RUTGERS

New Jersey Agricultural Experiment Station

Sampling Services & Procedures for the Hemp Industry, 2020

Jim Simon, Qingli Wu, Tom Gianfagna, Katharine Jaworski, Ariane Vasilatis, Theodore Brown, Martin Zorde

New Jersey Agriculture Experiment Station in concert with the New Use Agriculture and Natural Plant Products Program and the Center for Food Systems & Sustainability School of Biological & Environmental Sciences Rutgers University, 59 Dudley Road, New Brunswick, NJ 08901

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Hemp, CBDs and Hemp Seed Oil

Hemp is an aromatic plant:

- -High in fiber
- -Rich in a nutraceutical seed oil
- -Rich in CBDs and other bioactive compounds
- -Hemp usually also contains THC (challenge is to ensure it is <0.3% THC
- -Hemp usually also contains other aromatic terpenes
- -The secondary products are stress inducible
- -Plants must be sampled to ensure it is hemp, and not marijuana

A few critical parameters:

- Ensuring permissible limits of THC during all stages of value chain, especially at end
- Stability and shelf-life
- QC program needs to be in place at outset
- Proper packaging, security and tracking



Hemp in NJ

The 2018 Farm Bill:

- Further defined Industrial Hemp
- Removed from Controlled Substances Act (CSA)
 - Considered as an agricultural product
 - States and tribes can submit plans for primary regulatory authority over hemp production in their state/tribal territory

Alaska, Arizona, Kansas, Missouri, New Jersey, and Oklahoma enacted legislation for research and pilot programs

New Jersey

AB 1330 / SB 3145 (2018)

- Directs the New Jersey Department of Agriculture to create a pilot program to research industrial hemp cultivation.
- Exempts anyone participating in the agricultural pilot program from crimes and penalties relating to the purchase, sale, or cultivation of marijuana.



Hemp in NJ



https://d2azl42aua8mom.cloudfront.net/wp-content/uploads/2019/11/01171142/USDA-Hemp_fullwidth.jpg

NJ's Hemp Program was among the first three states approved by the USDA on December 27th, 2019

- NJ's Hemp Program complies to the 2018 Farm Bill
- License applications for the 2020 growing season are currently open and can be found on the NJDA website under the New Jersey Hemp Farming Act

From New Jersey Hemp Program N.J.A.C. 2:25-1 et seq.



Required Procedures

By the NJDA, NJ Hemp Grower License:

- Can be issued to an individual, or a business
- Handling In-Program, or unprocessed, material

NJ Hemp Processor/Handler License:

- Can also be issued individually or to a business, such as a third-party lab
- Handling compliance testing and production of Out-of-Program, processed, material



https://www.nj.gov/agriculture/divisions/pi/pdf/2020%20NJH%20Grower%20Application.pdf

From "Processor/Handler License Application", NJDA, New Jersey Hemp Farming Act

From "Hemp Grower License Application", NJDA, New Jersey Hemp Farming Act,

From "Instructions and Attachments for Participants", NJDA, New Jersey Hemp Farming Act



Program Materials

Table 1: In-Pro	ogram Materials (Unprocessed)	
Harvestable Component	Form of Material	
Fiber	whole stalks, including leaf and seed materials	
	bales of stalks	
Roots	raw	
Leaves or	fresh, unprocessed	
Floral Material	dried	
riorar Waterial	ground	
Grain (food product)	raw, unprocessed	
Seed (for planting)	whole seed, cleaned or uncleaned	
Transplants	rooted plants	
	cuttings	

Table 2: Ou	t-of-Program Materials (Processed)
Harvestable Component	Form of Material
Fiber	whole stalk stripped of leaf and seed materials decorticated fiber (bast and/or hurd)
Roots	dried ground
Leaves or Floral Material	cannabinoid extract All products derived from extracts
Grain (food product)	crushed, ground, etc. dehulled seed cake / meal roasted or toasted AND proven nonviable Seed Oil
and an annual segment of the second	ansfers of these materials are compliant with r state and federal laws may apply. Transfers are



