96-58 using hexane as a solvent with and without high-intensity ultrasound (47.6 W/cm^2)

Retention time (minutes)	Ultrasound-assisted (U)		Control group (C)		U/C ratio
	Peak area	Ratio	Peak area	Ratio	
8.02 ± 0.01 (16:0)	130.01	1	128.29	1	100.00
11.92 ± 0.02	38.6	0.2969	39.52	0.3080	96.40
12.35 ± 0.02 (18:1)	193.11	1.4852	197.27	1.5376	96.59
12.49 ± 0.02	15.96	0.1228	16.03	0.1250	98.24
13.34 ± 0.05 (18:2)	656.48	5.0491	651.14	5.0756	99.48
14.82 ± 0.02	90.46	0.6957	89.7	0.6992	99.50
16.46 ± 0.02	3.6	0.0277	3.83	0.0299	92.64
16.90 ± 0.02	2.4	0.01855	2.4	0.0187	99.20
22.33 ± 0.03	4.5	0.03461	4.77	0.0372	93.04

4. Conclusions

The results obtained in this study have implications for the edible oil industry. Ultrasound has the potential to be used in oil extraction processes to improve efficiency and reduce processing time. During commercial solvent extraction, a series of time-consuming preparation steps is necessary to achieve the maximum oil yield. These key steps involve cleaning, dehulling, moisture conditioning, flaking and heating. Our study demonstrated that a simplified, short term extraction procedure that utilizes ultrasound during the extraction process may be sufficient to obtain commercially acceptable yields. Careful consideration should be given to the choice of an appropriate solvent. The influence of the molecular affinity between solvent and solute is not the only parameter that impacts the suitability of solvent as is the case in classical extraction technologies. Factors that impact cavitation such as solvent vapor pressure and surface tension need to be considered as well.

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Decarboxylation of Δ^9-tetrahydrocannabinol: Kinetics and molecular modeling

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ABSTRACT

Efficient tetrahydrocannabinol (Δ^9-THC) production from cannabis is important for its medical application and as basis for the development of production routes of other drugs from plants. This work presents one of the steps of Δ^9-THC production from cannabis plant material, the decarboxylation reaction, transforming the Δ^9-THC-acid naturally present in the plant into the psychoactive Δ^9-THC. Results of experiments showed pseudo-first order reaction kinetics, with an activation barrier of 85 kJ mol^{-1} and a pre-exponential factor of 3.7×10^8 s^{-1}.

Using molecular modeling, two options were identified for an acid catalyzed β-keto acid type mechanism for the decarboxylation of Δ^9-THC-acid. Each of these mechanisms might play a role, depending on the actual process conditions. Formic acid proved to be a good model for a catalyst of such a reaction. Also, the computational idea of catalysis by water to catalysis by an acid, put forward by Li and Brill, and Churchev and Belbruno was extended, and a new direct keto-enol route was found. A direct keto-enol mechanism catalyzed by formic acid seems to be the best explanation for the observed activation barrier and the pre-exponential factor of the decarboxylation of Δ^9-THC-acid. Evidence for this was found by performing an extraction experiment with Cannabis Flos. It revealed the presence of short chain carboxylic acids supporting this hypothesis. The presented approach is important for the development of a sustainable production of Δ^9-THC from the plant.

1. Introduction

At present there is a growing interest in cannabis and its medicinal uses [1,2]. Cannabis contains more than 400 different ingredients, including at least 60 cannabinoids. The major active component, called (−)-Δ^9-tetrahydrocannabinol (Δ^9-THC), does not occur at significant concentrations in the plant, but is formed by decarboxylation of its corresponding acid upon heating.

As described in a patent [3], Δ^9-THC acid (Δ^9-THCA) is obtained from plant material by extraction into an aqueous solvent under basic pH conditions. After acidification of aqueous fraction, the aqueous fraction was extracted using a non-polar solvent, yielding the acid in high purity in organic solvent. Δ^9-THCA is then converted to Δ^9-THC which is further purified and combined with a carrier for pharmaceutical use. The total process includes seven different steps and four purification steps and requires a lot of energy, while producing a lot of inorganic/organic contaminated water. The contaminations are mainly inorganic salts and organic waste; principally organic solvents such as heptane and isopropyl ether. To improve this production process, reducing the number of process steps, energy consumption, water consumption and waste production, is crucially important. In a recent patent [4], both Δ^9-THCA and Δ^9-THC are extracted into an organic solvent followed by decarboxylation with aqueous base in the same solvent. Despite the obvious improvement presented, many process steps are still needed to obtain pure Δ^9-THC. In our view, the ideal process would start from a solid plant source with the highest level of Δ^9-THCA, which then is extracted, decarboxylated, and purified in the minimum number of steps, avoiding water, inorganic salts, and organic solvents.

As most cannabinoids in the plant, including Δ^9-THC, are present as their acid precursor, decarboxylation in the solid phase (i.e. in the plant material) followed by extraction into a neutral solvent, might be considered a viable option. Previous work on the decarboxylation of cannabinoids in the solid phase has been performed in closed reactors [5,6], open reactors and on a glass surface [7]. However, little research has been performed to understand the kinetics and the mechanism of solid state reaction in cannabis, despite the fact that these are crucial for scale-up.

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The first section of this paper presents experimental work to determine the best reaction conditions (i.e. temperature and time) for decarboxylation and its kinetics. Molecular modeling is then used to support or justify proposed mechanism and kinetics parameters for this solid state reaction in accordance with available literature and experimental data herein.

2. Experimental

2.1. Materials

Methanol was HPLC grade and was purchased from J.T. Baker (Deventer, The Netherlands). Medical grade cannabis plant material (female flower-tops) was obtained from Bureau Medicinale Cannabis (The Hague, The Netherlands). It had a Δ^9-THCA content of about 18%, and virtually no free Δ^9-THC. The water content was ~3.6%. The standards of Δ^9-THC (4.2 mg mL^{-1} in methanol – ref number 130-151205x) and Δ^9-THCA (1.0 mg mL^{-1} – ref number 380-250407), with purity higher than 98%, were kindly donated by PRISNA B.V.

2.2. Method

A sample of around 400 mg cannabis was blended in a mixer, and heated at different temperatures in vacuum conditions for a certain time. The temperature range studied was from 90 to 140 °C. To follow the reaction rate, a sample was taken every 5 min for the first hour and then every half an hour until the conversion of Δ^9-THCA to Δ^9-THC was complete. Each solid sample was extracted with 50 mL methanol and sonicated for 15 min before being analysed with HPLC. In a series of extraction experiments it was determined that the extraction process was essentially complete. Calibration lines were determined for both Δ^9-THCA and Δ^9-THC. By this method the solid samples were inherently corrected for weight loss (up to ~30% at 140 °C) during thermal treatment. Balances during the experiments, based on the molalities of Δ^9-THCA and Δ^9-THC, are >95%, indicating that the decarboxylation process itself proceeds with ~100% selectivity. Some skeletal rearrangements however cannot be excluded.

2.3. HPLC analyses

The HPLC profiles were acquired on a Chromapack HPLC system consisting of an Isos pump, an injection valve and a UV–VIS detector (model 340 – Varian). The system is controlled by Galaxie Chromatography software. The profiles were recorded at 228 nm, as absorption by the solute is at its maximum at this wavelength. The analytical column was a Vydac (Hesperia, CA) C_{18}, type 218MS54 (4.6 × 250 mm^2, 5 μm). The mobile phase consisted of a mixture of methanol–water in a concentration gradient containing 25 mM of formic acid (pH ± 3). The methanol/water concentration ratio was linearly increased from 65% to 100% over 25 min, and then kept constant for 3 min. Then the column was re-equilibrated under initial conditions for 4 min, so the total running time was 32 min. The flow rate was 1.5 mL min^{-1} [8].

2.4. Molecular modeling

The Spartan '06 package [9] was used for all calculations. All structures were optimized using DFT B3LYP, level (6- 31G**), starting from PM3 optimized geometries. Transition states were identified and characterised using its unique imaginary vibrational frequency or Internal Reaction Coordinate. Thermodynamical corrections were applied; however activation energies were based on Total Energies, corrected for Zero Point Energy contributions (ZPE-contributions).

3. Results and discussion

3.1. Experimental results

Decarboxylation is a rather common chemical reaction in which a carboxyl group splits off from a compound as carbon dioxide. The reaction for Δ^9-THCA shown schematically in Fig. 1, can be induced by light or heat during e.g. storage or smoking. This reaction transforms the acidic cannabinoids to their psychoactive forms Δ^9-THC. In this article, only thermal decarboxylation will be considered. As described above, the decarboxylation reaction has been studied in the range of 90–140 °C. Under the experimental conditions, the highest yield to Δ^9-THC was obtained at 110 °C and 110 min. Analysis of the data leads to the conclusion that this solid state reaction surprisingly obeys a first order rate law. Raw kinetic data are presented in Fig. 2. Related k values are reported in Table 1. The corresponding ln k versus $1/T$ plots are shown in Fig. 3. This is a straight line, described by the formula:

$$\ln k = \ln k_0 - \frac{E}{RT}$$

from which E and k_0 are determined to be 84.8 kJ mol^{-1} and 3.7×10^8 s^{-1} respectively.

3.2. Literature results

In the literature, only a few liquid phase thermal decarboxylation reactions of carboxylic acids, both aromatic as well as non-aromatic, can be found [10–13]. Li and Brill reported experimental activation energies for the first order decarboxylation of a series of OH substituted benzoic acids under acidic conditions, ranging from 82 to 97 kJ mol^{-1} for 2,4,6-trihydroxybenzoic acid and 2,3-dihydroxybenzoic acid. Their k_0-values range from 3.61×10^{10} s^{-1} to 3.58×10^8 s^{-1}, the latter being similar to the one observed by us [13].

In addition, by applying computational chemistry techniques (B3LYP/6-31G*), Li and Brill found that intra-molecular decarboxylation of the acids via a four membered ring transition state yielded a very high activation barrier, thus suggesting that a real first order process is very unlikely. The calculated activation barriers for four-membered transition state for a series of caboxylic acids ranged from 213 kJ mol^{-1} for 2,4-dihydroxybenzoic acid, to 225 kJ mol^{-1} for 2-hydroxybenzoic acid, and with a constant value of 260 kJ mol^{-1} for 3-hydroxybenzoic acid, 3,5-dihydroxybenzoic acid, and benzoic acid itself.

Li and Brill also found that the addition of one molecule of water in the mechanism transformed the preferred transition state from a four membered ring to a six membered ring with concomitant reduction in the activation barrier to 130 kJ mol^{-1}, a value much closer to the experimental values. However, these values are still far too high, especially if it is realized that these barriers are based on the ~28 kJ mol^{-1} energetically unfavorable anti-conformer of the acid [10–13] which acts as a highly reactive intermediate.

Recently, Chuchev and BelBruno [14] published a study on the mechanism of the decarboxylation of ortho-substituted benzoic acids, wherein they supported the work of Li and Brill that a single water molecule is a potential model for an aqueous environment. In addition, they concluded that the presence of a water molecule forces the reaction through a keto-intermediate in the case of 2-hydroxybenzoic-acid. The keto-intermediate then intramolecularly decarboxylates to yield phenol and CO_2. The overall process is illustrated in Fig. 4. However, their calculated activation barrier for the decarboxylation of salicylic acid is ~150 kJ mol^{-1}, which is still

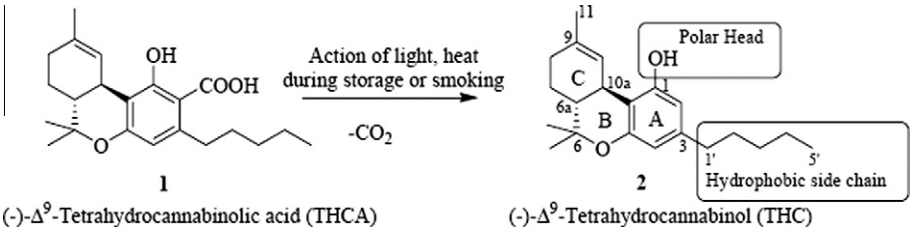

Fig. 1. Model of the decarboxylation reaction of Δ^9-THCA.

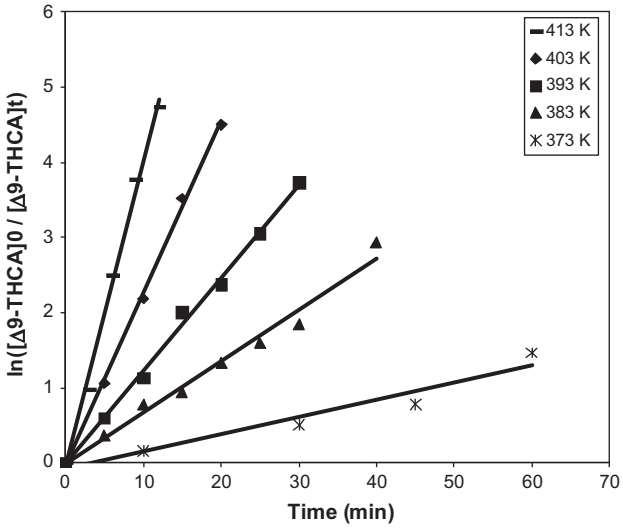

Fig. 2. Plot of $\ln[\Delta^9\text{-THCA}]_0/[\Delta^9\text{-THCA}]$ as a function of time at different temperatures.

Table 1
Values of the constant rate k and of the regression coefficient at different temperatures.

T (K)	$10^3\ k$ (s^{-1})	R^2
413	6.7	0.9949
403	3.8	0.998
393	2.1	0.9958
383	1.1	0.982
373	0.5	0.9426

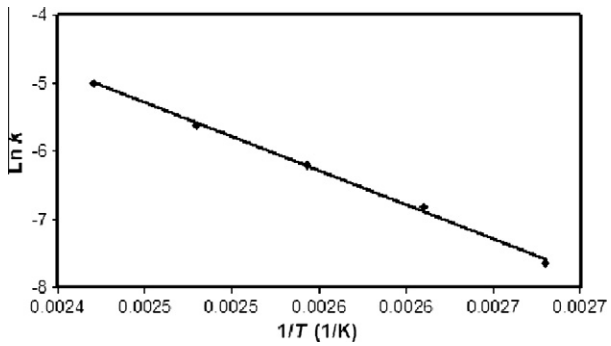

Fig. 3. ln k as a function of $1/T$ – Arrhenius' law.

significantly too high. Hence, it should be noted that the observed first order reaction can only be understood in terms of a pseudo-first order reaction on a molecular level.

For Δ^9-THCA in Cannabis Flos, the reaction takes place in a *solid* phase with a large amount of Δ^9-THCA (18 w% = 0.57 mol kg^{-1})

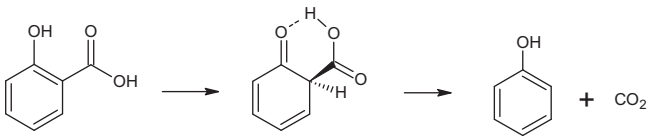

Fig. 4. Decarboxylation of 2-hydroxybenzoic acid via the β-keto acid pathway.

and a low amount of water (3.6 w% = 2.0 mol kg^{-1}). The low value for k_0 might be explained by the fact that it is a solid state reaction, or a catalytic process, leading to a pseudo-first order process. A molecular modeling study has been performed to test this hypothesis.

3.3. Molecular modeling results

Δ^9-THCA is a large molecule and therefore computationally intensive with respect to memory and time. 2-Hydroxybenzoic acid has been used as a suitable, simplest model for Δ^9-THCA. Furthermore both experimental and computational studies have been performed with 2-hydroxybenzoic acid. To allow a meaningful comparison between our work on Δ^9-THCA, and the existing literature on 2-hydroxybenzoic acid, the different options were investigated for 2-hydroxybenzoic acid first.

