Co-Products and Applications of Industrial Hemp for New Mexico Agriculture

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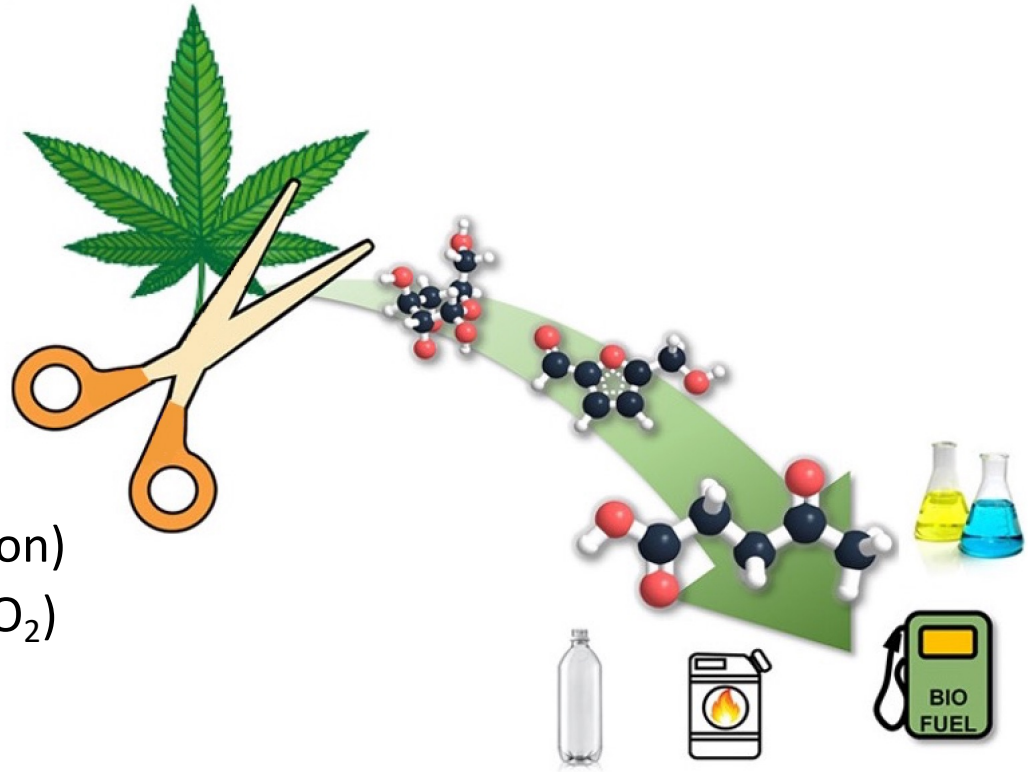
Overview

- **Hemp Background**
- **2022 Field Trial**
- **Flower Characterization**
  - Essential Oils (Steam Distillation)
  - Cannabinoids (Supercritical CO_2)
- **Lignocellulosic Waste**
  - Knowledge Gap: Fibers from High-CBD Crop
  - Compositional Analysis
- **Conclusions & Future Work**
  - Hydrolysis & Fermentation