Program Materials

"NJDA is <u>not responsible</u> for ensuring product quality or product compliance with other regulating authorities, such as the Federal Food and Drug Administration (FDA) and the New Jersey Health Department. <u>Licenses issued by the NJDA do NOT offer any legal protections from these governing bodies and do not provide waivers from their regulations."*</u>





https://www.fda.gov/media/99788/download

https://www.nj.gov/health/assets/img/NJDOH_logo_sp otlight.jpg



Required Procedures

- License agreements must be signed <u>before</u> <u>taking possession</u> of any viable hemp seeds or in-program harvested hemp materials
- When applying, <u>a colored map</u> and <u>GPS</u>
 <u>coordinates for all processing, handling, and</u>
 <u>growing locations</u> are required:

"You are required to provide to NJDA a photographic aerial map of all growing, handling, and storage locations. This requirement applies to all applicants and License Holders, and will assist with the NJDA's required reporting to law enforcement."



https://cdn.britannica.com/89/211689-050-628DD5DF/hemp-plantation.jpg



Fees for Growers

Licen	ise	Fee	
GRO	W	ER	

\$300 plus \$15 per acre

NOTE: GPS coordinates for all growing locations (fields and greenhouses/indoor growing sites) and storage locations are submitted on the application. Changes to growing locations will incur a Site Modification Fee (see below).

@ time of application

Important to note regarding GPS coordinates:

- Include GPS coordinates for each field or building
- GPS coordinates should be provided in DEGREES DECIMAL MINUTES (dd° mm.mmm'; example: lat: 38° 9.919'N, long: 84° 49.267'W)
 - This can be done through:
 - Web based mapping such as Google Earth
 - GPS coordinates from a smartphone
 - https://www.geoplaner.com/



Mapping

Google Earth is NJDA's preferred method for mapping, and they provide a <u>free code</u> within "**Instructions for creating maps for submissions to NJDA**"

- Farm Service Agency (FSA) maps <u>are generally not sufficient as they</u>
 typically are not in color and do not identify the roadway
- Maps must include:
 - Outline of each separate field to be used for <u>contiguous</u> <u>planting</u>
 - Greenhouses, indoor growing structures, storage buildings, or handling facilities and the location ID/name of each structure

Refer to "Instructions for creating maps for submissions to NJDA" in Instructions and Attachments for Participants for all details

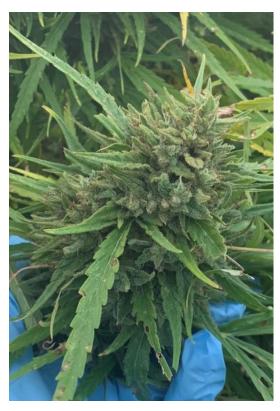


NJDA Mapping Example





Hemp Grower Documents



Katharine Jaworski

- If applying as a business, only one signing authority can represent
 - Can be changed later through a new Signing Authority for Corporate Entities form
- Reports are required throughout the growing season, including preplanting reports, planting reports, pre-harvest reports, and one annual production report

From New Jersey Hemp Program N.J.A.C. 2:25-1 et seq.

From "Hemp Grower License Application", NJDA, New Jersey Hemp Farming Act, https://www.nj.gov/agriculture/divisions/pi/pdf/2020%20NJH%20Grower%20Application. pdf



Hemp Grower Documents

Examples of reports:

- 2020 Planting report(s) are due:
 - For each address in your Licensing Agreement and each field and or every indoor growing address that was identified prior
 - Within 10 days following the first day of each planting
- 2020 Harvest/Destruction Report(s) are due:
 - For every field or indoor area planted
 - At least 30 days prior to harvest or destruction
 - An NJDA inspector will schedule an appointment to collect sample(s) unless you indicate your intent to hire a third party lab
 - Written approval required before harvest or disposal From "Instructions and Attachments for Participants", NJDA, New Jersey Hemp Farming Act, https://www.nj.gov/agriculture/divisions/pi/prog/nj hemp.html

From "Hemp Grower License Application", NJDA, New Jersey Hemp Farming Act, https://www.nj.gov/agriculture/divisions/pi/pdf/2020%20NJH%20Grower%20Application. pdf



Fees for Processing/Handling

License Fee – PROCESSOR / HANDLER (Due annually)

Fees per Processing Type

@ time of application

- Handlers \$450 annual fee
 - An example of a "handler" includes any private lab or service provider, such as a seed cleaner.
- Grain Processor \$450 annual fee
- Fiber Processor \$450 annual fee
- Floral, Oil or CBD Processor \$1,000 annual fee

NOTE 1: Processors working with multiple harvestable components will be required to pay an annual fee for EACH component. Ex. Grain (\$450) + CBD (\$1,000) = \$1,450 Production* Fee

Site Modification Fee (SMF) NOTE 2: GPS coordinates for all processing, handling, and storage locations must be submitted with the application submission. Changes to processing sites following execution of the *Processor Licensing Agreement* will incur a Site Modification Fee (see below).