As a starting point we initially confirmed the computational work of Li and Brill [13], and Chuchev and BelBruno [14] with respect to the geometries of the transition states both for the direct uncatalyzed and one water molecule catalyzed pathways. The geometries look very similar, and important bond lengths are similar within 0.01 Å.

Next, a mechanism was developed in which an organic acid was used as a catalyst to assist in the decarboxylation reaction. This allows the adaptation of the actual acid strength of the catalyst or implicitly the pH of the environment, while avoiding computationally intensive calculations. A disadvantage might be that thermodynamic corrections become meaningless in most cases, except for the ZPE. However, this is already the case, particularly for the entropy contributions, as experiments were carried out in solid phase, but not in the gas phase.

To obtain a good computational model catalyst for the decarboxylation reaction, several acids were investigated and compared in Table 2, for the case of 2-hydroxybenzoic acid. For the decarboxylation of Δ^9-THCA catalysis the work was limited to formic acid and trifluoroacetic acid. As can be seen in Table 2, the differences in activation energies for 2-hydroxybenzoic acid in both pathways with acetic acid, formic acid and trifluoroacetic acid are within 5 kJ mol^{-1}. Thus the acid strength of the catalyst does not seem to be a large discriminator in the calculations. Using formic acid as a model catalyst, two different transition states, shown in Fig. 5, could be located, both leading to the previously mentioned keto-intermediate.

The structure of the transition state with a value of 93 kJ mol^{-1} resembles the geometry of the transition state proposed by Chuchev and BelBruno and illustrated in Fig. 6 [14], with the hydrogen of the acid of the substrate in anti-position. Churchev

Table 2
Calculated activation energies of salicylic acid and Δ^9-THCA with different acids as catalyst.

Acid catalyst	E_a 2-hydroxybenzoic acid (kJ mol^{-1}) Direct keto-enol	E_a 2-hydroxybenzoic acid (kJ mol^{-1}) Indirect keto-enol	E_a Δ^9-THCA (kJ mol^{-1}) Direct keto-enol
Acetic acid	105	89	Not determined
Formic acid	104	93	81, 58^{indirect}
Trifluoroacetic acid	100	88	71

et al. reaction pathway presented in [14], shows in fact a three proton transfer process, starting with protonation of the α-C next to the COOH-group, followed by the transfer of the proton in anti-position of the substrate COOH-group to the catalyst, and finally proton transfer of the phenol group to the carboxylate group of the substrate. This mechanism will be referred to as indirect keto-enol pathway.

The pathway with an activation barrier of 104 kJ mol^{-1} resembles a direct keto-enol pathway. Fig. 7 shows the IRC-plots of the formation of the keto-isomer of 2-hydroxybenzoic acid with formic and trifluoroacetic acid as catalyst via the keto-enol pathway. The

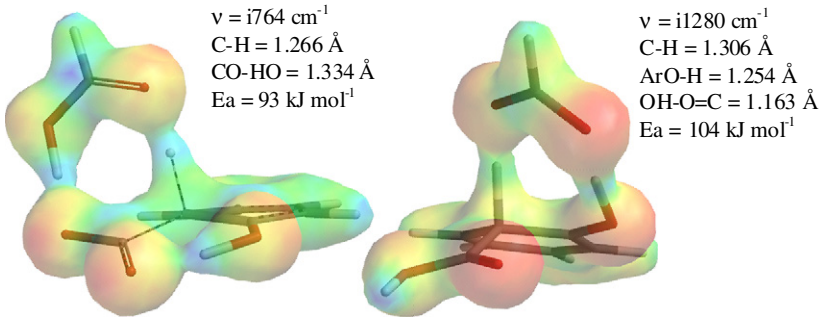

Fig. 5. The two transition states for formic acid catalyzed decarboxylation of 2-hydroxybenzoic acid.

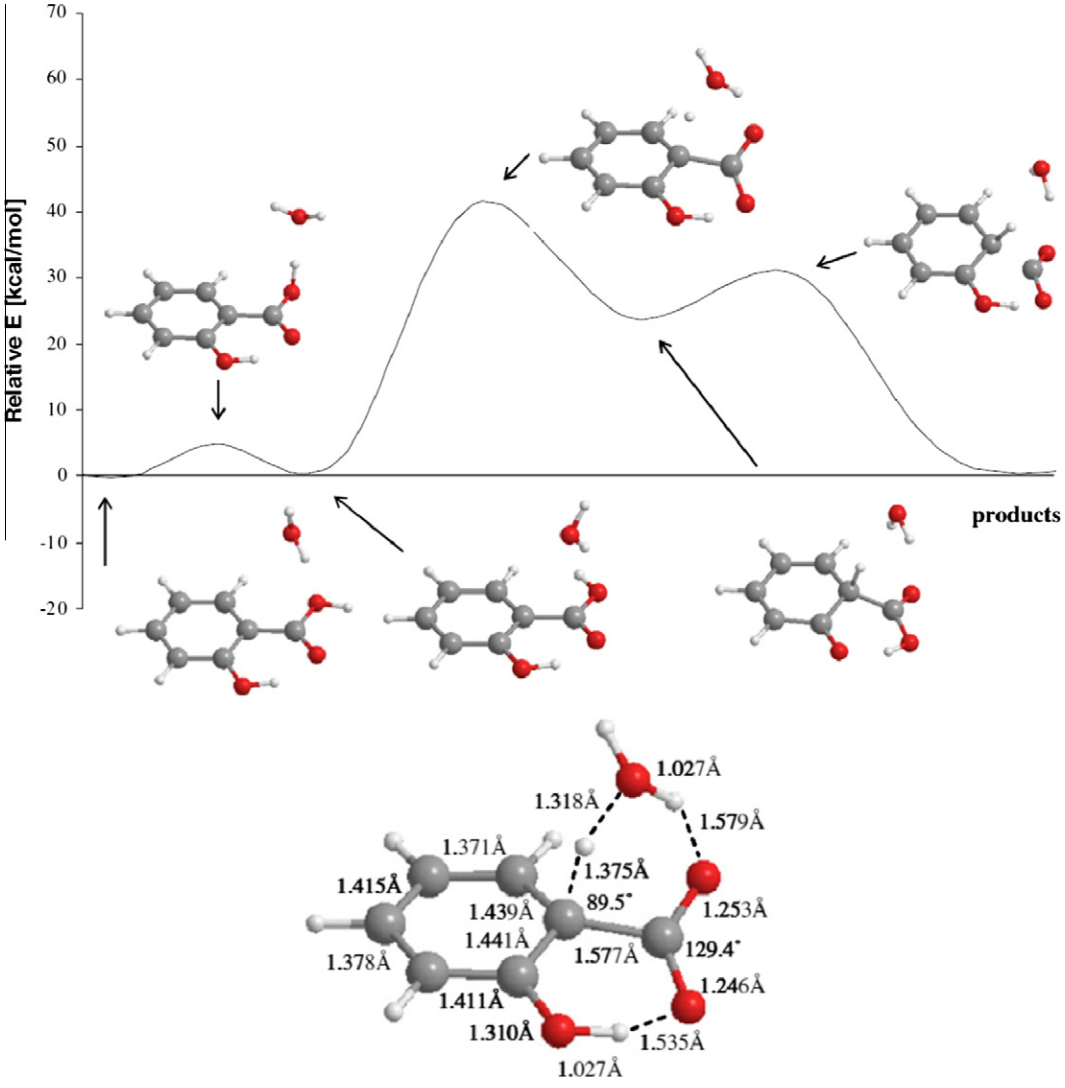

Fig. 6. Indirect keto-enol pathway according to Churchev and BelBruno [14]; structural details of the keto-enol transition state are listed below.

distance between the phenolic O–H atoms was taken as a measure for the reaction coordinate. The reaction starts from the phenol and ends with the keto-isomer. The geometries of the transition states change only slightly.

For Δ^9-THCA decarboxylation, the computed activation barrier for the catalyzed direct keto-enol route with formic acid (81 kJ mol^{-1}) compared well with the experimental value (85 kJ mol^{-1}). However, the computed activation barriers for trifluoroacetic acid (71 kJ mol^{-1}) and the indirect keto-enol pathway (58 kJ mol^{-1}) are much lower than the experimental values. Fig. 8 shows the IRC and the transition state of the first step of the formic acid catalyzed decarboxylation of Δ^9-THCA. Fig. 9 shows the overall reaction energy profile of the entire reaction, including the second step, the intra-molecular proton transfer of the acid to the keto-function.

3.4. Discussion

Aliphatic and aromatic acids are usually present [15] as plant constituents in cannabis. Inspired by the results of molecular modeling, the presence of acids other than Δ^9-THCA was verified experimentally. A sample of around 400 mg of cannabis was blended in a mixer, and extracted with distilled water after sonication for 10 min. The pH of the resulting aqueous solution was 6.1. A sample of 1600 mg of cannabis, yielded an aqueous solution with pH = 5.5. Under these conditions, Δ^9-THCA does not dissolve into water but short chain carboxylic acids do. Thus, acetic acid or formic acid not only can be used as a *model* for acid catalysis, but might be a realistic case from an experimental point of view as well. Furthermore, it offers a plausible explanation for the low value of k_0, as the experimental acidity is low.

To get a better overall understanding of the two different mechanistic options in acid catalyzed decarboxylation, Table 3 shows the comparison of experimental values with computational results obtained for a series of 2-hydroxybenzoic acids with formic acid as catalyst. Experimental data are scarce but, fortunately, well documented [13,14]. For the decarboxylation of 2-hydroxybenzoic acid two experimental activation energies are reported: 97.4 kJ mol^{-1} in catechol (weak acid), and 92 kJ mol^{-1} as an average of two distinct values: 91.4 kJ mol^{-1} in an HCl-solution of pH = 1.3, and 92.7 kJ mol^{-1} in an HCl-solution of pH = 2.7, thus showing a marked influence of both solvent and pH. A similar observation can be made for 2,6-dihydroxybenzoic acid. Here three values are reported: 111.1 kJ mol^{-1} in catechol, 92.7 kJ mol^{-1} at pH = 1.4 and 100.7 kJ mol^{-1} at pH = 2.0. Again, the dependence of the experimental activation energy on solvent type and pH is remarkable.

As can be seen from Table 3, the lowest value for the activation energy of 2-hydroxybenzoic acid, obtained experimentally in a strongly acidic environment, corresponds computationally with the indirect keto-enol pathway yielding an activation barrier of 92 kJ mol^{-1}. The latter requires the presence of a proton (in anti-position) of the substrate acid function. Under strongly acidic conditions this requirement is fulfilled. Under less acidic conditions this is not the case, and then the direct keto-enol pathway comes into play, resulting in an activation barrier of 104 kJ mol^{-1}.

The case of 2,6-dihydroxybenzoic acid is more complicated. It is a significantly stronger acid than 2-hydroxybenzoic acid, so the requirements for the indirect keto-enol pathway are no longer fulfilled in an HCl-solution of pH = 1.4. The direct keto-enol pathway leads to an activation barrier of 92 kJ mol^{-1}, close to the experimental value. The next experimental value of 101 kJ mol^{-1} at pH = 2.0, can be understood as a loss of coordination of one of the phenolic groups to the adjacent acid group due to the higher pH. Calculations for these systems lead to an activation barrier of 102 kJ mol^{-1}. With respect of the experimental work in catechol, computations with either formic acid or catechol itself as an acid catalyst, the indirect keto-enol pathway leads to an activation barrier of 114 kJ mol^{-1} close to the experimental value. The indirect pathway here is rationalized by the fact that 2,6-dihydroxybenzoic acid in catechol will not dissociate. Furthermore, it shows that formic acid can even act as a reasonable model for catechol.

From the computational results obtained it would be tempting to speculate what the activation barrier would become if strongly acidic conditions were applied in the case of Δ^9-THCA. However, the application of strong acids, containing halogens or sulfur would not contribute to the sustainability of the overall process.

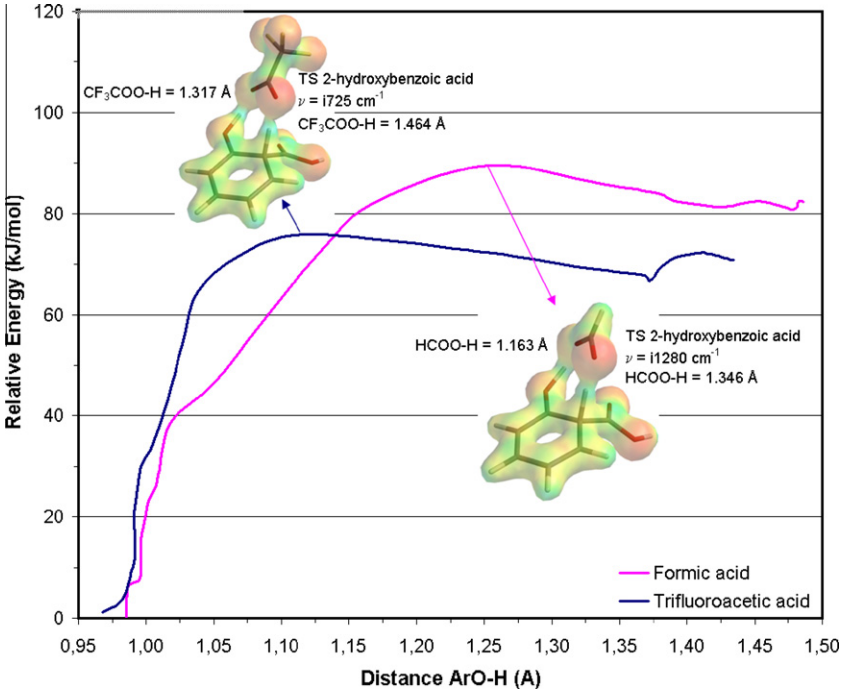

Fig. 7. IRC's of the formation of the keto-isomer of 2-hydroxybenzoic acid decarboxylation catalyzed by trifluoroacetic acid and formic acid via the direct keto-enol pathway.

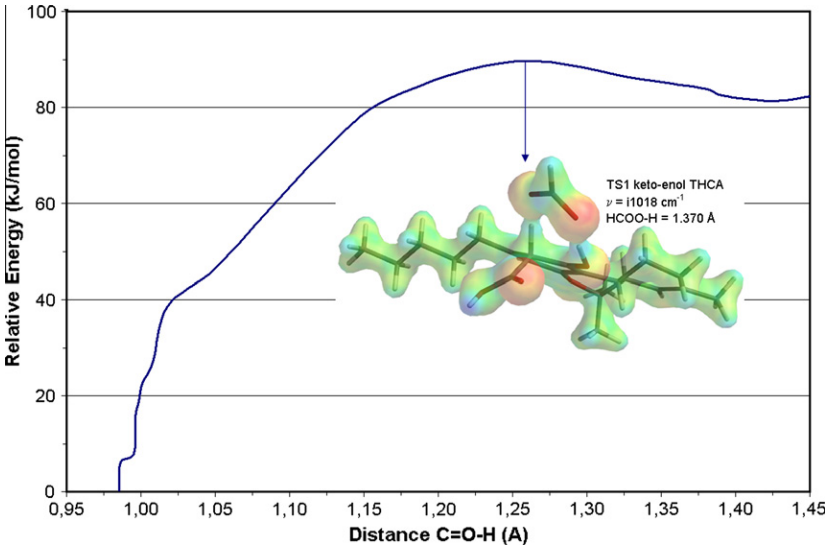

Fig. 8. IRC of Δ^9-THCA decarboxylation catalyzed by formic acid via the direct keto-enol pathway.