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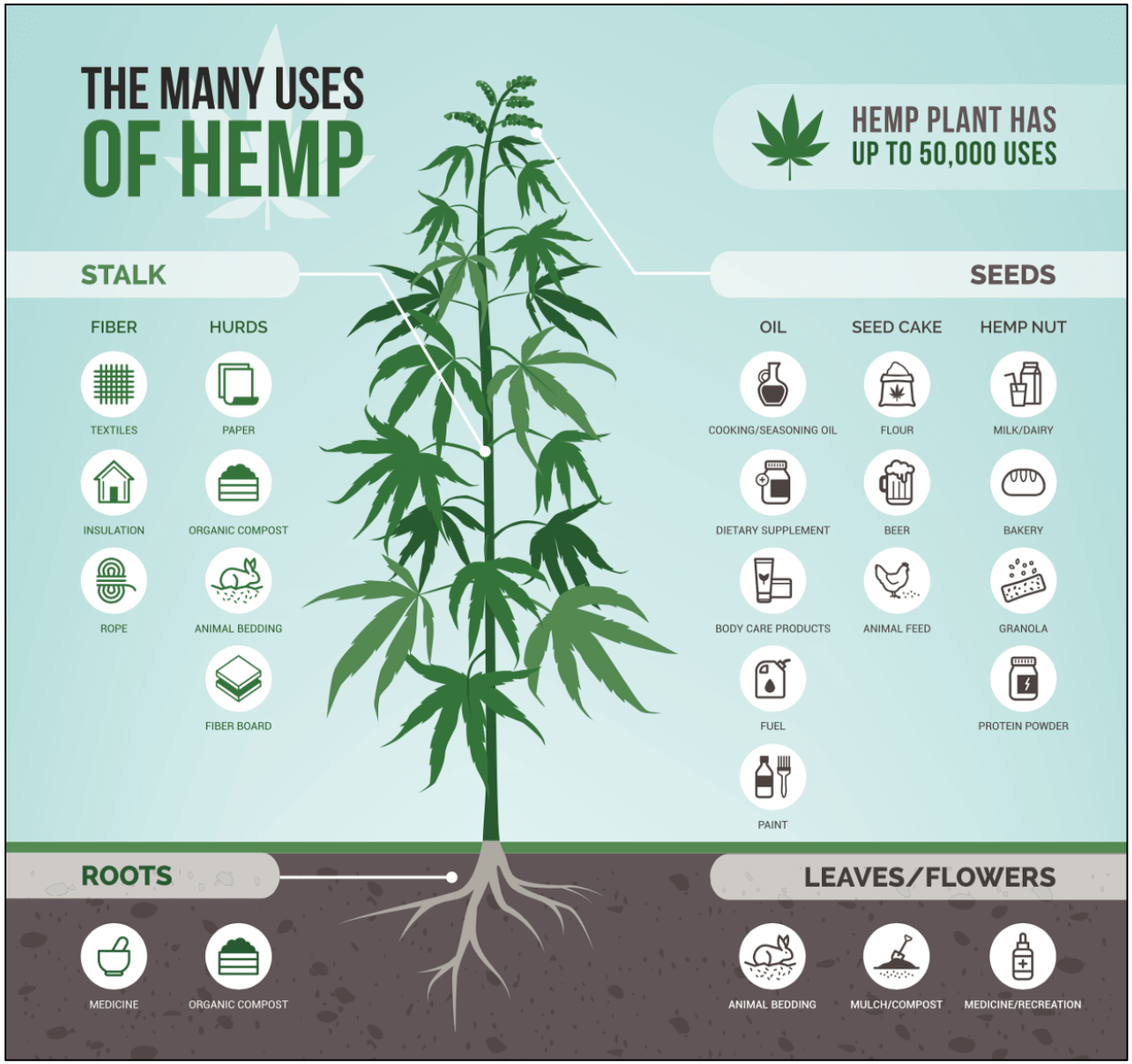
What is Hemp?

- Hemp is defined as *Cannabis sativa* L. with ≤ 0.3% total tetrahydrocannabinol (THC)