\$300 plus \$15 per acre each change or addition of GPS coordinates

Defined as any change to the GPS coordinates for processing or growing locations at an existing address on the *Licensing* form *Agreement*, or for the addition of a GPS coordinate not already on the *Licensing Agreement*). Storage location changes or additions will not incur the SMS.

@ submission of site modification request form



From Field to Lab to Public

In-Program material, <u>must remain in the</u> <u>Hemp Program</u>

Only to be handled by:

- 1. An individual or business with a NJDA licensing agreement as a handler or processor
- 2. or legally operating within another program authorized by federal law

Once processed, finished products can be transferred or sold to the general public, those with or without licenses



Source: Botanacor.com (not recommending but using only As an illustrative example



Transferring Samples to Labs

- Transfers of hemp material for phytocannabinoid level analysis are not to exceed 1 lb (0.45 kg) per sample
- Samples must be labeled properly and directly sent to the company address of the chosen lab



https://ritterspencer.com/wp-content/uploads/2019/11/USDA-Hemp-Production-Plan-Part-II-Sampling-Testing-of-Hemp.jpg



Transferring Samples to Labs

- For all samples being transferred/delivered to labs for testing, program participants (growers and handlers) must be prepared to produce a copy of the Licensing Agreement upon request by:
 - NJDA Hemp Staff,
 - The New Jersey State Police,
 - Or, to any law enforcement agency
- Additionally, a copy of the Licensing Agreement, along with the address of origin, must accompany any hemp material in transit



Inspection and Compliance:

- Sampling carried out by the DEA or a DEA registered third-party lab shall occur within 15 days prior to the anticipated harvest date
- Additionally, an annual inspection, at minimum, will be carried out to randomly sample and this inspections may be random and withour advance notification to grower and/or processor
- A <u>minimum of two samples from each variety</u> planted during the growing season and before harvest to ensure compliance with the federally defined THC level
- Hemp producers must agree to grant entry to the DEA into premises where hemp is grown, processed or handled for inspections and may be required to be present



Testing of In-Program Material:

- Quantitative determination of delta-9 THC levels
- THC testing procedures must use decarboxylation or other reliable methods such as High performance liquid chromatography (HPLC) or Ultra-Performance Liquid Chromatography (UPLC)
- The method used for sampling from the flower material of the cannabis plant must obtain a confidence level of 95 percent to ensure no more than one percent (1%) of the plants in the lot would exceed the federally defined THC level for hemp

From New Jersey Hemp Program N.J.A.C. 2:25-1 et seq.



Required Testing Results for In-Program Material:

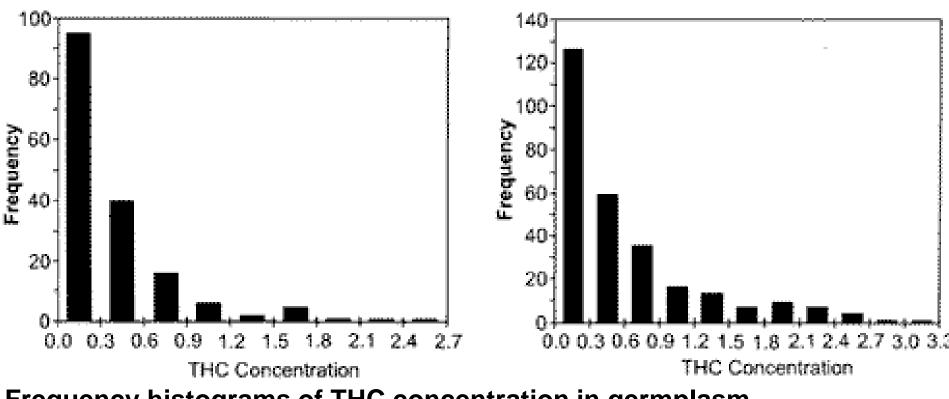
- Ensure that all hemp grown and processed maintains the federal delta-9 THC concentration limit of 0.3 percent on a dry weight basis*
- "All hemp with a delta-9 THC concentration of more than 0.3% must be destroyed, but it will only be considered a negligent violation pursuant to these rules if the hemp has a delta-9 THC concentration of more than 0.5% on a dry weight basis." A hemp producer who violates these rules with a culpable mental state greater than negligence may be subject to criminal law enforcement actions.
- Results are subject for review by the DEA, who are authorized to retest and collect samples
- A \$150 fee shall be assessed for all Departmental testing, including but not limited to, retests and pesticide residue quantification tests, unless inconsistent, then it may be waived