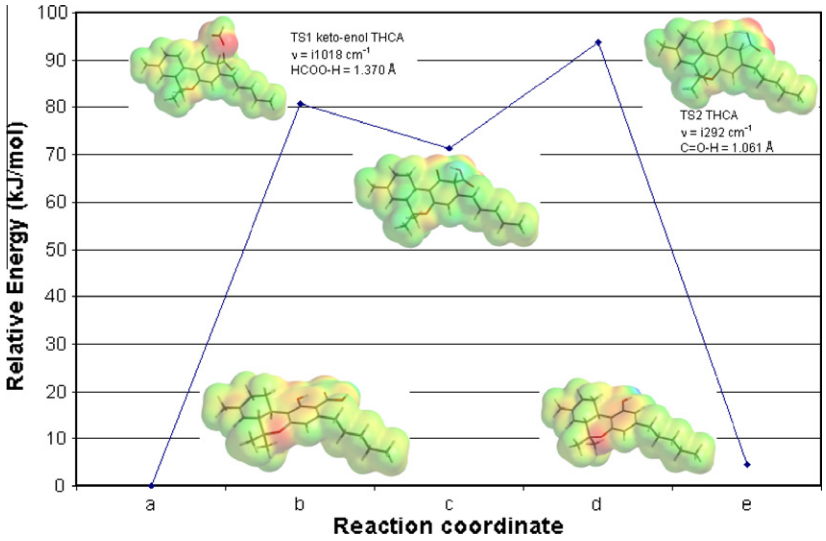

Fig. 9. Energy profile of formic acid catalyzed decarboxylation of Δ^9-THCA.

Table 3
Activation energies of substituted 2-hydroxybenzoic acids with formic acid as catalyst.

Compound	E_a-exp (kJ mol^{-1})	E_a-comp (kJ mol^{-1})
2-Hydroxybenzoic acid	97 [10], 92 [13]	104[a], 92[b]
2,6-Dihydroxybenzoic acid	111 [10], 101, 92 [13]	114[b], 102[c], 92[a],
Δ^9-THCA	85	81[a]

[a] Direct keto-enol pathway.
[b] Indirect keto-enol pathway.
[c] Direct keto-enol pathway with one phenolic OH group not forming an hydrogen bridge with the acid function.

4. Conclusions

Decarboxylation of Δ^9-THCA can be described as a pseudo-first order reaction catalyzed by formic acid, as a model for short chain organic acids present in the flowers of the cannabis plant. The presence of such acids was verified in a series of extraction experiments. Also, the computational idea of catalysis by water to catalysis by an acid, put forward by Li and Brill, and Churchev and Belbruno was extended, and a new direct keto-enol route was found. This route offers the best explanation for the experimental results obtained with Δ^9-THCA, both with respect to the activation barrier and the pre-exponential factor. However both routes can play a role, depending on the exact experimental conditions, as an analysis of available experimental and computational results shows.

Acknowledgment

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Beyond Buds

Next Generation

Marijuana Concentrates and Cannabis Infusions

Ed Rosenthal with Greg Zeman

VAPORIZING

Most early adopters of cannabis vaporizing were focused on reducing the harms associated with smoking and the by-products of combustion: Cannabis is an exceptionally safe substance, but burning and inhaling smoke is not a lung-friendly practice. The concept of vaporizing is to activate and release the cannabinoids and terpenes but leave the inert plant matter unburned.

In a way, the practice of vaporizing buds is rooted in the same basic idea behind extraction; accessing the cannabinoids and terpenes without consuming the inactive and potentially harmful plant material. The method of consumption targets desirable elements for vaporization at temperatures too low to combust the plant matter.

The first wave of products created to achieve this or attempt it were inconvenient to use. Few products from this era remain, notwithstanding a few originals on the shelves of smoke shops with nostalgic or optimistic owners. The first was the Tilt Pipe: It resembled a desktop gumball machine, but instead of candy, the glass dome housed a small metal dish that heated up. Put the bud in the dish, turn it on, and inhale warm, bud-flavored air through a length of rubber aquarium tubing. It had fans but never really took off. Why? Inefficient conduction and the War on Drugs, which forced may early cannabis entrepreneurs from the business.

CONDUCTION VS. CONVECTION

All vaporization is characterized by the absence of combustion, the chemical process behind "burning." This largely self-sustaining process is a simple molecular exchange in which carbon is oxidized, yielding carbon dioxide.

Because inhalation of the resulting smoke, while pleasurable, isn't ideal from a respiratory health standpoint, some cannabis consumers prefer methods that provide the instant impact and easy titration of inhalation while reducing contact with harmful smoke. Because heat is required to achieve decarboxylation, some kind of heat transfer is required. There are three kinds: conduction, convection, and radiation. No practical method of vaporization utilizes radiation, so we'll focus on the first two.

CONDUCTION

Conduction is used in vape domes; the material is placed on the heating element, which transfers heat through direct contact — the same way an electric stove heats a skillet. This type of heating works well for concentrates because they melt and continuously recycle the surface area in contact with the heating element. For example, the coil in a reloadable "dab pen," which in its earliest incarnation was physically very similar to the BC Vape, down to the (albeit much smaller) glass dome. But at this early point in the history of vaporizing cannabis, people were more or less exclusively vaping buds. And because conduction relies on direct contact with the material being vaporized, it isn't particularly efficient for dry herbs, which offer limited and static surface area.

BLASTING BASICS

HOW IT WORKS

The ultimate goal of all concentrate extraction is the same: Separate the resin glands from the buds. What makes solvent extraction different from other methods is the precision with which it targets the desirable elements — cannabinoids and terpenes, both of which are contained within the resin glands, also called trichomes.

Those macro-lens super close-ups of cannabis plants featured in magazines? Those are showcasing the trichomes — the little translucent mushrooms clinging to the buds, what many people used to call "crystals."

Instead of relying on physical agitation to remove the glands, butane extraction dissolves them, creating a "resin" — the removed cannabinoids and terpenes and the liquid solvent. Because its boiling point is so low, much of the residual butane will evaporate at room temperature, but some will still be trapped inside the resin. At that point, the use of a vacuum oven will be required to remove the residual solvent and determine the style of BHO created.

Calyx - Relic Seeds Photo by Professor P. / Dynasty Genetics

Regardless of which style of BHO you're planning to produce or which method you intend to use, the fundamentals of the initial process are always the same; "run" butane through raw cannabis buds to dissolve the cannabinoids, terpenes, and other active ingredients from the plant matter, then evaporate the solvent. What's left behind is highly concentrated resin with trace amounts of solvent that still need to be purged. Depending on the process used for the purge and the physical qualities of your plant matter and resulting resin, your end product could look like any of the styles listed we explore in this book.

There are two primary methods for handling this process — open blasting and closed-loop extraction — but before choosing which is ideal, it's crucial to understand and account for the substantial hazards associated with both approaches.

Shattered Sour Diesel shatter, Pissing Excellence Photos: Fred Morlege

VACUUM OVEN BASICS: SHATTER, WAX, BUDDER, AND DISTILLATE

Temperatures and vacuum settings can vary widely. Torr or mm Hg is a measure of pressure: the ratio of force to the area over which that force is distributed; mm Hg refers to milligrams of mercury, but a more common measure in America would be pounds per square inch, or psi. Pressure measurement converters are available online and -600 mm Hg (Torr) equals -11.6 psi.

SHATTER

The tastes of cannabis consumers are ever changing. One month the market demands crystal clarity; the next, consumers want terp crystals. There's no reliable way to predict which way the cannabis market will shift, so it's best to dial in an effective process and master it.

With that in mind, shatter is the most difficult consistency to achieve, but it's one that consistently enjoys steady demand; get this style right, and the demand will be a reliable safe haven from the always shifting tastes for other styles.

HOW TO MAKE SHATTER

As with all styles of BHO, start by placing the pre-purged resin on parchment paper and place it on a rack in a specialized vacuum oven set to 98˚F with a minimum pressure setting of -600 mm Hg — use more pull if possible. Do not use a normal vacuum oven — butane will degrade typical vacuum pumps, so use one specialized to withstand exposure to liquid butane. The purge process will take from 24 to 36 hours and should be interrupted at least twice by "slab flipping," which will mechanically release more solvent through exposure of alternating surfaces to heat.

The residual butane in the solution is too low to present any fire hazard, but as the pressure drops and the temperature rises, the material will visibly "loaf up." This is normal. You can drop the "muffin" by opening the mantle. If the extractor hasn't pre-purged enough residual butane, the process must be stopped. You can get transparent clarity by pulling the vacuum harder, but it will reduce the operating life of your pump.

Another key aspect of the purge process is lowering the viscosity of the slab to allow more solvent to escape. The key to getting this right is to monitor the bubbles; keep an eye out for big, thin bubbles that pop themselves without assistance. If the bubbles are thick and don't pop, the viscosity hasn't been reduced enough and the temperature setting should be boosted. Or, if there is no expansion or off-gassing the heat may be set too high — the entire process is a delicate balancing act.

Even within the classification of "shatter" there are several subsets of texture, ranging from flexible saps to malleable and brittle "snap 'n' pull" shatters. For strains that yield a sappy consistency, the heat setting could be as low as 68˚F, but for the snap 'n' pull, it will measure between 85˚F and 100˚F. Classic shatter — the hard golden resin with solid stability and crystal clarity — will heat to 95˚F to 115˚F. Some strains will require up to 120˚F, but over 112˚F will cause most strains to "budder."

WAX AND BUDDERING

Leaving shatter in the vac oven at 110˚F–120˚F for several hours results in a honeycomb-like wafer. More butane is removed when the temperature is raised and the pressure lowered, but this also removes more terpenes.

But if shatter turns from clear to opaque, a process called nucleation (or "buddering") has occurred. The process is more or less a one-way street: Once a shatter "budders up," it must go through another process to get it back to true shatter.

Shatter turns to budder when heated too long or exposed to contaminants like residual water. Even the finest shatter turns to budder eventually during storage: "Buddered" extracts are the result of a process called nucleation. An example of this process can be seen in an old milk chocolate bar: the surface takes on a powdery white patina from the separation of milk fats and

sugar, and the consistency is chalky and crumbly. In shatter, heavier fats and lipids precipitate out of the solution. Buddering often starts in one corner of the resin patty and spreads across the entire piece.

Today's market is fickle. One day clarity and stability are the most desirable trait; the next, it might be terpene concentration. Nucleated shatter often has a much "louder" terpene profile than the shatter it started as, so don't worry too much if nucleation strikes a shatter slab. As long as the BHO has been extracted and purged properly, retaining the flavor profile and cannabinoid content while removing residual hydrocarbons, the other physical characteristics are a matter of taste.

DISTILLATE

Distillate is enjoying an increase in popularity because of its potency — upward of 90% cannabinoid concentration — and because it's flavorless, though the addition of food-grade or reclaimed cannabis terpenes allows for customization. The process is simple enough — winterized oil is poured into a short-path distillation system, which allows for "fractional distillation." For more in-depth information on short path and fractional distillation, see the "Distillate" chapter.

DECARBOXYLATION OF BHO

Because it is an extraction of THCa, BHO is not psychoactive in its raw form. It is completely harmless in this form if it is accidentally ingested by a pet, a child, or an unaware adult. However, THCa offers many medical benefits to people who want relief without euphoria.

If the BHO is to be used in edibles, it must first be decarboxylated—a process through which THCa and/or CBDa turns into THC and CBD. This starts happening at 222˚F (106˚C).

Thankfully, it's quite easy to decarboxylate purged BHO — just double boil it in a water bath set to above 222˚F (106˚C). The BHO will start producing CO_2 bubbles when it exceeds the target temperature, at which point it must be stirred. When the bubbles taper off, the BHO is decarboxylated.

But be careful: The same heat that turns THCa into THC also turns THC into cannabinol (CBN), which produces a more sedative effect than THC. When THCa is 70% decarboxylated into THC, the rate of THC-to-CBN production eclipses the rate of decarboxylation from THCa to THC. That is, when the bubble formation tapers off, the oil has reached the maximum level of THC, and further heat will only increase CBN and make it more sedative.

Of course, BHO destined to be dabbed or vaped doesn't require decarboxylation — the heat used during consumption will take care of that process.

Gravity pulls the oil down, and the blades turning around the coil wipe the cannabis to four millimeters. This distills the oil over a very short distance and, as the gas evaporates and condenses onto the coil, the different fractions are made.
Photo: Ed Rosenthal

DISTILLING CANNABIS OIL

There are a number of parallels between liquor distillation and cannabis oil distillation, making it an excellent starting point for those wholly unfamiliar with the concept as it relates to cannabis. First and foremost, distillation is a secondary process that refines material extracted through some other method. When distilling bourbon whiskey, one starts by making a "mash," a concoction of water and grains that ferments, creating a mixture with a relatively low alcohol content. Similarly, when distilling "clear" distillate, you start with the product of solvent extraction — usually BHO, CO_2, or ethanol extract, which is distilled to further refine the THC fraction.

In whiskey distillation the mash is the product of fermentation; in cannabis distillation the "mash" is the product of a previous (often hydrocarbon) extraction process.

Like solvent extraction, fractional distillation is an industrial process that requires relatively expensive equipment, but as we've just covered, the organic chemistry at play is not particularly advanced. With a safe laboratory setup and a working understanding of the process, you can take subpar BHO, CO_2, or any other concentrate and separate out highly desirable elements, like specific terpenes and crystalline CBDa or THCa. Distillate can also be produced "from scratch" using buds or trim, without the intermediate step of making shatter or wax, but most producers do not use this approach because of the widespread availability of cheap, cannabinoid rich "crude" oil.

When applied to cannabis, the science of fractional distillation is the same as with petroleum products, but at a much smaller scale and using a different source material; cannabis "oleo resin," a broad term that encompasses all next-generation extracts that can be distilled. Instead of fuels and oils, fractional distillation of cannabis is targeting the usual suspects — terpenes and cannabinoids — but with a razor focus. Where solvent extraction removes and

concentrates the terpenes and cannabinoids from raw flower, fractional distillation takes the concentrated cannabis resin created by solvent extraction and targets individual fractions. When performed properly, this process can create large crystals of solid THCa or CBDa with purity exceeding 99%. This process can also be used to isolate specific terpenes, which can be added back to the solids or used to flavor other products.

CBD isolate from Harmony Extracts

Theoretically, fractional distillation can be used to isolate any compound present in your starting material. Practically speaking, there are really only three salable products you can create using this process: crystalline THCa, crystalline CBDa, and terpenes. There are companies who isolate CBN, but mostly as an experimental novelty. There is no marked demand for any crystalline cannabinoids other than THC and CBD, but like all things related to cannabis extraction, that can change at any time.

CHAPTER 7

Hash to the Future

From Dry Sift to Machine Hash

Kief, also known as "Dry Sift," is composed of the unpressed glands scraped from dried mature flowers and leaves using a screen. It is very popular because it is easily gleaned from leaves and trim.

Kief is the easiest marijuana product you can make. Tiny resin-filled glands cover the buds and leaves. These tiny stalked glands, known as trichomes, are the only part of the plant that contain significant amounts of cannabinoids, such as THC and CBD, as well as the pungent terpenes that give each marijuana strain its distinctive aroma, taste, and medical and psychoactive qualities. Making kief consists of collecting those trichomes. There are a number of techniques for separating them from the plant material and sorting them.

Kief can be smoked just as it is collected; you can add the kief to your pipe without further processing or preparation. It is often pressed to make hash. It can also be used to produce tinctures or cooking ingredients. Those uses are discussed in their respective chapters. This chapter explains various screening techniques to produce kief, as well as methods using ice, dry ice or CO2 to enhance the process.