- U.S. re-legalized crop in 2018, following nearly 70 years of prohibition



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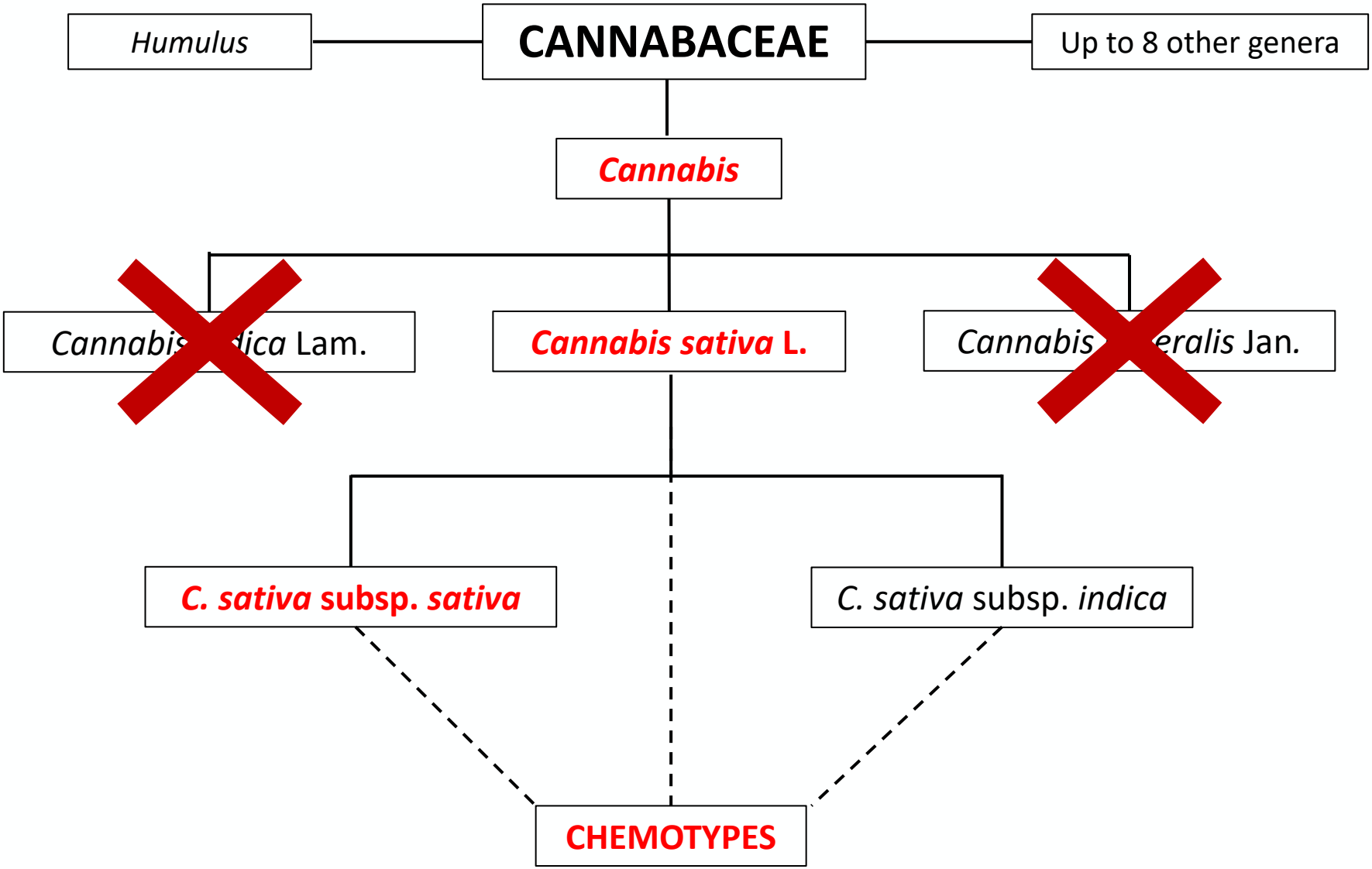
Humulus
CANNABACEAE
Up to 8 other genera
Cannabis
Cannabis sativa L.
C. sativa subsp. sativa
C. sativa subsp. indica
CHEMOTYPES

Cannabis Chemotypes

Total THC = THC + (0.877)*THCA
Total CBD = CBD + (0.877)*CBDA

- Chemotype (or chemovar) = chemical phenotype

Chemotype	Dominant Cannabinoid	Major Composition	THC:CBD
I. Medicinal / Drug (Marijuana)	THC	> 0.3% THC; < 0.5% CBD	5:1 – 30:1
II. Intermediate	Balanced	> 0.3% THC; > 0.5% CBD	1:1 – 1:4
III. Hemp	Cannabidiol (CBD)	< 0.3% THC; > 0.5% CBD	1:8 – 1:25
IV. Hemp	Cannabigerol (CBG)	> 0.3% CBG; < 0.5% CBD	-
V. Hemp	None	< 0.1% all cannabinoids	-

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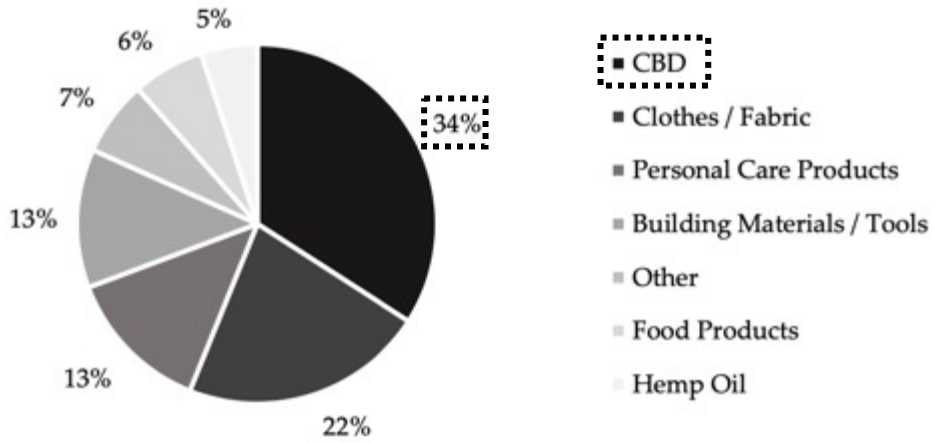
Types of Hemp by Product Category