From New Jersey Hemp Program N.J.A.C. 2:25-1 et seq.

*Seed lots may not be genetically homogenous- don't assume fixed THC levels!



Why is there a concern about THC with Hemp?



Frequency histograms of THC concentration in germplasm collections.

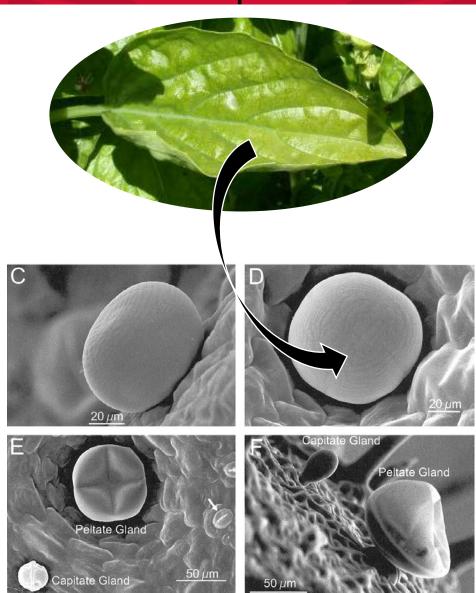
Left, collection of E. Small and D. Marcus; 43% of the 167 accessions had THC levels >0.3%.

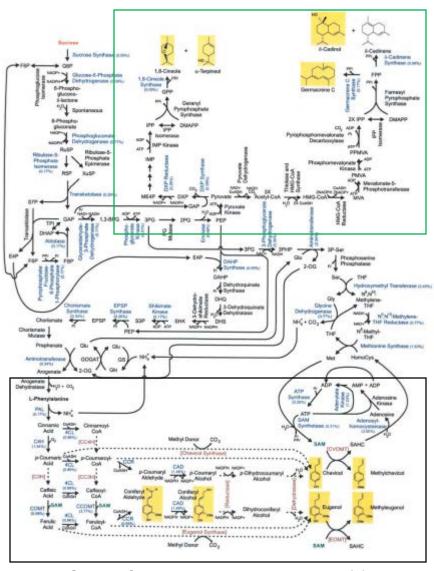
Right, Vavilov Institute, St. Petersburg; of the 278 accessions about 55% had THC levels >0.3%. (Small & Marcus, 2002)

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Our Rutgers lab studies and profiles plant volatiles and bioactive terpenes



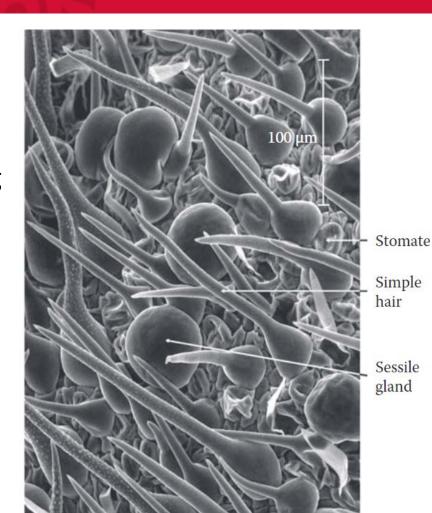


Gang & Simon 2001. Plant Physiol.125 (2): 539-555.