Because kief is so easy to collect from dried cannabis it is one of the oldest marijuana preparations and is known in many corners of the world. Alternatively spelled as *kif*, *kief*, *kef*, or *kiff*, the word appears in many languages. The origin of the word is the Arabic *kayf*, which means well-being or pleasure. The term was historically used in Morocco and elsewhere to mean a mixture of marijuana and tobacco, not unlike modern-day spliffs or blunts, though it was typically smoked using hookahs. In Amsterdam and other parts of Europe, kief is sometimes called pollen or polm, and many of the screens and devices used to separate kief from other plant material are called pollen screens or pollen sifters.

The marijuana plant produces three basic types of resin-rich glands that grow to different sizes expressed in microns or micrometers, which is a metric measurement equal to one millionth of a meter. Marijuana glands or trichomes range from as small as 15 microns to as large as 500 microns. That lets you easily separate the different glands by using screens of corresponding sizes.

The bulbous glands are the smallest, ranging from 10 to 15 microns. These tiniest glands perch atop equally tiny one-cell stalks that cover the leaves of vegetative plants.

The capitate-sessile glands are the middle size, ranging from 25 to100 microns, and are more numerous than the bulbous glands. "Capitate" means globular, and that's what they look like—spherical globs of resin that lay on the leaf and flower surfaces.

Capitate-stalked glands are the ones most visible on the buds of mature, flowering marijuana plants, as these rich resin balls are the largest at 150–500 microns, and they sit high on stalks that can reach 500 microns. These are the glands that hold most of the cannabinoids and terpenes and are found most abundantly on the upper leaves, flowers, and bracts (the tiny leaves surrounding the flowers) of unfertilized female plants. These are the glands that are captured to make kief.

The maturity of the plant and its variety and environmental conditions all affect gland size. For instance, many Moroccan varieties may have glands that are under 80 microns. Many sativa varieties also have small glands. "Hash plant" varieties often have glands that are 120 microns or larger. Most sinsemilla is in the mid-range, between 80 and 110 microns.

To give you a sense of these sizes, a human hair is about 70 microns or a bit more; the finest beach sand is 100 microns; playground sand is roughly 250 microns and the eye of a needle is more than 1200 microns.

To measure the size of the glands with precision use a microscope and a slide with a micron scale etched on it. Some microscopes come equipped with a scale called a reticule built into one of the eyepieces to measure microns. Count the number of hash marks the gland spans and multiply by the conversion factor for the magnification power.

HOW KIEF SCREENING WORKS

THC and other cannabinoids and terpenes are concentrated in glands that cover many parts of the marijuana plant, but they're concentrated in the upper leaves, flowers, and flower bracts of unfertilized female plants. They are also found on the seed covering and surrounding areas of pollinated plants. Screening cured plant material is one of the easiest ways to rescue these glands for use.

There are several different ways to prepare the plant material for screening or sifting. In countries close to the 30th parallel, such as Nepal, Afghanistan, and Lebanon, small amounts of kief have traditionally been made using a silk scarf stretched tightly over a bowl. Dried marijuana, frequently cured for as long as six months, is rubbed on the taut silk cloth. The cloth's fine weave allows the small glands to pass through to the bowl, leaving the vegetative material on top. Silk scarves are still used in parts of the world, but the nylon or metal mesh screens used for printing (still often called silk screens) are more durable and come in a variety of dimensions and mesh sizes.

One of the simplest methods of making kief is by gently rubbing the plant material over a fine screen. The size of the openings in the screen determines which size glands and how much residual plant material will make it through. The vigor used in rubbing the material on the screen has a profound effect on the quality of the final product. Different grades of kief are produced by varying the amount of time the material is sifted, the screen's gauge, and the pressure used. Sifting the same material a few times yields more kief, but each sift results in a

Rubbing ground bud across a metal screen in a kief making box.
Photo: Lizzy Fritz

Isolating the resin glands that contain the flavors and effects of cannabis from the largely inert plant material is far from a new idea. The old methods of concentration are used to create hashish, often called hash. The practice began millennia ago, probably in Asia near the Hindu Kush region. But there is a rich historical tradition of cannabis extraction across Asia, the Middle East and North Africa, home to historical hash capitals including India, Nepal, Afghanistan, Pakistan, Lebanon, and Morocco.

Perhaps the oldest way of making hash is hand-rubbing fresh cannabis plants and collecting the resin that accumulates on your fingers and palms, then rolling it into balls or coils. This is called charas. The resulting product is prized globally and the legends of its unique potency still attract travelers to the northern Himalayas, where it's made on a large scale. The yields from charas are low. It requires much more material and far more labor than other methods. The process can produce extremely high-quality hash, but because the resin is collected fresh from the plant and is still very sticky, it is pressed by working it with palms and fingers into a ball or patty until it dries a bit.

Another classic method of hash production involves suspending dried cannabis plants over tarps and collecting the glands that fall naturally and pressing them into hashish. Many contemporary methods for "dry sifting" hash still exist, ranging in complexity from small boxes lined with screens for collecting kief from a personal use stash, to mechanical tumblers that agitate the cannabis and screen out the glands.

The techniques outlined in this chapter can all trace their lineage directly to hash makers of antiquity, because the core physical principles are unchanging; manually removing the resin glands using cold and physical agitation, then concentrating the resin using heat, pressure, and time.

DRY ICE KIEF — THE MANUAL METHOD

Perhaps the cheapest, simplest way to concentrate cannabinoids is also one of the newest. Since 2009, hash makers have been turning to dry ice — which is frozen carbon dioxide—to yield an impressive amount of kief. Dry ice is the fastest way to turn trash into gold. Manual dry ice sieving is very inexpensive to set up, results in very little mess or cleanup, and doesn't involve explosive chemicals like BHO, or require expensive machinery like CO_2 and other methods of extraction.

One-Minute Dry Ice Kief is very smooth and contains a lot of terpenes because it's made cold and not mixed with anything, even water, preserving the natural terpenes. It has very little vegetation so you're inhaling only gland products.

EQUIPMENT

- Cannabis (1 ounce, dry trim or fresh frozen)
- Bubble Bags (durable 160- and 220-micron water bubble bags)

PRESSING BY HAND METHOD

Pressing by hand is a method for transforming kief into hashish a few grams at a time. Pressing by hand is convenient since it requires no additional equipment but it takes considerable energy and the results are better with a practiced technique. Those unaccustomed to hand pressing may find it difficult to make the material bind together. The considerable work it takes to get well-pressed hash can easily result in sore hands.

This method works best using freshly sieved medium to high-quality kief. If the kief contains a significant amount of vegetative material, it's harder to mold into hash and may not stick together properly. To hand press, measure out a small mound of fresh kief that will fit comfortably in the hand, usually a few grams at the most. Work this material with one hand against the other until it begins to cohere into a solid piece. Then rub it between the palms, or between palm and thumb. After 10 minutes or more of working the material, it begins to change density. Dry, aged kief lacks some of its original stickiness and may take longer to stick together, but if it was stored properly, it should cooperate, though it may require more kneading. When a piece of hashish has not been pressed properly, it crumbles easily at room temperature.

If the kief is particularly stubborn and won't stick together to form a mass, mildly heat it. Wrap the material in food-grade cellophane, ensuring that it is completely sealed and all the air is squeezed out. Wrap this package in several layers of thoroughly wetted newspaper, cloth or paper towels. Turning frequently, warm in a skillet that is set on the lowest heat. It doesn't need to be heated as long as other methods because the only point of heating is to get the material to stick together so it can be kneaded into a solid piece.

Another method is to wrap it the same way and press it for a few seconds on each side with an iron that is set on a very low heat setting.

CHAPTER 8

Rosin

Rosin is a concentrated blend of terpenes and cannabinoids extracted using a method sometimes called "rosin tech." It's the simplest, least expensive way to extract concentrate from raw buds or hash for more effective dabbing. Instead of a chemical process, rosin tech relies on heat and pressure to squeeze cannabinoids and terpenes from the source material. It is a very fast process: A batch of rosin can be produced in moments and consumed immediately. Another advantage of rosin production is that it poses minimal risk of physical injury.

The physical science of rosin is simple: Applying heat melts the terpenes and cannabinoids into a pliable resin. Then it is squeezed using a press. Some lipids and waxes melt at the same temperatures. Thus the finished product is generally not as refined as the results of some other methods. One trade-off is the speed and ease of extraction.

There is a wide range of tools and equipment that can be used to make rosin. The choice depends mostly on the quantity being pressed. On the hobby level you can use household items. Industrial processors use pneumatic or hydraulic presses.

No matter the size of the project, the start-up costs of this method are very low compared to chemical extraction, where just the cost of the safety equipment and laboratory modifications exceeds the cost of even an elaborate large-scale rosin operation. However, the costs for the processes of running a solvent extraction setup are lower and the yields higher.

Starting materials used for rosin making. Hash, bud, pressed hash, and kief.
Photo: Fred Morledge

A WORD ON MATERIAL SELECTION AND ROSIN CONSUMPTION SAFETY

A few chapters back we noted that butane extraction is dangerous but BHO is not. Rosin is the inverse because you must be careful about screening material for mold, residual pesticides, and other contaminants. They stick with the resin so they become concentrated in the process. This can cause serious repercussions for the end product and, more importantly, the end *user.*

One feature of hydrocarbon extraction is its ability to strip away or neutralize biological impurities including bacteria, mold, and other contaminants. Moldy but potent trim processed with butane results in a safe product. However, when processed for rosin, there is a buildup of dangerous microbials. Even if you're growing your own starting material, it should be tested for you to know exactly what is present in the rosin.

Many people choose to consume rosin over solvent-extracted products because of perceived health concerns. Some of them do this because of an immunodeficiency or some other medical condition, while others are caught up in a cloud of alarmist "reefer madness" surrounding solvent extraction.

Bottom line: It's crucial that you ensure clean, high-quality source material, whether you're pressing rosin from trim, buds, or hash.

ROSIN 101: THE FLAT IRON TECHNIQUE

The easiest way to understand rosin is to make a small batch on your own. It's simple and requires very little equipment. Let's begin by pressing out some flower rosin — here's what you'll need to get started:

EQUIPMENT FOR BASIC FLOWER ROSIN PRESS:

- **Buds** — for our purposes, use 1-7. Our goal is to learn the process and taste your first homemade product.
- **Tong-style hair-staightener / flat iron** — there are several factors to

consider here, but the biggest obstacles are heat and durability. Some popular models like the Remington have minimum settings too hot to leave the device on during pressing, meaning that you have to warm it, turn it off, and use a laser thermometer "heat gun" to ensure ideal temp. This tool is inexpensive and fun to use. If you don't have access to a heat gun, something inexpensive like the 2-inch model from Conair will allow you to "set and forget" the heat, because the lowest setting is generally cool enough for rosin extraction. However, part of the lower cost comes from a more brittle plastic housing for the heating plates, meaning that the Conair is more susceptible to physical cracking and breakage. A model with a digital temperature readout is also a good choice for irons that do have temp settings low enough for rosin.

- **Parchment paper** — but NEVER wax paper, because you don't want wax to melt into your final product. This will happen if you use wax instead of parchment paper. You can also use silicone mats and other heat-resistant material, but for your first press, parchment is fine.
- **Bar clamp (optional)** — pressure is half of the magic behind rosin, so you have to ensure that you have enough. When pressing small quantities, manual pressure is generally adequate, but for a more efficient press and a higher yield, clamps can be applied to the outside of the iron.
- **Micromesh/silkscreen filters (optional)** — pressing rosin tends to spread the extracted concentrate outward from the buds being pressed, meaning that screens aren't always strictly necessary to keep plant material out of the final product. However, to ensure a product free of particulates, you can wrap your bud in silkscreen or micromesh material. Some people also use unbleached tea bags for these smaller batches of flower rosin.
- **Collection tool** — this can be anything with a flat edge and a roughly nonstick (or easily heatable) surface, so a razor blade or other scraper works well.
- **Protective work gloves** — It's pretty difficult to injure yourself making rosin, especially using this method, but it's not impossible. Wearing work gloves protects your hands from painful burns, which a hair straightener is more than capable of inflicting.

PRESSING FLOWER ROSIN

Plug in the flat iron and set it to the target temperature. If you've selected the basic 2-inch Conair model, set it to "1." If you have a model with a digital temperature display, set it between 280 and 330°F. Check model.

Place your bud inside the tea bag or filter (if applicable), and fold it inside folded parchment paper.

Ensure the iron is still at the appropriate temperature and that the bud is secured in its envelope, then clamp the envelope with the flat iron, focusing the pressure on the buds in the middle. If you're using clamps, tighten them for 3-8 seconds — you'll know you're done when you hear the sizzle sound of resin escaping and interacting with the heat.

Unclamp the iron, open the envelope, and pluck the buds out — this step is another reason many people use bags and filters, because it reduces the opportunity to contaminate an otherwise clean rosin batch while removing plant material.

Take the envelope of warm rosin, refold it, and roll or spread out your rosin as desired. Then place the envelope on a cool surface for a minute or so before opening and collecting the rosin.

Now it's time to dab the rosin! If you have any left when you're done dabbing, keep it in a cool, dark place inside a nonstick container. The main drawback to rosin is that it's best consumed fresh and it doesn't retain its terpenes as well as other cannabis concentrates, so it goes stale quicker, especially when it isn't kept in a cool environment. This is something to consider when deciding how much rosin to make at a time.

THE ROSIN REVOLUTION

Rosin has blossomed in popularity over the last few years because you can quickly make tasty, potent extracts using inexpensive equipment. With training and experience, the product can rival solvent extracts in potency and flavor.

Rosin processing, though not a cold process, occurs below the volatilization point for *most* of the terpenes, and doesn't reach the temperatures needed for decarboxylation, so the rosin is mostly a concentration of THCa and/or CBDa, the acidic precursors to the cannabinoids. The result: the material will not be intoxicating if eaten.

Rosin can also be used to infuse edibles if preparation involves a high enough temperature to decarboxylate the acids. For an edible that doesn't require a hot enough temperature for decarboxylation), predecarboxylate it in the butter or oil being used in the recipe. Place the rosin and the oil or butter on simmer for 15 to 20 minutes to activate the cannabinoids.

STARTING MATERIAL

There are three basic types of material you can press rosin from: buds, hash and kief. Within those categories there are different types and grades.

BUDS

The higher the cannabinoid content of your starting material, the higher yields you can expect. For the best rosin, press the best buds. Small buds and trim can also be pressed into rosin.

After the movie, brownies took hold as the preferred method for eating cannabis. One reason might be that the rich chocolate flavor masked the less tasty plant flavors. Practically every person who's eaten cannabis at some point in their life has tasted a pot brownie.

"Magic brownies" were still largely perceived as a novelty item in the United States, generally reserved for festivals, parties, and other occasions for celebration. There was no "dosing" in those days; you just ate some, waited, ate some more, and sometimes ended up taking a longer, stranger trip than anticipated.

Brownies also played a central role in San Francisco's medical cannabis revolution, which planted the seeds for Proposition 215 and the dawn of a golden age for medicinal cannabis. Mary Jane Rathburn earned a hallowed space in cannabis activism history (and the moniker "Brownie Mary") for her groundbreaking activism, which began when she started providing cannabis-infused brownies to AIDS patients at San Francisco's General Hospital at the peak of the AIDS crisis. From then on, cannabis edibles became a cornerstone of California medical cannabis, providing potency and discrete consumption for those who can't or simply don't smoke buds.