- Types of hemp:
  1) CBD/essential oil;
  2) grain;
  3) fiber

Different hemp types: (left) CBD-type; (right) industrial-types

- Different morphology, physiology, and chemical profile between types

- Current US market is dominated by cannabidiol (CBD) products



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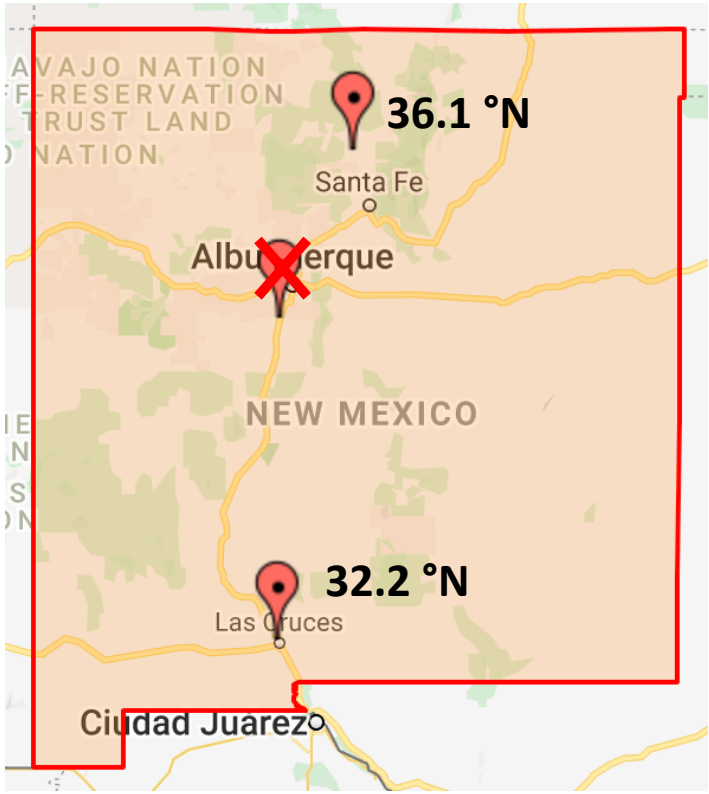
PLANT PRODUCTION

2022 Field Trials

Plot Management Details		
Location	***Treatment Plot***	***Common Plot***
Leyendecker Plant Science Research Center	Water stress: watered with 50% lower frequency than standard plot	Bi-weekly fertilizer application (12-4-8)
Sustainable Agricultural Science Center at Alcalde	Organic fertilizer: treated with OMRI certified organic fertilizer (11-3-8)	Water application minimum once per week



Varieties & Planting Density	
Variety	***Density***
The Wife Sweetened	Transplants, 3ft spacing

Planting & Harvest Dates		
Location	***Planting***	***Harvest***
Plant Science Research Center at Leyendecker	April 18	Sept. 13
Sustainable Agricultural Science Center at Alcalde	May 13	Sept. 28

Challenge: Fiber/Grain Production at Low-Latitudes

- ***PREMATURE FLOWERING***
  - Reproductive structures appear as early as 2 weeks after seeding for some varieties