Revisiting hemp glandular trichomes

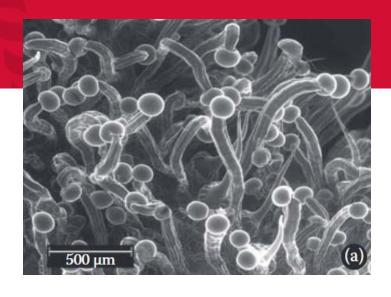
- Genetic and environmental factors affect the distribution of trichomes and biosynthesis of cannabinoids
- Manipulating this factor is one approach with selection and breeding that can have impact on breeding for high or low levels of cannabinoids
- Glandular trichomes, plant's drug factories
- 30% of flowering plants possess glandular trichomes
- Produce secondary, bioactive, metabolites
- Three classes of epidermal secretory glandular trichomes





Type of epidermal structures

- Scanning electron micrographs from a high THC variety of *C. sativa*
- Stalkless or sessile type, may have a very short stalk (c)
- Long-stalked glands (a) and (c), at the center of the image (c)
- Non-glandular hair, on the background of (c)

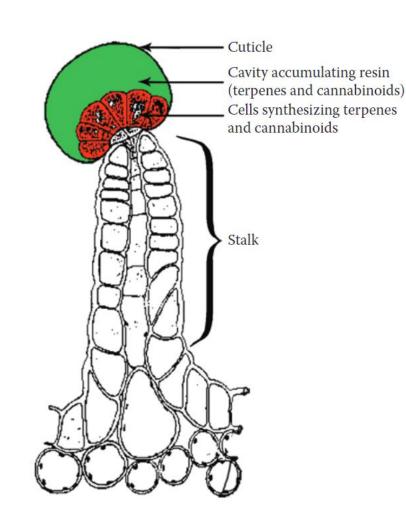






Site of Cannabinoid synthesis

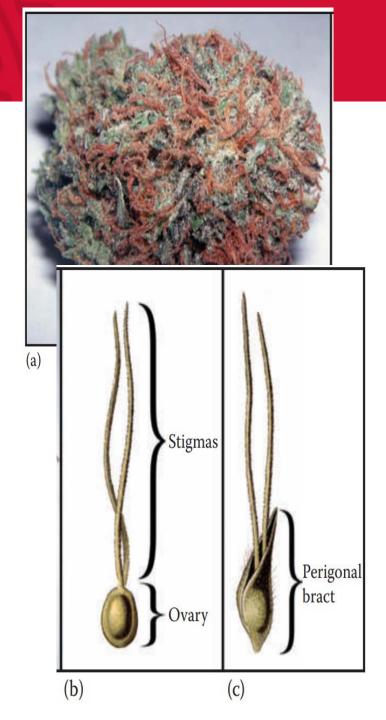
- Anatomy of a long-stalked secretory gland
- Glandular head and stalk
- The cells of the glandular head produce the resins (red), that contains terpenes and cannabinoids
- Resin is excreted and accumulated in the cavity (green)
- That resin is what you touch and feel with the 'buds'





Site of THC accumulation

- a. Buds
- b. Actual female flower, devoid of THC, so defining or characterizing marijuana as the flowers not quite botanically correct.
- c. The bracts (modified specialized leaves associated with flowers) are the structures that accumulates most of the THC.
- Female flowers of cannabis lack sepals and stamens lack typical petals.
- A female flower is inside a surrounding perigonal bract.
- The perigonal bracts contain the majority of the bud's THC but are not visible in (a) as they are nestled deeply amidst tiny leaves. The reddish-brown threads in (a) are dried, overmature stigmas, shown in the fresh, green stage in (b).



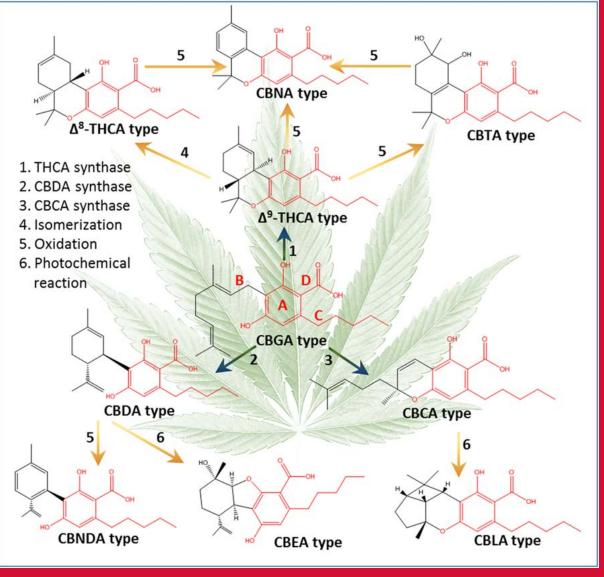


THC content in the plant

- Absolute cannabinoid content varies in different parts of the plant
- 10–12% in pistillate flowers
- 1–2% in leaves
- 0.1–0.3% in stalks
- < 0.03% in the roots
- None in actual seed