BEYOND BROWNIES

Brownies were just the beginning. Any food item you can think of is likely to exist in a cannabis-infused form, from potato chips to chicken wings. In this new age of cannabis acceptance, edibles are no longer just something college kids furtively scarf while standing in line for festival tickets; they're also part of an expanding salon culture focused on the intersection of gourmet cuisine and ingested cannabis.

As the world of cannabis edibles grows larger in both scope and focus, the demand for specialty products that cater to specific dietary needs and culinary trends is creating a new discussion around the marriage of food and cannabis. Breaking bread has always been a foundational element of any culture, and cannabis culture is no exception.

The American cannabis culture of the late 20th century viewed edibles as a ticket to adventure and excitement, but with a new set of laws, the early 21st century has seen the rise of a more holistic, health-minded perspective regarding the use of edibles. Instead of eating a 100 mg cookie and zoning out for hours, people are increasingly drinking 5–15 mg of cannabinoids in their cup of tea with breakfast and perhaps chewing a 10 mg stick of gum on the subway on the way to work. New cannabis users are looking to enhance their daily life with cannabis.

New regulations regarding potency limits on edibles, including potency caps on edible portions, such as the 10 mg dose cap imposed by California's regulatory framework, are changing the way people ingest cannabis. New regulations on manufacturing facilities ensure quality, consistency, and purity. Because of this, a professional edibles culture has emerged, which has drawn capital into various sectors of the edibles market. Just a few decades ago the notion of a commercial cannabis product was considered absurd; there was no real demand for such a

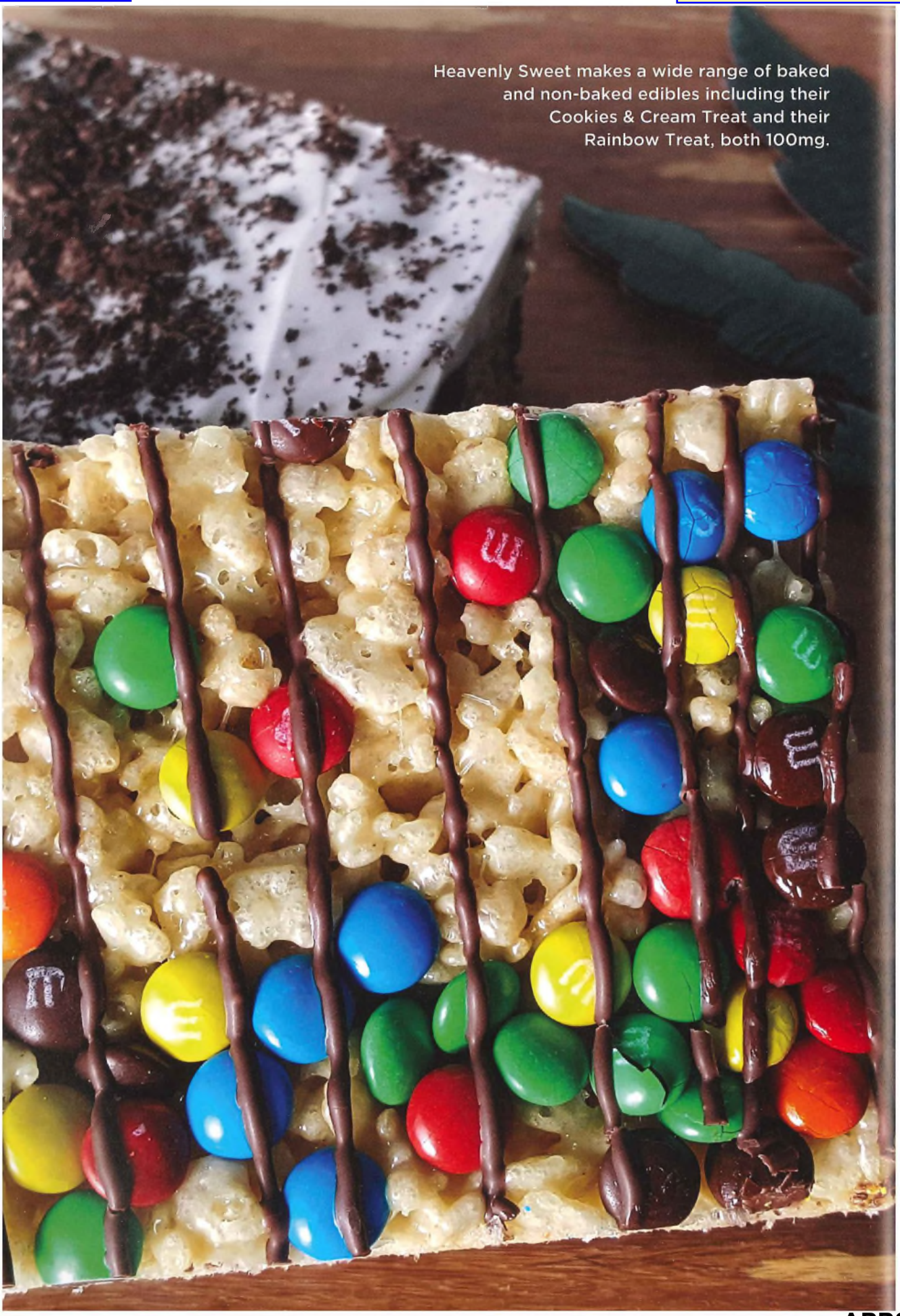
Chocolate in bulk, before melting.

Automated tempering machine heating, stirring, and circulating the melted chocolate.

Medicated chocolate bars after being removed from the refrigerator and molds.

Paul and Candi move chocolate into the corners of the chocolate bar mold while it's on a shaker, assuring consistent dosing and even chocolate throughout the bar.

Wrapping finished chocolate bars. Caligold produces strain specific 125 mg chocolate bars with Himalayan salt. Photos: Darcy Thompson


Heavenly Sweet makes a wide range of baked
and non-baked edibles including their
Cookies & Cream Treat and their
Rainbow Treat, both 100mg.

thing and nobody was making it. Now, cannabis product marketing is beginning to mirror wine and fashion industry trends. In this new market, companies are required to put more effort into branding, packaging, and placement.

THE SCIENCE BEHIND WHY THAT BROWNIE RUINED YOUR NIGHT

There is a vast and expanding body of research that supports the broad physiological benefits of responsible cannabis consumption and confirms the numerous effective applications of cannabis medicine. And while clinical research is absolutely crucial to further standardize and understand the mechanisms at play, the fundamental efficacy of medical cannabis is a matter of scientific fact.

Humanity's trust in cannabis medicine stretches across millennia: Hundreds of thousands of generations of human beings agree — cannabis medicine works. And though there are undoubtedly distinct medical benefits and applications for inhaled cannabis, particularly concentrates, much of the human history of cannabis medicine has been characterized by people ingesting it. Why? Because of cannabis metabolites.

Most cannabis users, even those with elevated tolerances, report that eating cannabis edibles provides a more intense, longer high than smoking or dabbing. The reason for this is simple: Eating cannabis edibles actually does provide a more intense, longer high than smoking or dabbing.

One of the main benefits of edibles over smoking (apart from the obvious health benefits of not setting plant matter on fire and pulling the smoke into your lungs) is that ingested cannabis is metabolized by your liver before entering the bloodstream, which transforms its chemical makeup, producing THC metabolites, namely 11-OH-THC. This metabolite is more potent than regular THC (Delta-9THC), and while it's created in the body when cannabis is inhaled, the levels of 11-OH-THC can be over 10 times higher when it's ingested.

THC'S JOURNEY — FROM ACID TO METABOLITE

Many people still use THC as a catch all when discussing the potency of buds, as in "this strain generally tests around 23 percent THC." But from a technical standpoint, the cannabis plant doesn't actually produce THC, not the delta-9 THC people are thinking of when they say THC. It actually produces THCa, the precursor acid to delta-9; the process of decarboxylation converts the acid to the "active" delta-9 form, which is itself converted to the THC metabolite 11-hydroxy-THC, which metabolizes into the brain more quickly than delta-9. The final stage of THC's journey is the conversion of 11-hydroxy-THC to 11-Nor-9-carboxy THC, an essentially inert secondary metabolite that possesses an exceptionally long half-life, which is why it's the primary target of most blood- or urine-based cannabis drug tests.

Over-the-Stove Method: Infusing Butter, Vegetable Oil, Olive Oil, and Coconut Oil

Ingredients:

1 cup of ground cannabis flower (or less for milder potency)

1 cup of oil or butter of your choice

You'll need:

Strainer or cheesecloth

Grinder works best or an appliance like a food processor, blender, or coffee grinder to pulverize the cannabis. Once again, not too small of a grind, as it can result in too much plant matter in the oil.

Double-boiler, slow cooker, or saucepan

Directions:

1. Grind the cannabis with a food processor or blender, but not too small, as anything too small will go through the strainer. You may include the entire flower, leaf, and trim, depending on your preference.
2. Combine oil and cannabis in your double-boiler or slow cooker, and heat the two together on low or warm for at least 4 to 6 hours. This allows for the cannabis to be decarboxylated and activate the THC in the cannabis. Low and slow will add to the potency of the infused oil, but if you heat too high, it will destroy the THC content. Stir occasionally throughout the process. If on the stove top, a small amount of water can be added to the mixture to help avoid burning.
3. Once the infusion is completed, let it cool down and then strain in a strainer or cheesecloth. Do not squeeze the cheesecloth; this will simply add more plant matter to your oil. All remaining plant material can be discarded or used in other dishes. The oil's shelf life is at least eight weeks and should be refrigerated.

NOTE: Be cautious when using the oil to prepare dishes that require heating. Do not microwave and choose low heat whenever possible. Whatever method you choose, temperature of the oil should not exceed 245°F.

Start out low and slow; five to ten milligrams of THC in one dose of an edible is a safe starting point.

Canna Balm from Doc Green's is their strongest most potent topical with over 500mg of active cannabinoids per jar. Made with pure, raw, solventless CO2 concentrate, bee propolis and pollen, as well as essential oils for increased healing.

Skin is the human body's first line of defense against the elements and potentially harmful microbial contaminants, but this huge organ (roughly 20 square feet all told) is also involved in several crucial physiological processes like detecting and regulating body temperature and circulation, sensing pain and other vital stimuli, and retaining or expelling moisture. The epidermis consists of four to five layers of tissue that encompass various glands, ducts and membranes, all of which have distinct physical processes that allow them to function properly. Like the rest of your body, the efficiency of these processes can be directly improved by activating the homeostasis regulating mechanisms of the Endocannabinoid System (ECS). In addition to CB1 and CB2 receptors, skin cells contain enzymes responsible for endocannbinoid metabolism. So the skin essentially contains its own version of the ECS, and that system plays a very important role in skin physiology.

TOPICAL OR TRANSDERMAL?

Because many of the ECS receptor sites are located in the epidermis, cannabis infused "topical" products are applied directly to the organ being targeted; the skin. But skin is semi-permeable, which means cannabinoids can also be absorbed into your system through your skin — this is called "transdermal" application, which is often achieved by using a transdermal patch. Because topical applications of cannabis, like balms or liniments, absorb into the epidermis and don't breach the blood-brain barrier, they have no psychoactive effect. In contrast, transdermal products use the skin as a conduit to the bloodstream and depending on the dosage and formulation may make you feel "high."

TRANSDERMAL APPLICATION

With transdermal application we're seeking to impact systems beyond the epidermis. Transdermal therapies are able to pass through the skin and target muscles deep below the surface, decreasing inflammation and thereby pain.

No matter what the end result is, the goals of transdermal treatments are to get the active

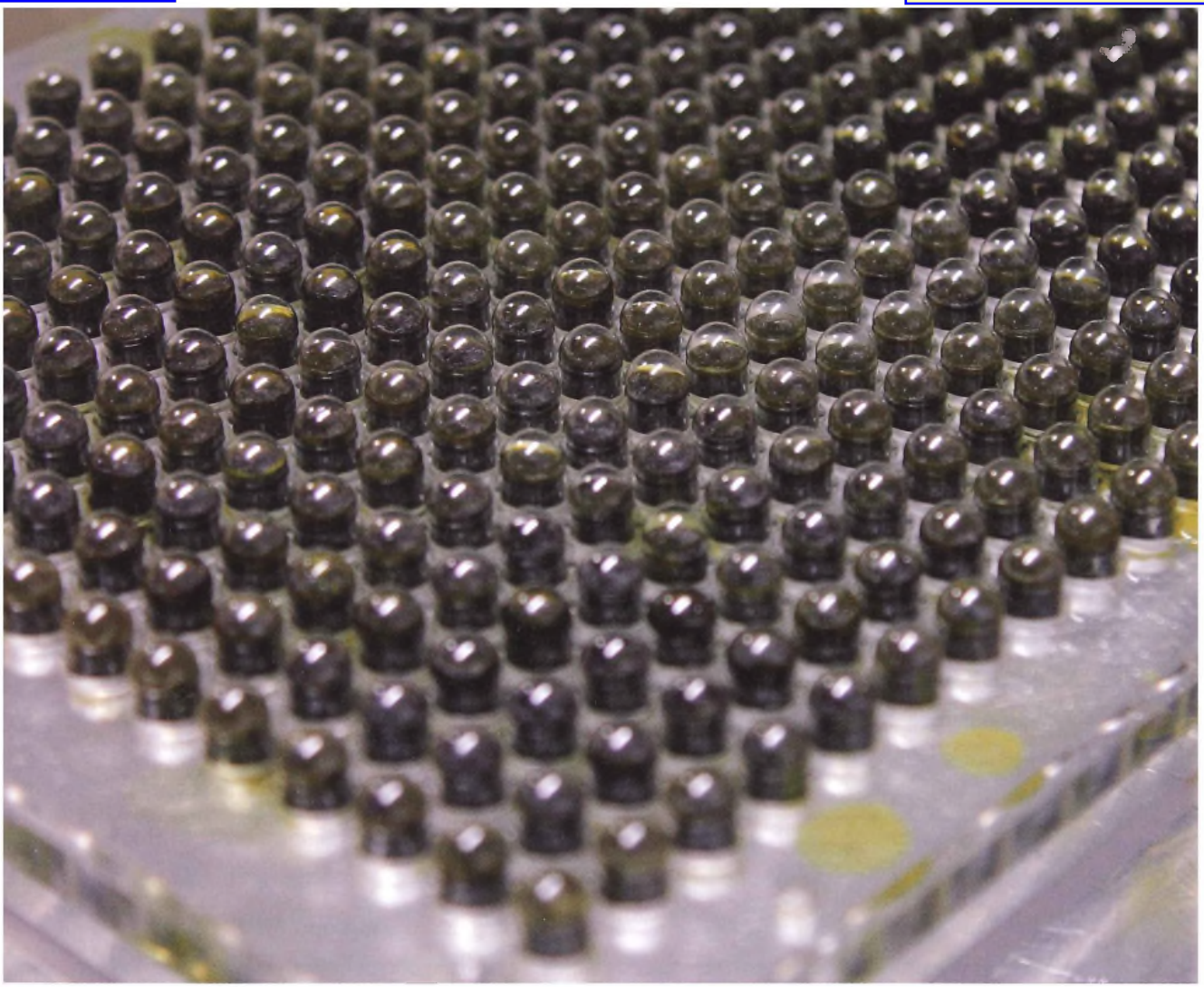
CHAPTER 11

Tinctures, Capsules and Beyond

TINCTURES

Before cannabis prohibition, tinctures were the most common way of buying and consuming marijuana in America. Recently, they've been making a comeback. Commercially prepared tinctures are now available in dispensaries in many states. Tinctures are discreet to use and are quite easy to make at home.