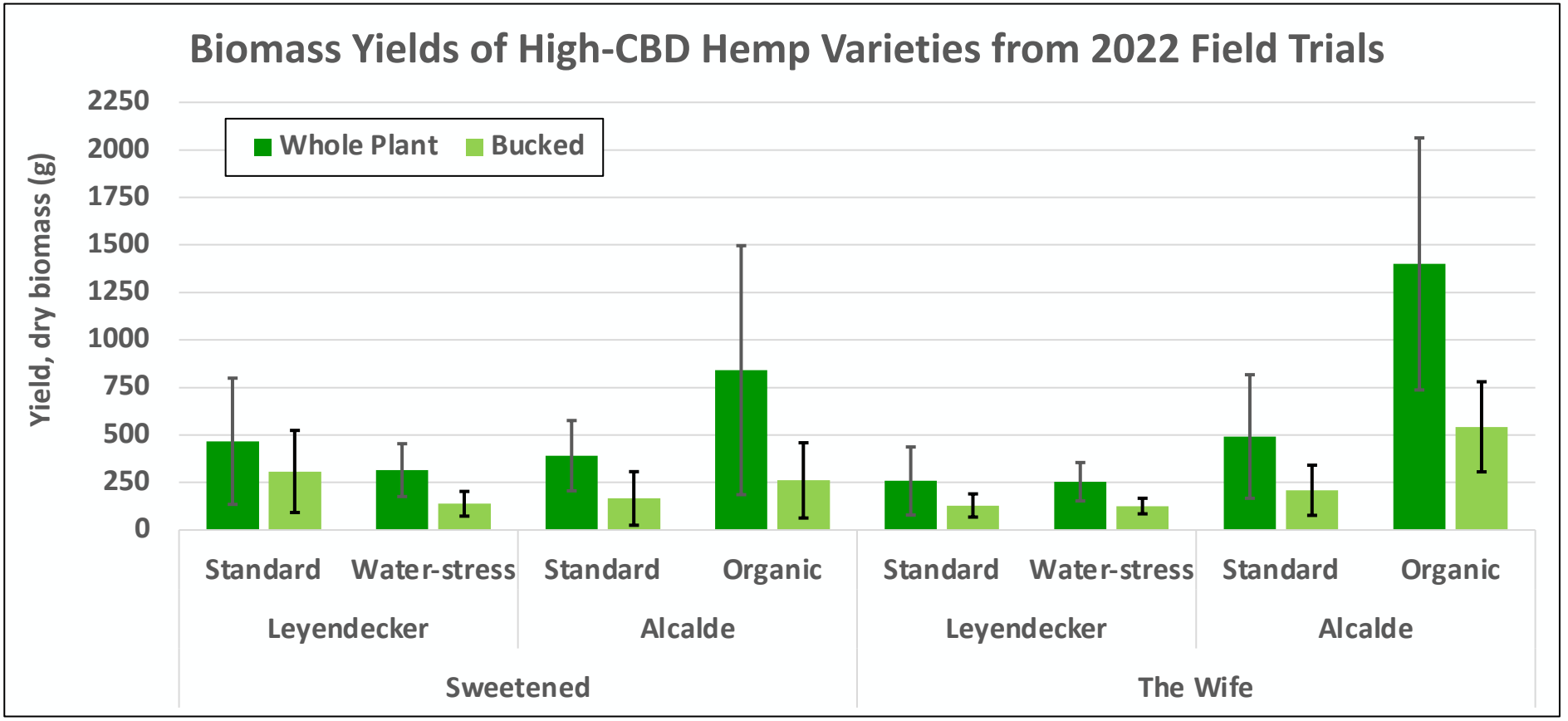
- Hemp is photoperiod sensitive

- Most industrial genetics are sourced from northern latitudes

Plants exhibiting premature formation of female (top) and male (bottom) reproductive floral structures

Total vs. Bucked Biomass

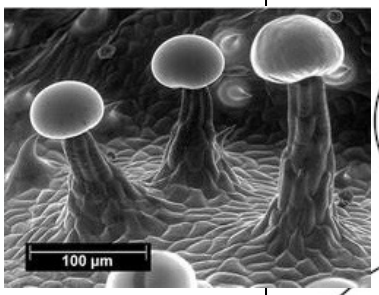
- 34-69% of total crop weight was fiber across all samples

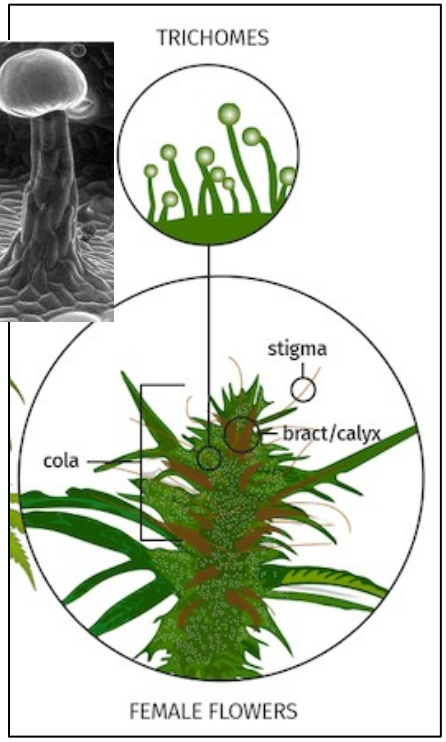


Bucking: to strip herbaceous biomass from stalks/stems
(bucked yield = leaf + flower)