[Actual procedure in sampling rather critical]

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Phytocannabinoid biosynthesis and degradation routes and products. The most prevalent acid components are presented for each type of phytocannabinoid. In general, CBG, $\Delta 9$ -THC, CBD and CBC phytocannabinoid subclasses are biosynthesized in *Cannabis* plants, while the <u>other</u> subclasses are probably the result of decomposition either in the plant or due to poor storage conditions following harvest.

Of the >550 metabolic constituents in hemp, >140 cannabinoids of cannabis have been identified- with many more likely to be identified. Key ones include D9-THC (delta-9 tetrahydrocannabinol), the chief intoxicant chemical and predominates in intoxicant strains; the isomer D8-THC was previously considered to be found in trace amounts. CBD (cannabidiol) is the chief non-intoxicant chemical, and predominates in non-intoxicant strains- or in hemp. The non-intoxicant CBN (cannabinol) is a degradation/oxidation product. Non-intoxicant cannabichromene (CBC) is a trace compound in intoxicant strains. Non-intoxicant cannabigerol (CBG) is considered

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- > The principal phytocannabinoids appear to be delta-9-tetrahydrocannabinol (i.e. Δ9-THC, THC), CBN, and cannabidiol (CBD)
- > Other phytocannabinoids found in cannabis include cannabigerol (CBG), cannabichromene (CBC), tetrahydrocannabivarin (THCV) and many others
- In the living plant, these phytocannabinoids exist as both inactive monocarboxylic acids (e.g. tetrahydrocannabinolic acid, THCA) and as active decarboxylated forms (e.g. THC); however, heating (at temperatures above 120 °C) promotes decarboxylation (e.g. THCA to THC)



Required Testing Results for Out-of-Program Material:

- Ensure that the final product batch of any processed hemp, such as floral extracts, maintains the federal delta-9 THC concentration limit of 0.3 percent
- Seeds must have third-party germination study carried out, and render 0% germination level using roasting or toasting methods
- Processors need to label hemp products with the amount of oil or extract, the percentage of THC, and the percentage of CBD extract
- Additionally, labeling must distinguish between hemp extract,
 CBD, or hemp oil

From New Jersey Hemp Program N.J.A.C. 2:25-1 et seq.

From "2020 New Jersey Hemp Testing Guidelines", NJDA, New Jersey Hemp Farming Act, https://www.nj.gov/agriculture/divisions/pi/pdf/HempTestingGuidelines.pdf



Hemp Seeds

- Hemp seeds are a product of commerce
- Hemp seeds contain virtually no THC, but THC contamination results from contact of the seeds with the resin secreted by the epidermal glands on the leaves and floral parts, and also by the failure to sift away all of the bracts (which have the highest concentration of THC of any parts of the plant) that cover the seeds
- Hemp seeds can be cleaned, processed, ground and/or pressed for hempseed oil used in foods and beverages

KUIGEKS	Fatty Acid Composition of Hempseed of	di
New Jersey Agricultural		
Experiment Station	Fatty said composition (0/)	

Futura

77

5.6

0.2

3.1

12.8

54.6

1.5

19.2

0.8

0.4

0.6

0.2

1.3

The fatty acid of the seeds compare favorable to that of canola,

various anti-oxidants, including tocopherols and sterols (Blade et

and also contain low amounts of GLA. The oils also contain

Beniko

5.5

0.2

2.8

12.2

56.4

2.1

17.8

0.8

0.4

0.3

0.2

1.3

Fedora 19

5.5

0.2

2.8

12.0

56.1

2.0

18.1

0.8

0.4

0.3

0.2

1.6

Felina 34

5.4

0.2

2.8

11.8

56.1

2.1

18.5

0.8

0.4

0.3

0.2

1.4

Fatty acid

Palmitic (16:0)

Stearic (18:0)

Oleic (18:1)

Linoleic (18:2)

Linolenic (\alpha 18:3)

Arachidic (20:0)

Eicosenic (20:1)

Behenic (22:0)

Others

Erucic

Lignoceric (24:0)

al., 1999).