A tincture is a concentrated extract of any herb in liquid—usually alcohol, oils like medium-chain triglyceride (MCT) oil, or sometimes glycerin—that is taken by mouth as a drop on or under the tongue. Alcohol is used to separate the cannabinoids, terpenes, and other essential oils from the marijuana plant material and acts as a preservative. In herbal medicine, tinctures are commonly 25% alcohol, which is achieved by diluting the mixture with water. People who do not want to consume alcohol may opt for glycerin or oil-based tinctures.



Capsules that have just been filled by hand at Kind Medicine's facility near Santa Cruz, CA.

CAPSULES

We've all been in situations where it's just not cool to smoke. Maybe you've wondered if it's possible to take a marijuana pill. Popping a pill in your mouth with a gulp of water to enjoy the therapeutic and mind-enhancing effects of cannabis would sure be easier and more discrete than firing up a spleef. Turns out you can. Marijuana capsules, also called "maripills" or "canna caps," are very effective and quite easy to make. What's more, they will produce a longer-lasting and somewhat different high than smoking or vaping.

A pill and a pipe won't produce the same effects, even if they contain the same variety and amount of marijuana. The digestive process creates somewhat different metabolites from inhaled marijuana, and those have different effects than the smoked form.

One difference is time: how long it takes to be effective and how long the high will last. Take a puff, and the effects are felt within seconds, letting you easily judge how high you're getting. Take a pill, and you won't know for a while. Anything that gets into your system through your stomach takes much longer to be felt, and that can make knowing how much you have on

Patient perceptions of an inhaled asthma medication administered as an inhalation powder via the Diskus or as an inhalation aerosol via a metered-dose inhaler

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Objective: To evaluate patient preference, ease of use, and correctness of use of fluticasone propionate administered as inhalation powder via the Diskus (GlaxoSmithKline, Research Triangle Park, NC) and as inhalation aerosol administered via metered-dose inhaler (MDI).

Methods: In 154 patients 12 years of age and older with asthma and a history of MDI use, the Diskus and the MDI were compared in a randomized, open-label, 7-week crossover study.

Results: In patients who had used both devices, more found the Diskus easier to use (59%) and preferred it overall (60%) compared with the MDI ($P \leq 0.025$). Ninety-eight percent (for the MDI) vs 91% (for the Diskus) of patients were able to correctly perform all the maneuvers necessary to use the devices correctly by either viewing a single demonstration and/or reading the instructions for use. Ninety-four percent of all patients found it easier to tell the number of residual doses with the Diskus ($P < 0.001$), and 59% of patients indicated that they would most likely request the Diskus from their physician ($P = 0.025$). Compliance was significantly better with the Diskus; 91.1% of patients used the Diskus as directed compared with 78.6% for the MDI ($P = 0.013$).

Conclusions: In patients exposed to both devices, the majority preferred the Diskus and found it easier to use compared with the MDI. Ninety-one percent of patients used the Diskus correctly with minimal training, and when given a choice, most indicated they would likely request the Diskus from their physicians. Together, these data indicate a significant level of acceptance of the Diskus device in this patient population.

Ann Allergy Asthma Immunol. 2003;91:55–60.

INTRODUCTION

Asthma is a serious, chronic disease that affects an estimated 17 million people in the United States.[1] Symptoms associated with asthma include breathlessness, wheezing, chest tightness, and cough. These symptoms are a result of air flow limitation caused by bronchoconstriction, inflammation, and increased bronchial hyperresponsiveness.[2]

The main goals of asthma therapy are to control daytime and nighttime symptoms, prevent recurrent exacerbations, normalize lifestyle, maintain normal activity levels, restore normal or near-normal lung function, and avoid adverse effects from asthma medications.[2]

Because most asthma medications are administered via the inhaled route, it is inherent that the features of the inhaled delivery system are important to medication effectiveness and acceptance. Inhaled medications, in general, have an advantage in that they deliver medication directly to the site of action. Ideally, the inhaled delivery system should provide consistent dose delivery to the lungs across a wide range of inspiratory flows, deliver an optimal particle size for lung deposition (2 to 5 μm), and have a multidose capability.[3] In addition, these devices should also be small, easy to use, cost-effective, and have an ability to track the number of residual doses, as these features are important to patients.[4] Chlorofluorocarbon-containing metered-dose inhalers (MDIs) are the most commonly used devices for delivering inhaled asthma medications; however, some patients find them difficult to use.[5–7] For example, some patients may find it difficult to coordinate device actuation with inhalation. Likewise, patients may forget to shake the canister or prime the device after prolonged periods of non-use. Residual doses are also hard to track with current MDIs, and environmental concerns with the use of chlorofluorocarbons as propellants make them less desirable. Other patients, however, use the MDI effectively and are comfortable with its operation.

Recent advances in dry powder technology have lead to introduction of dry-powder inhalers (DPIs) such as the Aerolizer (Novartis Pharma AG, Basel, Switzerland), Turbuhaler

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(AstraZeneca, Wilmington, DE), Diskhaler (GlaxoSmithKline, Research Triangle Park, NC), and Diskus (GlaxoSmithKline). These powder devices have an advantage over the MDI in that they are breath-actuated and require no propellants.[8–11] Unlike MDIs, residual doses can be easily tracked with DPIs.[11]

The Diskus, a new multidose DPI, takes into account the shortcomings of MDIs and other DPIs.[12] Each Diskus device contains a 1-month supply of medication, with each dose individually wrapped, as opposed to being delivered from a reservoir. In addition, the Diskus device incorporates a dose counter, which allows patients to keep track of remaining doses, thereby eliminating the possibility that patients will unknowingly run out of medication. The purpose of this study was to examine patient preference, ease of use, and acceptance of the Diskus device compared with the MDI.

METHODS

Patients

Male and female subjects 12 years of age or older with a medical history of asthma were included. Patients were enrolled if they had used an oral or inhaled short-acting β-agonist for at least 2 months before enrollment and had not been treated with an inhaled asthma controller medication for at least 2 months before study entry.

Patients were excluded from the study if they had only mild-intermittent, exercise-induced, or seasonal asthma. Patients were also excluded if they had life-threatening or unstable asthma; hypersensitivity to β_2-agonists, sympathomimetics, or corticosteroids; had a respiratory infection within 2 weeks before study entry; were pregnant; currently used tobacco or had a $\geq$10 pack-year history of smoking; or had used an investigational drug within 30 days before study entry. Patients with other clinically significant, uncontrolled diseases (eg, coronary artery disease, malignancy, diabetes) were also excluded.

The use of any inhaled, oral, or systemic corticosteroids, long-acting β_2-agonists, cromolyn, nedocromil, anticholinergics, antibiotics, leukotriene modifiers, or other medication that might affect the course of asthma or interact with sympathomimetic amines or corticosteroids was not allowed during and 2 months before the study. Medications for the treatment of rhinitis, including intranasal corticosteroids, were allowed. Maintenance immunotherapy was also allowed if the patient's regimen remained constant throughout the study.

Study Design

This was a multicenter, randomized, open-label, crossover study conducted at 14 centers in the United States. Conduct of this trial conformed to the human experimentation guidelines of the declaration of Helsinki and title 21, parts 50 and 56, of the United States *Code of Federal Regulations*. An institutional review board for each clinical center approved protocols, and all patients (or parent/guardian) gave written informed consent. In addition, assent was obtained for all subjects younger than 18 years old.

The treatment outline is shown in Figure 1. Medication doses were either fluticasone propionate (FP) 100 μg, twice daily, via the Diskus or FP 88 μg, twice daily, via the MDI. A screening/randomization visit was followed by two 3-week treatment periods separated by a 3- to 7-day washout. Subjects attended the clinic a total of four times: at screening/randomization and at weeks 3, 4, and 7. At the screening visit, eligible patients replaced their oral or inhaled short-acting β_2-agonists with albuterol (Ventolin Inhalation Aerosol, GlaxoSmithKline). Treatment assignments were computer-generated in blocks of four; each treatment was represented twice in random order. At screening/randomization (visit 1) and visit 3, patients were instructed in the use of the assigned device. Diary cards were collected and correctness of use testing was conducted after 3 weeks' use of the assigned device (weeks 3 and 7). Patient preference questionnaires related to patient perceived ease of use, overall device preference, and which device the patient would most likely request from his/her doctor, were administered at visit 4 (week 7).

Patients maintained diary cards and recorded morning peak expiratory flow rates using a hand-held Astech Peak Flow Meter (Center Laboratories, Port Washington, NY) and the number of puffs of albuterol used. Combined asthma symptom scores, including chest tightness, wheeze, and shortness of breath, were rated each evening using a six-point scale (0, no symptoms to 5, symptoms that caused discomfort and prevented normal daily activities) and recorded on the diary card. Likewise, use of study drug was recorded daily on the diary card. Subjects were instructed to return completed diary cards at each clinic visit.

Efficacy Assessments

The primary efficacy endpoint was the comparison of the proportion of patients indicating a preference in terms of ease of use for the MDI vs the Diskus. Secondary measures included correctness of use of the devices, overall device

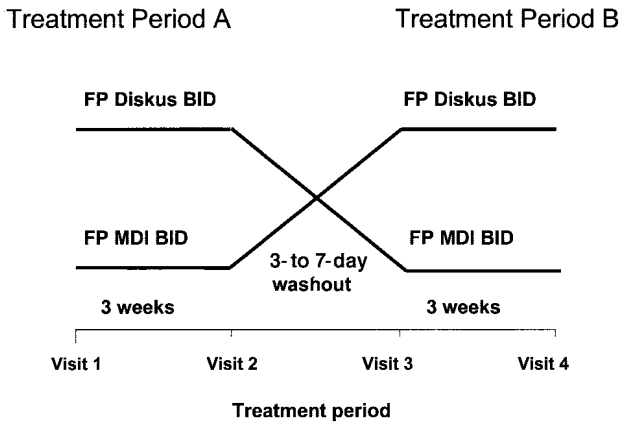

Figure 1. Treatment outline. FP, fluticasone propionate; BID, twice daily; MDI, metered-dose inhaler.

preference, and evaluation of the teaching necessary to use each device correctly. Related measures were preference, in terms of which device was easier to be taught to use, which was easier for the subject to tell how many doses were left in the inhaler, and which device the patient would most likely request from his or her doctor.

Safety Assessments

Safety was evaluated using clinical adverse event (AE) monitoring, asthma exacerbations, and physical examination findings. An asthma exacerbation was defined as any event that required treatment with any asthma medication other than study medication or albuterol. Patients who had an asthma exacerbation were withdrawn from the study. For the purpose of this study, exacerbations were not classified as AEs, unless the exacerbation met the definition of a serious AE.

Statistical Analyses

All statistical analyses were performed using the intent-to-treat population, which consisted of all subjects randomized to study drug (n = 154).

Primary endpoint analysis. The proportion of subjects who indicated a preference for Diskus vs MDI in terms of ease of use was compared using a one-group χ^2 test.

Secondary endpoint analysis. The correctness of use analysis was based on McNemar test of equality of paired proportions. A one-group χ^2 test was used to test for a difference in the proportion of subjects with an overall device preference for the Diskus compared with the proportion of subjects with a preference for the MDI. Preference in terms of easier to be taught to use, easier to tell how many doses were left in the inhaler, and the device one would most likely request from his/her doctor were analyzed in the same manner as device preference. A sign test was conducted to compare compliance rates between devices.

For each device, subjects were rated as to the level of training necessary to teach correct use of the device. The level of training was categorized as reading the instructions only, reading plus 1 training and demonstration session, reading plus 2 training and demonstration sessions, or did not successfully complete all steps after reading the instructions with two training and demonstration sessions. To evaluate the amount of training necessary to teach the correct use of each device, the categories described above were scored as follows: 1, reading the instructions only; 2, reading the instructions plus training and a live demonstration; 3, reading the instructions plus 2 training and demonstration sessions; and 4, did not successfully complete all steps after reading the instructions with two training and demonstration sessions. For each subject, the difference in the score for the Diskus and the MDI was calculated. A sign test on this difference was used to evaluate the amount of training necessary to teach correct use of one device relative to the other.

RESULTS

One hundred fifty-four patients met the inclusion and exclusion criteria required for entrance into the treatment period of the study. Patient demographics are shown in Table 1. To be included in the primary analysis, a patient had to complete both crossover treatment periods (n = 145). Nine patients failed to meet this requirement. Three patients withdrew because of worsening asthma symptoms, 2 withdrew due to an AE (chest pain-Diskus/MDI sequence, bipolar disorder MDI/Diskus sequence), and 2 were lost to follow-up. Two patients were withdrawn early due to protocol violations. Two patients who completed all visits and questionnaires but were later found to have protocol violations were included in the analysis.

Device Preference

Sixty percent of all patients said that they preferred the Diskus overall compared with 40% of the patients for the MDI ($P = 0.016$). In subjects older than 40 years of age, an

Table 1. Summary of Demographic and Baseline Characteristics

	Diskus/MDI	MDI/Diskus	Total
Intent-to-treat population	N = 78	N = 76	N = 154
Age, mean (range), y	29.7 (12–61)	28.1 (12–54)	28.9 (21–61)
Sex, no. (%)			
Male/female	34/44 (44%/56%)	28/48 (37%/63%)	62/92 (40%/60%)
Ethnic origin, % (no.)			
White	78% (61)	71% (54)	75% (115)
Black	14% (11)	16% (12)	15% (23)
Asian	0	0	0
Hispanic	5% (4)	7% (5)	6% (9)
Other	3% (2)	7% (5)	5% (07)
Duration of asthma, mean (range), y	14.8 (1–53)	14.5 (1–44)	14.7 (1–53)
Baseline % predicted PEFR*, mean (range)	100.8 (59–173)	105.7 (63–204)	103.2 (59–204)

Abbreviations: MDI, metered-dose inhaler; PEFR, peak expiratory flow rate.
* The % predicted PEFR values are from treatment start day +1.

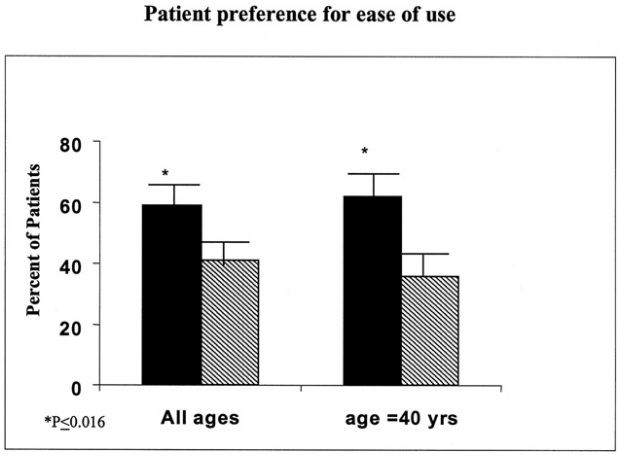

Figure 2. Patient preference for ease of use. Ease of use of the Diskus (solid bars) and the metered-dose inhaler (hatched bars) as perceived by the entire study population and by patients 40 years or younger.

even higher percentage of patients (62%) preferred the Diskus compared with the MDI (38%; Fig 2). In addition, 94% of patients found it easier to track residual doses with the Diskus ($P < 0.001$). The majority of patients (59%) also found the Diskus easier to use than the MDI ($P = 0.025$; Table 2). When patients were asked, if given a choice, which device they would most likely request from their doctor, 59% of the patients indicated that they would most likely request the Diskus ($P = 0.025$; Fig 2).