FLOWER CHARACTERIZATION

Hemp Flower





- Female flowers are the primary source of cannabinoids
  - Accumulated in glandular trichomes

- Male flowers contain pollen
  - Needed for seed production (grain)
  - Can adversely affect cannabinoid content

Leafly. 2020. *Marijuana plant anatomy and life cycles.* https://www.leafly.com/learn/growing/marijuana-plant-anatomy

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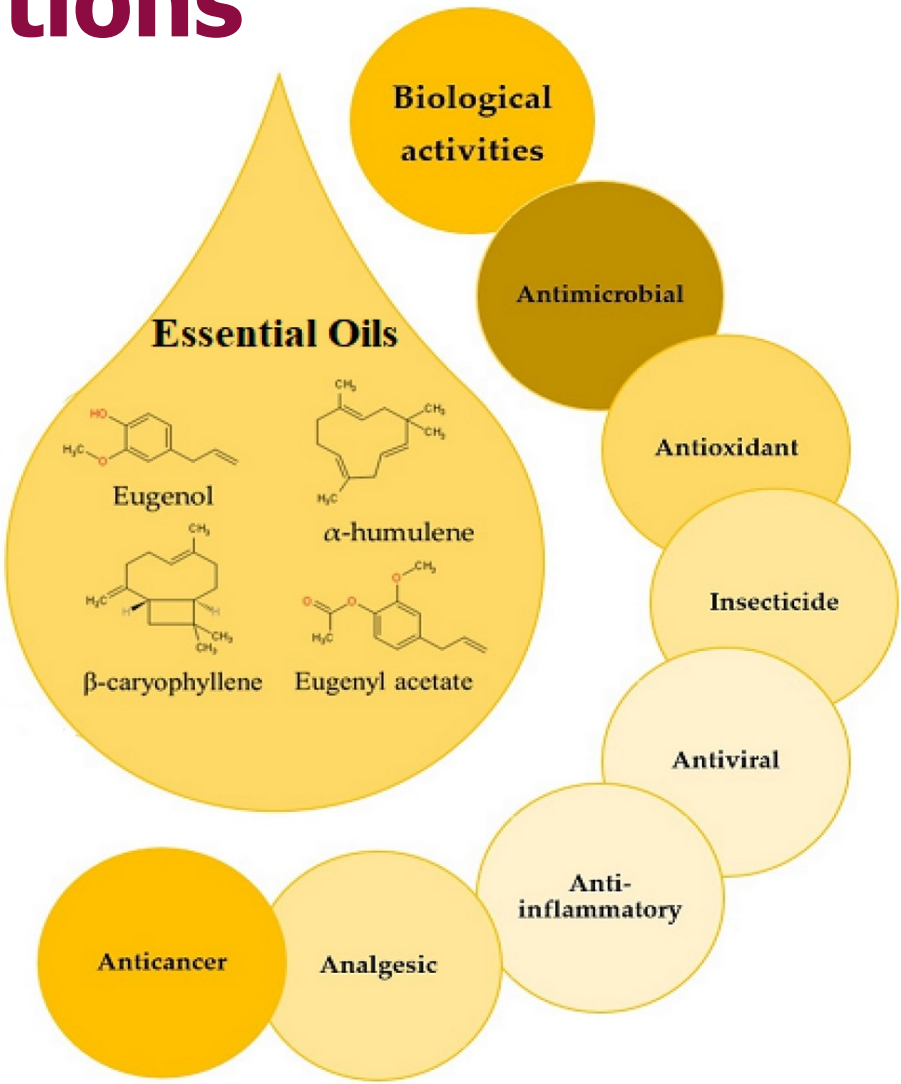
Essential Oil Applications

- Complex mixtures of low-molecular weight, volatile compounds
- Utilized as aromatic/flavoring ingredient; for nutraceutical and personal care products
- Effects related to profiles; synergy between compounds
- Over 100 unique compounds reported in hemp