Gamma linolenic (y18:3)

Palmitoleic (16:1)

Fatty acid composition (%)

Zolotonosha 11(LR)

5.4

0.2

2.9

12.1

55.6

2.2

18.4

0.4

0.4

0.3

0.2

1.5

Zolotonosha 13(LR)

5.6

0.2

2.9

12.2

55.6

2.2

18.1

0.8

0.4

0.3

0.2

1.5

Typical canola

3.2

0.2

1.2

55.6

21.7

12.9

0.6

2.2

0.3

0.4

1.5

MATGERS	Fattv	Acid	Comi	oosit	tion of	f H	empsee	d oil
New Jersey Agricultural								<u> </u>

RUTGERS	Fatty Acid	Composition	of Hemr	seed oil
New Jorgey Agricultural	i ally Acid	Composition	OI HEILI	Jaccu VII



Hemp Testing Laboratories are encouraged to adhere to ISO 17025, but are not required by the USDA

Laboratories Approved for THC testing MUST:

- Be registered with the Drug Enforcement Administration (DEA) to handle controlled substances under the Controlled Substances Act (CSA), 21 CFR 1301.13
- Be registered with the NJ Hemp Program as a Handler
- Comply with the Departments testing procedures
- Directly transmit results to the Department
- Submit to random quality assurance testing by the Department to validate the accuracy of testing results

From New Jersey Hemp Program N.J.A.C. 2:25-1 et seq.

From "2020 New Jersey Hemp Testing Guidelines", NJDA, New Jersey Hemp Farming Act, https://www.nj.gov/agriculture/divisions/pi/pdf/HempTestingGuidelines.pdf



NJAES Hemp Team Can Provide Support to Growers and the State by:

- Analyzing samples for THC & CBDs
 We have the state of art analytical instrumentation including High performance liquid chromatography (HPLC/MS and Q-TOF/HPLC/MS) and an Ultra-Performance Liquid Chromatography (UPLC/QQQMS) allowing us to comply with THC testing
- We can screen samples for cannabinoids in hemp (THC and CBDs) from field or greenhouse through postharvest handling and processing to final product evaluation/testing.

procedures that use decarboxylation

 Ensuring all samples tracked and archived using LIMS system and even subsampled for other certified labs.

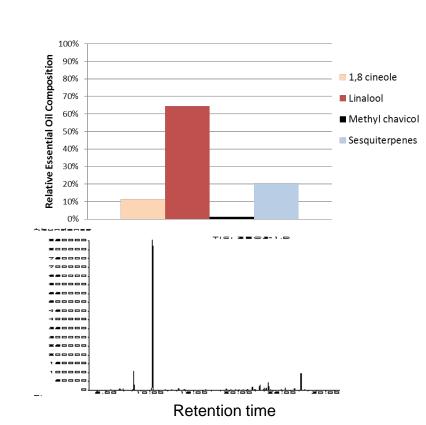
- We can analyze the fatty acid composition of hemp and seed oil using our GC/MS
- We can analyze the aromatic terpenes with our GC/MS
- Other groups at Rutgers may be able to provide analytical support for heavy metals, pesticide residues and mycotoxins.
- Other NJAES team members poised and ready to conduct supportive field studies to assist growers in evaluation of hemp production systems.

RUTGERS Chemically characterizing the aroma, flavor New Jersey Agricultural and fatty acids in Hemp: By GC/MS

- GC/MS, SPME or Headspace Volatile Capture and Analysis used to determine aroma composition and fatty acid composition in hemp and can analyze THC and CBD.
- SCFA and aromatic compounds can rapidly be analyzed using our Shimadzu GCMS-TQ8040 Triple Quadrupole MS interfeaced with a Shimadzu AOC6000 autosampler allowing us to us to detect very low concentrations.