Device Correctness of Use

Correctness of use testing demonstrated that 98% of patients in this study were able to use the MDI correctly after either reading the instructions only or after reading the instructions along with a single demonstration, vs 91% of patients with the Diskus ($P = 0.002$). Fifty-seven percent of patients perceived the MDI easier to be taught as compared with 43% for the Diskus; however, this difference was not statistically significant. The most commonly missed steps for both the Diskus and MDI were forgetting to exhale completely before inhalation of the dose. For the Diskus another common mistake was not holding the device level. Among patients who failed to use either device correctly after a single demonstration, all were successful after a second verbal demonstration.

Other Measures

Compliance, as evaluated by diary cards, was significantly better with the Diskus; 91.1% of patients used the Diskus as directed compared with 78.6% for the MDI ($P = 0.013$; Fig 3). No differences were seen between treatment groups in morning peak expiratory flow rates, supplemental short-acting β-agonist use, or combined asthma symptom scores (measured as the mean of the last week of treatment), regardless of the sequence of devices.

Safety

Overall, FP treatment was well tolerated when administered from either device. The most common AEs were common cold, headache, and sore throat (4, 7, and 3%, vs 5, 7, and 5% with the Diskus and MDI, respectively). Nine patients experienced drug-related AEs, which included headache (1% vs 3%) and sore throat (1% vs <1%), for Diskus and MDI, respectively. Other drug-related AEs that occurred in less than 1% of patients receiving either the Diskus or MDI included dry throat, hoarseness/dysphonia, oral pain, migraine, and chest pain. Seven exacerbations were experienced by two subjects in each treatment group and were either mild (5) or moderate (2) in severity. The suspected cause of three of the exacerbations was a respiratory tract infection; four exacerbations were of unknown etiology.

DISCUSSION

It is generally thought that for a long-term treatment such as asthma therapy to be successful, it must be effective, it must be used correctly, and patients must be willing to comply with its use. These factors are also important to physicians when prescribing long-term asthma treatments and are key factors in helping patients realize the goals of asthma therapy.

With respect to asthma maintenance therapy, it is reasonable to ask what patients with asthma actually prefer. One recent study suggested that patients did not have a strong preference for either oral or inhaled medication, but greatly preferred once- or twice-daily therapy to medications taken four times a day. In fact, most of these asthma patients stated

Table 2. Questionnaire Results*

Questions asked	Number (%) of patients who preferred the Diskus	Number (%) of patients who preferred the MDI	*P* value
Overall, which device did you find easier to use?	86 (59%)	59 (41%)	0.025
Which device did you find easier to be taught to use?	63 (43%)	82 (57%)	0.115
Which device made it easier to tell how many doses were left in the inhaler?	139 (94%)	9 (6%)	<0.001
Given a choice, which device would you most likely request from your doctor?	86 (59%)	59 (41%)	0.025
Overall, which device did you prefer?	87 (60%)	58 (40%)	0.016

Abbreviation: MDI, metered-dose inhaler.
Ease of use and preference for the Diskus or MDI are presented as number of patients (percent).
* The total number of patients surveyed was 154.

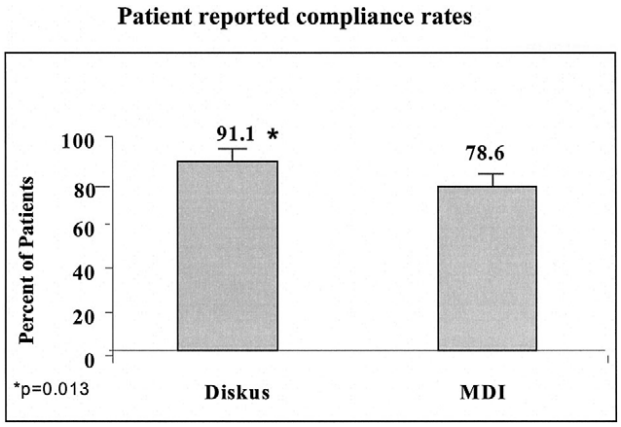

Figure 3. Patient-reported compliance rates. Subjects recorded use of study drug daily and were instructed to return completed diary cards at each clinic visit. MDI, metered-dose inhaler.

they would favor an inhaled drug taken once daily.[13] Although speculative, this may have been attributable to the familiarity asthma patients have with inhaled medications, since the first drug most asthma patients are given is an inhaled bronchodilator.

In a similar study, Camargo et al[14] showed that asthma patients visiting an emergency department had no preference for an oral medication vs an inhaled drug, if both were equally efficacious. However, the inhaled drug was highly preferred if patients were told it was twice as efficacious as the oral drug, suggesting that drug efficacy strongly influences patient preference.[14] Although it is essential for inhalation devices to deliver reproducible doses of the appropriate particle size (ie, between 2 and 5 μm) with each actuation,[3] compact device size, multidosing capability, ease of use, ability to track residual doses, and cost-effectiveness are other features that patients find extremely desirable.[4] These factors contribute significantly to patient acceptance and compliance.

The design of this study required that patients have a diagnosis of asthma that required the use of inhaled or oral β_2-agonists before study entry. Since both devices contained similar doses of the same steroid medication, any potential drug or efficacy bias affecting patient preference was eliminated. This observation was supported by the diary data results. The average patient in this study reported having had symptoms of asthma for an average of 14 years (Table 1) and, therefore, was familiar with the use of the MDI, at least as a rescue medication. This familiarity was reflected in the fact that 98% of the patients were able to accurately use the MDI after either reading the instructions or by viewing a single demonstration. However, only 41% of these patients indicated that the MDI was easier to use and would actually request it from their physician even if an alternative such as the Diskus were available. Other studies have shown varying abilities to effectively use the MDI. A study by Manzella et al[5] found that most patients use MDIs incorrectly, despite training from their physicians on proper technique. Thompson et al[15] found that the rate of misuse of an MDI by hospitalized patients approached 80%, even after house staff had received training on how to teach proper inhaler use. In another study,[16] 28% of adult asthmatic patients could not use the MDI device correctly after a 1-year self-management program. Furthermore, when Newman et al[17] evaluated the repercussions of incorrect MDI use, they found that patients who had poor technique only achieved a mean improvement in forced expiratory volume in 1 second (FEV_1) of 10% after inhaled albuterol (baseline FEV_1, 57% predicted). However, when patients were instructed on correct MDI use, the mean improvement in FEV_1 after inhaled albuterol increased to 30%. In addition, when used incorrectly, medicine is less likely to be deposited in the lungs; therefore, patients are more likely to compensate by taking more puffs than actually needed.[17] Since this was a device comparison between the use of the MDI and the Diskus, spacers were not allowed. Spacers, although recommended in clinical practice, would have added an additional device into this study. With the training involved, this would have added another variable into the study.

Although the Diskus is a relatively new device compared with the MDI, 91% of patients performed all the maneuvers correctly after reading the instructions and/or viewing a single demonstration. The remainder of patients successfully used the Diskus after an additional demonstration. Fifty-nine percent of the patients thought the Diskus was easier to use and would request the device from their physicians. Sixty percent of the patients preferred the Diskus overall to the MDI. These data are consistent with several other studies that have shown the Diskus to be easy to use and easy to teach.[4,12,15]

In comparative studies of DPIs, the Diskus was the preferred device. Diskus was preferred over the Diskhaler,[4,18–20] with more patients using the Diskus correctly and more patients content with the device. Similarly, when the Diskus was compared with the Turbuhaler it was also the preferred device, with more patients using the Diskus correctly and more patients preferring it to the Turbuhaler.[4,16,19,20]

This study also demonstrated that patients preferred the Diskus as a delivery device for long-term asthma therapy. Patients found the device easy to use, particularly liked the dose counter, and most reported that, if given the choice, they would request it from their physicians. Thus, this device appears to be preferred by a majority of patients compared with the MDI, the former standard device for inhaled asthma therapy.

In this study compliance was significantly higher with Diskus (91.1%) compared with MDI (78.6%). This is the first study to show increased compliance with the Diskus device when compared with an MDI. Other studies comparing DPIs such as the Turbuhaler have not shown better compliance compared with the MDI (87% vs 95%, respectively).[21] The overall preference and perceived ease of use of the Diskus may also have contributed to the significantly better compli-

ance compared with the MDI. The importance of improved compliance should not be underestimated. Several studies[22,23] have shown that increased use of inhaled corticosteroids results in better treatment outcomes, including fewer emergency department visits, hospitalizations, and deaths. Should the use of this delivery device result in higher patient compliance in the clinical setting, better treatment outcomes may be realized. Taken together, these data indicate a significant level of Diskus acceptance in this patient population.

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Extraction of Seed Oils with Liquid and Supercritical Carbon Dioxide

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Vegetable oils can be extracted from crushed seeds with liquid or supercritical carbon dioxide. The yields obtained depend upon the pressure and the temperature employed during extraction as well as the size and shape of the seed particles. Oil fractions differing in color, taste, and odor can be recovered at various pressures and temperatures. Parameters influencing the extraction and fractionation of soybean, sunflower seed, and rapeseed oils are described.

Pressing as well as extraction with organic solvents is used widely in the production of vegetable fats and oils. The yields obtained by pressing are not as high as those achieved by extracting oil seeds. Therefore, pressing of intact or ground seeds, a most convenient process, is often followed by extracting the resulting press cake with hot organic solvents, such as petroleum hydrocarbons, for nearly quantitative recovery of the seed oils. Solvent extraction alone is used, e.g., in the commercial production of soybean oil.

The present communication describes the results of studies aimed at substituting organic solvents by liquid or supercritical gases, particularly carbon dioxide, for the extraction of oils from soybeans, sunflower seeds, and rapeseeds at fairly low temperatures.

The complete removal of organic solvents used for extracting seed oils is mandatory, if the oil is to be used for human consumption. Liquid and supercritical carbon dioxide offer the advantage of being easily removable from the extracted oil. In contrast to organic solvents and some of their contaminating components, carbon dioxide is nontoxic, and it cannot easily lead to environmental pollution. Moreover, this inexpensive gas is available on an unlimited scale both from renewable organic resources and from inorganic material including various minerals.

As in the extraction with organic solvents, the efficiency of extraction with liquid and supercritical carbon dioxide is dependent upon its amount and the time it is in contact with the ground seeds. The yield of oil is also influenced by the size and physical structure of the seed particles. In working with liquid and supercritical gases, pressure and temperature during extraction and recovery of the oil are parameters that should receive special attention.

The principle of the equipment used for the extraction of seed oils with liquid and supercritical carbon dioxide, as shown in Figure 1, is simple. Gaseous carbon dioxide is condensed in a diaphragm compressor, C, to a pressure of 350 bar, p1; even higher pressure, up to 700 bar, can be obtained by employing a second compressor. The liquid or supercritical carbon dioxide flows through an extraction vessel, E, containing crushed seeds. The extracted oil is recovered from its solution by lowering the pressure in two stages, in a first trap, S1, to ~200 bar, p2, and in a second trap, S2, to 30–65 bar, p3, that is, below the critical pressure of carbon dioxide. The gas released is again condensed in the compressor, C, thus closing the cycle. Further details of the construction and operation of the equipment used are described under Experimental Section.

EXPERIMENTAL SECTION

Materials. Seeds of soya, *Glycine max* var. Corsoy, were obtained from the American Soybean Council, St. Louis, MO, those of sunflower, *Helianthus annuus* var. Fransol, from the International Sunflower Association, Zevenaar, The Netherlands, and those of rape, *Brassica napus* var. Rapora, from Norddeutsche Pflanzenzucht Hans-Georg Lembke K.G., Hohenlieth, Germany.

Analytical Procedures. Oils were extracted by treating the ground seeds (60–100 mesh) in a Soxhlet apparatus with hexane for 5 h. After evaporation of the solvent, the oil content of the seeds was determined gravimetrically.

The oils extracted with hexane as well as those obtained by extraction with liquid or supercritical carbon dioxide were analyzed by thin-layer chromatography on silica gel

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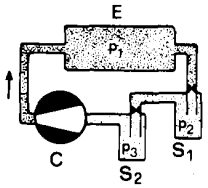

Figure 1. Principle of the experimental design employed in extracting seed oils with liquid and supercritical carbon dioxide. C, compressor; E, extraction vessel; S1 and S2, separators (traps); p1, p2, and p3, pressure in various parts of the apparatus.

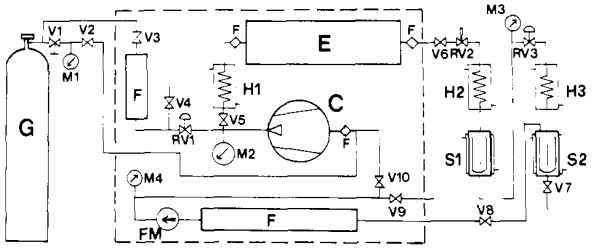

Figure 2. Apparatus used for extraction with liquid or supercritical carbon dioxide. For details of construction and operation, see the text.

G (E. Merck A.G., Darmstadt, Germany) with hexane–diethyl ether–acetic acid (80:20:1 v/v) as the solvent and a 0.1% aqueous 2′,7′-dichlorofluorescein solution or iodine vapor as the indicator (Mangold and Malins, 1960).

Aliquots (10 mg) of the oils extracted with hexane or liquid or supercritical carbon dioxide were subjected to methanolysis (Chalvardjian, 1964); the resulting methyl esters were purified by thin-layer chromatography in the above solvent system. The fatty acid composition of the various oils was determined by gas chromatography with a Perkin-Elmer F22 instrument using a glass column, 2 m × 2.5 mm, packed with 10% Silar 5 CP on Gas-Chrom Q, 80–100 mesh, at a temperature of 200 °C, with nitrogen as the carrier gas.

Apparatus. The design of the equipment used is shown in Figure 2. Gaseous carbon dioxide of high purity is obtained from a storage tank, G, via a reducing valve, V1; its pressure is indicated by a manometer, M1. A membrane compressor, C (Type MK 35002), (Andreas Hofer Hochdrucktechnik GmbH, Mühlheim-Ruhr, Germany) is used up to a pressure of 350 bar. By the addition of a second compressor (Type MK 1000; Nova Werke AG, Effretikon, Switzerland), pressures up to 700 bar can be obtained. The second compressor, the extraction vessel, and some connecting parts are enclosed in a thermostated and soundproof insulated casing.

The jackets of the heat exhangers H1, H2, and H3, as well as those of the separators S1 and S2 are filled with water that is kept circulating by a pump. Several filters, F, are used to purify the gas stream.

The pressure used during extraction, p1, is adjusted by a pressure regulator, RV1 (Circle Seal, Anaheim, CA) and checked by the manometer M2. The excess output of the compressor is fed back into the gas tank, G. The flow rate of carbon dioxide through the extraction vessel, E, is regulated by the valve RV2 (C.T. GmbH, Hamburg, Germany) through which the gas is allowed to expand. The pressure in the preseparator, S1, is kept constant by the pressure regulator RV3; it can be read off the manometer M3. The flow rate of carbon dioxide is measured by a flow meter FM (Brooks Instrument GmbH, Pinneberg, Germany) that is installed after the two separators S1 and S2. The oil dissolved is precipitated from the gaseous phase, which means that the temperature in the

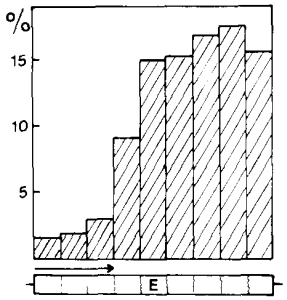

Figure 3. Distribution of residual oil (percent) in various segments of a bed of ground soybeans contained in the extraction vessel E, after treatment with carbon dioxide. For experimental conditions, see the text.

preseparator, S1, is kept above the critical temperature of carbon dioxide and in the final separator, S2, above its liquefaction temperature. Thus, extracts free of water can be obtained at temperatures exceeding 40 °C.