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Average Yield of Essential Oils (mL/100g)

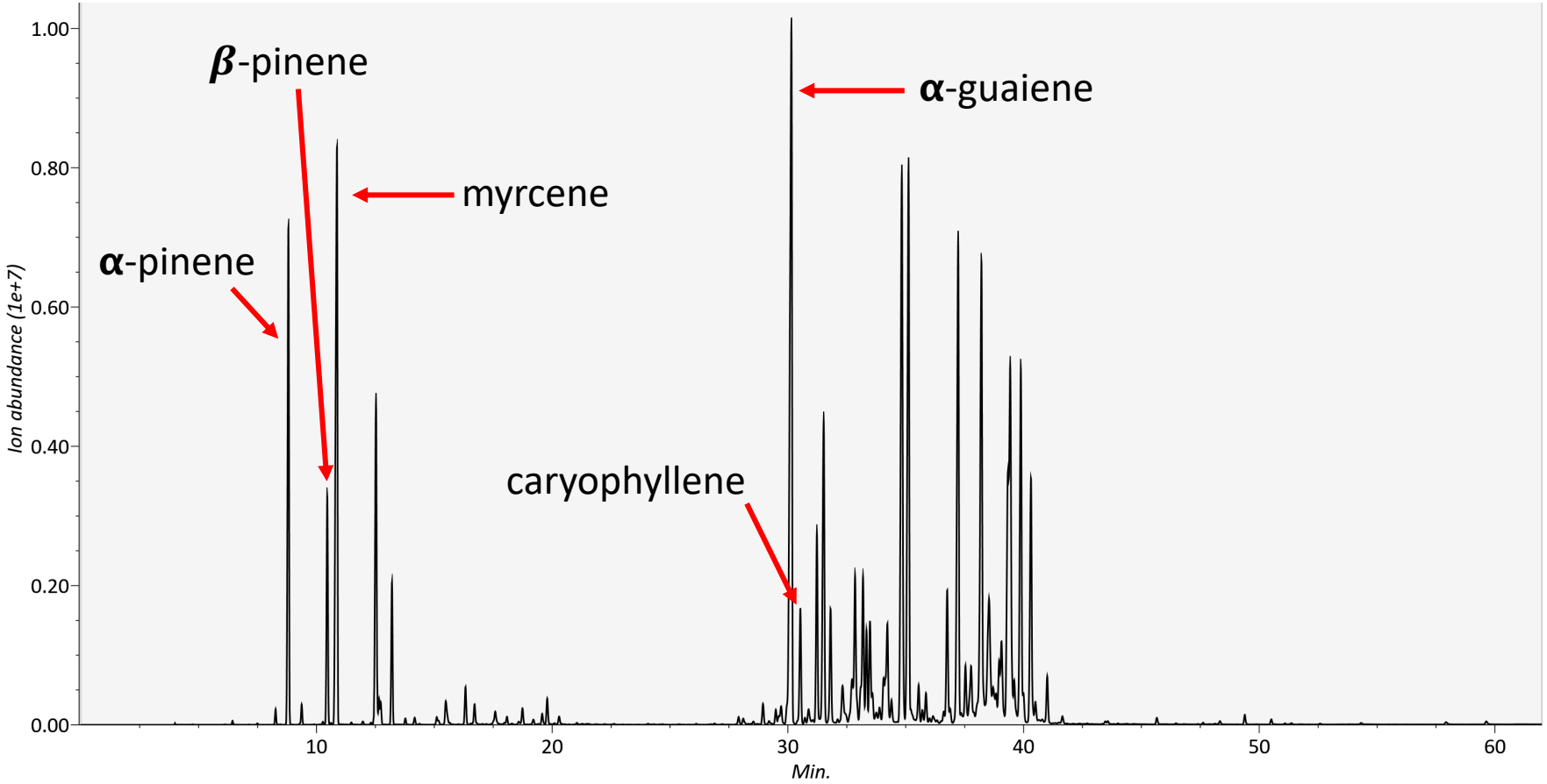
- Steam Distillation
  100 g in 3 L for 4 h

Location	Variety	Treatment	Yield
Leyendecker	Sweetened	Standard	1.01
		Water-stress	0.72
	Wife	Standard	1.26
		Water-stress	1.12
Alcalde	Sweetened	Standard	1.25
		Organic	1.21
	Wife	Standard	1.75
		Organic	1.40