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New Jersey Agricultural Experiment Station

Our lab: Searches for new bioactives,

then profiles and screens



Our analytical Instruments can quantitate THC & cannabinoids



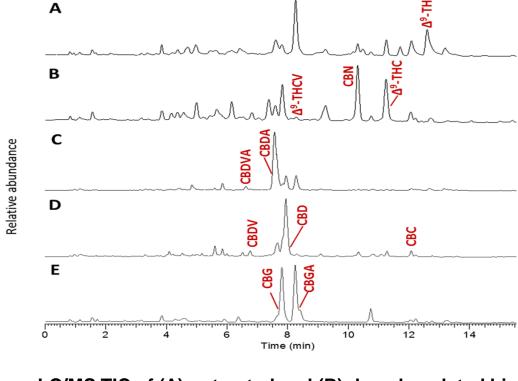
- **-**The D8 location is thermodynamically more stable than the D9 location
- -D8-THC and D9-THC show a similar profile of activity on cannabinoid receptors, with D8-THC being only slightly less active than D9-THC
- -Compounds from the D8 series can be converted into their D9 isomers by addition of hydrochloric acid and base-mediated dehydrohalogenation
- -This reaction is of great relevance, since D8-THC is much easier to synthesize than D9-THC

$$\begin{array}{c|c} & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & &$$

Conversion of D8-THC into D9-THC



Illustrative example of quantitation of hemp bioactives



LC/MS TIC of (A) untreated and (B) decarboxylated high-Δ9-THCA; (C) untreated and (D) decarboxylated high-CBDA; and

(E) partly decarboxylated high-CBGA Cannabis strains

ura nple

urate quantitative analysis of natural products in plant or in biological nples: Agilent 1290 Infinity II UHPLC interfaced with an Agilent 6470 Triple and rupole MS with ESI.



Microbial contamination

- Microbiological contaminants pose a potential threat and processors need to check for microbes and mycotoxins
- Bacteria and fungi may cause opportunistic infections in immunocompromized individuals
- Total aerobic microbial count (TAMC): <100 CFU/g
- Total yeast and mold count (TYMC): <10 CFU/g
- Total coliforms
- Escherichia coli (toxin producing <1CFU/g)
- Salmonella spp. (<1CFU/g)



Heavy metals

From Health Canada:

- Arsenic (0.14 μm/kg body weight per day),
- Cadmium (<0.09 μm/kg),
- Lead (<0.29 μm/kg),
- Mercury (<0.29 μm/kg).



Product Specification sheet: *Cannabis* ssp. sativa, variety Bedrocan (hemp flowers)

Market: to be sold on the pharmaceutical market. Strength: THC: approx. 22%

cannabidiol: <1.0%

Take Home Message:

TEST!

And

Ensure you keep all your

documentation!

	Method	Specification		
P. aeruginosa, S. aureus and Bile tolerant gram neg bacteria	5.1.41.	Absent		
Absence of heavy metals				
lead	Ph. Eur (current ed.)	max. 20.0	ppm	
mercury	"Heavy metals in herbal	max. 0.5	ppm	
cadmium	drugs and fatty oils"	max. 0.5	ppm	
arsenic (indicative)	(monograph)	-		
nickel (indicative)		-		
zinc (indicative)		-		
Absence of aflatoxines	Ph. Eur (current ed.) "Determination of aflatoxins B ₁ , B ₂ , G ₁ and G ₂ in herbal drugs" (2.8.18)	<4	μg/kg	
Loss on drying	Ph. Eur (current ed.) "Loss on drying" meth. C (2.2.32)	≤10.0	%	
Assay (UPLC)				
fingerprint	Monograph	similar		
dronabinol (THC)	Monograph	approx. 22	%	
cannabidiol (CBD)	Monograph	<1.0	%	
Related substances (UPLC)				
cannabinol (CBN)	Monograph	<1.0	%	



Acknowledgements

- New Jersey Agriculture Experiment Station
- Rutgers Natural Plant Products Program (NUANPP)
- Center for Food Systems Sustainability, Institute for Food, Nutrition & Health (IFNH)
- Rutgers Center for Sensory Sciences and Innovation (CSSI)
- Shimadzu Analytical Instruments
- Agilent Analytical Instruments

For information on analytics, postharvest & quality control:

Profs. Jim Simon, Qingli Wu, T. Gianfagna jimsimon@rutgers.edu