Extraction with Liquid or Supercritical Carbon Dioxide. The ground seeds to be extracted (50–300 g) are filled in the extraction vessel, E, and stamped down carefully. In order to remove air, the entire installation is rinsed repeatedly with carbon dioxide at a pressure of ~10 bar. During this operation, the valves V5, V6, RV2, and V8 are kept open, whereas V7, V9, and V10 are closed; the gas is released via V9 while V2 is kept closed. When the apparatus has reached the desired temperature, it is filled with carbon dioxide in several steps. First, the pressure adjusted at V1 is established throughout the installation by opening V2 and V10. The compressor, C, is then started, V5 being closed and V3 being open, and its output pressure is increased by the pressure regulator RV1. When the desired pressure is reached, V5 is opened and thus the extraction vessel, E, is filled with carbon dioxide. If the oil is to be precipitated in two steps, V6 and RV2 are kept open, for a while, and the pressure desired for trapping it in S1 is adjusted at RV3. The valve V6 is closed when this pressure is reached.

Extraction is started by opening valve V6 and adjusting the flow rate of carbon dioxide at RV2. The extracted oil is collected in test tubes that are placed in the separators S1 and S2. For fractional recovery of the oil, the extraction process can be interrupted for a few minutes. Alternatively, the extract can be withdrawn through valve V7. Such a valve may be installed also at S1; in this case, the gas outlet is fitted on top of the separator. After separation, the gas is released through the sorption filter F and the flow meter FM. After the extraction process is terminated, the valve V5 has to be closed, thereby reducing the output pressure of the compressor to a pressure slightly above that prevailing in the gas tank, G, and pumping the excess carbon dioxide back into this tank. Valves V5 and V10 are then closed, and the gas remaining in the apparatus is released through V9.

RESULTS

Extraction of Seed Oils with Carbon Dioxide. In order to assess the solubility of seed oils in carbon dioxide, ground soybeans were treated at a pressure of 200–250 bar and a temperature of 40 °C with supercritical carbon dioxide for 60 min. The contents of the extraction vessel were cut into nine segments of equal length, and, after extraction with hexane, the oil that had remained in each section was determined gravimetrically. Figure 3 shows the distribution of oil over the nine zones.

It is evident that, after 60-min extraction with carbon dioxide, the oil content of the first two zones amount to

Table I. Extraction and Stepwise Recovery of Seed Oils

extraction pressure, bar	temp, °C	first fraction: pressure, bar	first fraction: temp, °C	first fraction: yield, %	second fraction, yield, %	total yield of oil, %
		Decrease in Pressure (Rapeseeds)				
300	50	180	50	28.4	4.4	32.8
		Increase in Temperature (Sunflower Seeds)				
300	40	300	75	34.5	11.7	46.2
200	20	200	75	36.7	4.3	41.0
		Decrease in Pressure and Increase in Temperature (Sunflower Seeds)				
300	40	200	60	36.7	8.8	45.5
350	40	90	50	30.7	0.6	31.3

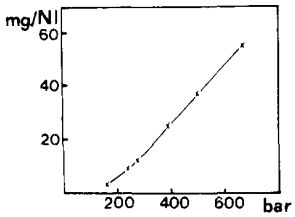

Figure 4. Solubility of sunflower seed oil in carbon dioxide at various pressures and a temperature of 40 °C.

less than 2%, each, whereas from the fifth zone on the oil level remaining in the ground seeds amounts to 15–17%. Thus, it can be presumed that in the initial phase of extraction a saturated solution of oil in carbon dioxide is attained.

The initial treatment with carbon dioxide yielded 6% of oil and the subsequent extraction of the ground soybeans afforded another 10.1%.

Additional experiments showed that over a wide range of flow rates the concentration of oil in carbon dioxide is independent of the flow rate of the gas. The pressure of carbon dioxide during extraction, however, is of great significance. By use of sunflower seeds as an example, Figure 4 shows that, at a temperature of 40 °C, the concentration of oil in supercritical carbon dioxide increases with increasing pressure during extraction. From ~300 bar on, this increase in the concentration of the oil as a function of the pressure of carbon dioxide proceeds in a linear fashion; at 700 bar it reaches a value of 55 mg/NL, which corresponds to ~3%, by weight (1 NL = 1 L of gas at 293 K and 1 atm).

Not only the solubility of an oil in supercritical carbon dioxide but also the solubility in liquid carbon dioxide increases with pressure, though at a different rate. As an example, Figure 5 shows the increase in concentration of soybean oil when the seeds are treated either with supercritical (curve 1) or with liquid carbon dioxide (curve 2).

Obviously, at pressures below 250 bar, the concentration of oil is higher in liquid carbon dioxide whereas above 250 bar its concentration is higher in supercritical carbon dioxide. Apparently, the effect of pressure on the solubility of a seed oil is much more pronounced when supercritical carbon dioxide is used for its extraction. The concentration of oil in supercritical carbon dioxide is a decisive factor in the amount of carbon dioxide needed for the extraction of the oil from a certain amount of seeds. By use of sunflower seeds (100 g) as an example, Figure 6 shows the yields of oil at pressures of 660–700 and 250–280 bar, respectively, and at a temperature of 40 °C, as well as the concentration of oil in carbon dioxide at these pressure ranges.

It is obvious that, at a pressure of 660–700 bar and a temperature of 40 °C, the extraction of 40 g of oil requires

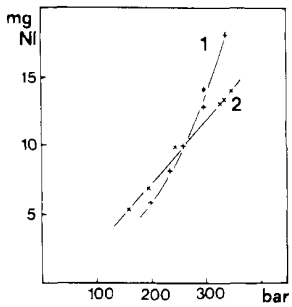

Figure 5. Solubility of soybean oil in supercritical (curve 1) and liquid (curve 2) carbon dioxide at various pressures and at temperatures of 40 and 20 °C, respectively.

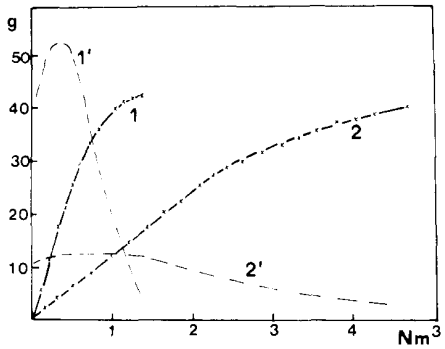

Figure 6. Yields of sunflower seed oil at pressures of 660–700 bar (curve 1) and 250–280 bar (curve 2) and a temperature of 40 °C. The concentration of oil in carbon dioxide at the two pressure ranges is given by curves 1′ and 2′.

~1.1 Nm^3 of carbon dioxide (curve 1) whereas at 250–280 bar and 40 °C the extraction of 40 g of oil requires more than 4 times this amount of carbon dioxide (curve 2) (1 Nm^3, at 293 K, 1 atm, corresponds to ~1.8 kg of carbon dioxide). At the higher pressure, the concentration of oil in carbon dioxide decreases rapidly after ~0.7 Nm^3 have flown through the bed of ground seeds (curve 1′) whereas at the lower pressure the concentration of oil in carbon dioxide remains fairly constant until ~1.5 Nm^3 have been used (curve 2′). Thus, an increase in pressure to over 300 bar can obviously be of advantage, a fact which is in contrast to our experiences so far. We assume the following two reasons to be responsible: a different solubility behavior of the lipids and a relatively high oil content of the seeds.

As an increase in the pressure of carbon dioxide leads to an increase of the solubility of an oil, a decrease in pressure after extraction should be useful for recovering the oil. It should be possible to achieve the same effect by increasing the temperature of the solution. The data given in Table I show how a stepwise decrease in pressure, an increase in temperature, or both can be used to recover

Table II. Extraction and Recovery of Seed Oils

seeds	extraction with hexane,[a] yield, %	extraction with carbon dioxide: pressure, bar	temp, °C	time, min	yield, %	residual oil %
soybeans	19.9					
		280	20	150	16.6	3.3
		300	40	120	16.4	3.1
sunflower seeds	38.4					
		250-300	20	150	35.0	1.5
		320-350	40-50	150	36.0	2.3
rapeseeds	40.1					
		320	17	180	38.2	4.5
		350	40	180	39.3	3.9

[a] Extraction was carried out in a Soxhlet apparatus.

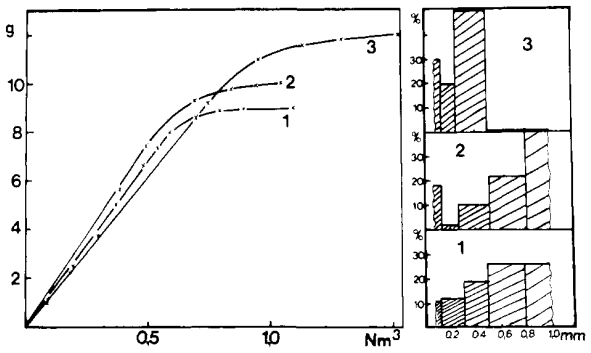

Figure 7. Yields of oil extracted from ground soybeans of different size and shape (1, 2, and 3) (left). The distribution of particle sizes in samples 1, 2, and 3 is given (right). 1, 83 g of beans crushed in a laboratory grinder; 2, 80 g of beans crushed in a cross-beater mill; 3, 80 g of beans crushed in a laboratory grinder.

an oil in fractions. In all cases, final recovery was achieved below the critical pressure and at a temperature that yielded an oil free of water, that is, 30–65 bar and 40–55 °C.

Experiments with sunflower seeds showed that even at supercritical pressures the oil can be recovered in a large amount by increasing the temperature. In a continuous technological process, recovering an oil solely by increasing the temperature would offer the advantage of requiring less energy than would be needed if the same effect were accomplished by decreasing the pressure. The data given in Table II show that both liquid and supercritical carbon dioxides can be used to extract almost all of the oil that can be obtained from various seeds by conventional extraction with hexane.

As in all extraction processes, the seeds have to be ground to assure a satisfactory extraction of the oil. Thus, it is almost impossible to recover any oil by treating intact rapeseeds with liquid or supercritical carbon dioxide; between 96 and 98% of the oil can be extracted, however, from rapeseeds that have been ground to 40–120 mesh.

It should be noted that not only the size of the ground seed but also their shape affect the yields of oil. As an example, Figure 7 demonstrates the differences in the time course of extraction as well as the yield of oil when soybeans ground by different methods are treated with liquid or supercritical carbon dioxide.

Gas-chromatographic analyses of the methyl esters of the constituent fatty acids of oils extracted with hexane or carbon dioxide showed no significant differences.

Samples of soybean, sunflower seed, and rapeseed oils that were obtained by a stepwise process differed greatly in their color, taste, and odor. Some pertinent observations are summarized in Table III.

Table III. Characteristic Properties of Seed Oils Extracted and Recovered under Different Experimental Conditions

source of oil	fractions	characteristics
soybeans	(1) 200 bar, 40 °C	clear yellow oil, odorless
	(2) 40 bar, 40 °C	turbid yellow oil, maltlike odor
sunflower seeds	(1) 150 bar, 40 °C	clear light yellow oil, colorless
	(2) 40 bar, 40 °C	turbid brown oil, acidic odor
rapeseeds	(1) 180 bar, 50 °C	clear yellow oil, slightly bitter taste, odorless
	(2) 45 bar, 50 °C	turbid yellow oil, bitter taste, mustardlike odor

It is noteworthy that the fractions obtained by separation from the miscella at relatively high pressure were clear and less colored than those that were isolated at much lower presures. Moreover, the fractions isolated at "high" pressures had superior organoleptic properties as compared to those obtained at "low" pressures.

Instead of separating most of the oil from the solution at a fairly high pressure and the rest at a much lower pressure, the opposite sequence may be applied to the same end. Thus, extraction of sunflower seeds with carbon dioxide at 150 bar and a temperature of 40 °C yielded a turbid yellow oil having a definite odor; subsequent extraction of the major portion at 330 bar and the same temperature afforded a clear, yellow, and almost odorless oil.

The method can be used also for refining sunflower oil by treating it with carbon dioxide at a pressure of 150 bar and a temperature of 40 °C. The oil obtained as a residue was clear and light yellow whereas the fraction that had been washed out was turbid and brown.

These results demonstrate the efficiency of the use of liquid and supercritical carbon dioxide both for extracting and refining seed oils.

DISCUSSION

The method of extracting natural products with liquified or supercritical gases is still in a state of development. In a few cases the experience gained in work on the laboratory scale have been scaled up to pilot plant and even technical applications (Schneider et al., 1980).

The results of our study on the extraction of seed oils by means of liquid and supercritical carbon dioxide permit an evaluation of the factors that have to be considered in trying to apply the method on a large scale.

We have shown that the amount of carbon dioxide needed depends to a large extent on the pressure and

temperature used during extraction. In our experience, liquid and supercritical carbon dioxides at pressures of at least 250 bar and a temperature of 20 and 40 °C, respectively, are equally suitable for the extraction of oilseeds. At pressures over 300 bar, however, liquid carbon dioxide is inferior to supercritical carbon dioxide with regard to dissolving power. Moreover, still higher pressures reduce the amount of carbon dioxide required to extract a certain amount of oil. Thus, the extraction of 40% oil of sunflower seed at 260 bar requires 47 NL/g of seeds whereas at 700 bar only 11 NL of carbon dioxide/g of seeds is needed. Other natural products are, as a rule, best extracted at pressures below 200 bar.

The yields of oil that can be extracted with liquid or supercritical carbon dioxide are comparable to those obtained by conventional solvent extraction. The use of carbon dioxide offers the advantage, however, that the quality of the extracted oil can be influenced by varying the parameters of the extraction process. Moreover, the oils obtained by extraction with liquid or supercritical carbon dioxide are of course free of organic solvents, whose complete removal is a time- and energy-consuming process in present-day oil technology. Last but not least, extraction with carbon dioxide at ambient temperature is quite obviously of great advantage if the seed proteins are to be recovered because extraction with hot organic solvents invariably leads to pronounced denaturation of these proteins.

The use of liquid and supercritical gases including carbon dioxide for the extraction of compounds of "high molecular weight" has been suggested, more than 40 years ago, in some patents (Pilat and Godlewicz, 1936a,b). The same principle has been recommended for extracting fats and oils (Dickinson, 1947; Groll, 1953; Palmer and Fanwood, 1950), yet practical applications have not been described.

In the sixties, the idea of extracting lipids and other natural products with liquid or supercritical carbon dioxide has gained new impetus (Zosel, 1964). It has been demonstrated that lipids can be extracted from copra, sunflower seeds, soybeans, and shelled peanuts with carbon dioxide at pressures ranging from 280 to 350 bar (Vitzthum and Hubert, 1972). Moreover, it has been shown that the constituents of lipid mixtures that usually are separated by vacuum distillation at fairly high temperatures can be resolved under much milder conditions by fractional recovery from their solution in supercritical gases. The use of a "carrier" has been recommended for the efficient fractionation of complex mixtures (Peter et al., 1976).

Systematic studies have been facilitated by the development of an apparatus for the extraction of natural products with supercritical carbon dioxide on a microscale (Stahl and Schilz, 1976). By means of this piece of equipment, it has been found recently that in the pressure range from 80 to 200 bar a solubility can be reached which is sufficient for the extraction of many nonpolar compounds. For polar, almost insoluble substances, extending the pressure up to 2500 bar does not lead to substantial improvements (Stahl et al., 1978). However, we found that in a preparative extraction plant (Schütz, 1979) terpenes, for example, can be completely extracted in the range up to 160 bar (Stahl and Schütz, 1978). Contrary to these results, it has been shown that the solubility of lipids increases steadily in the range above 160 bar (Schilz, 1978).

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