- Both factors were significant for standard plots (variety, $P = 0.0485$; location, $P = 0.0433$)
- At Leyendecker for water-stress, variety was significant ($P = 0.0333$)
- At Alcalde for organic plots, no significance (factors: variety, treatment)

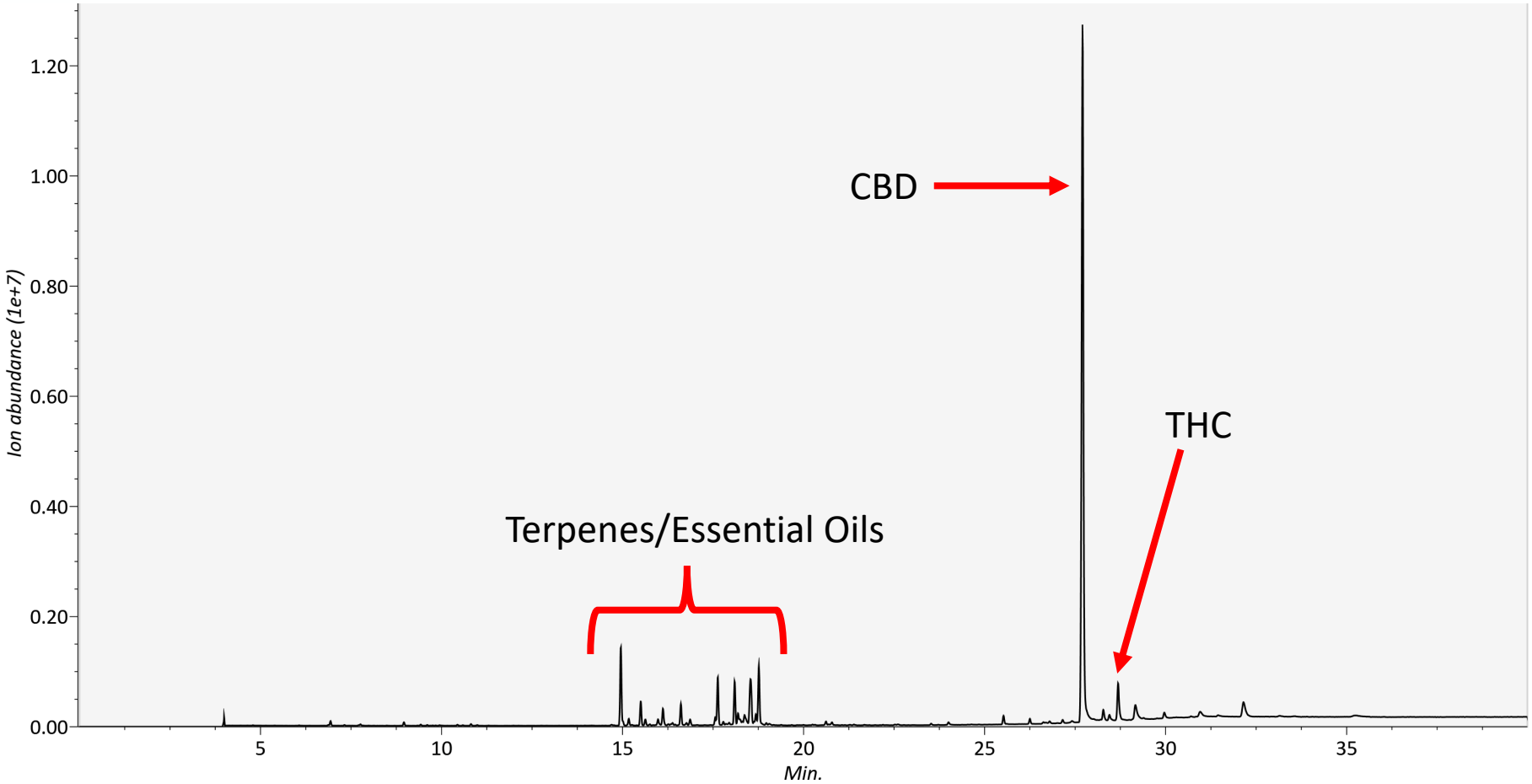
Essential Oils: Steam Distillation

- Gas Chromatography-Mass Spectrometry analysis
  DB-5 column
  Ramp: 60-246 °C, 3 °C/min



Cannabinoid Extract: Supercritical CO_2

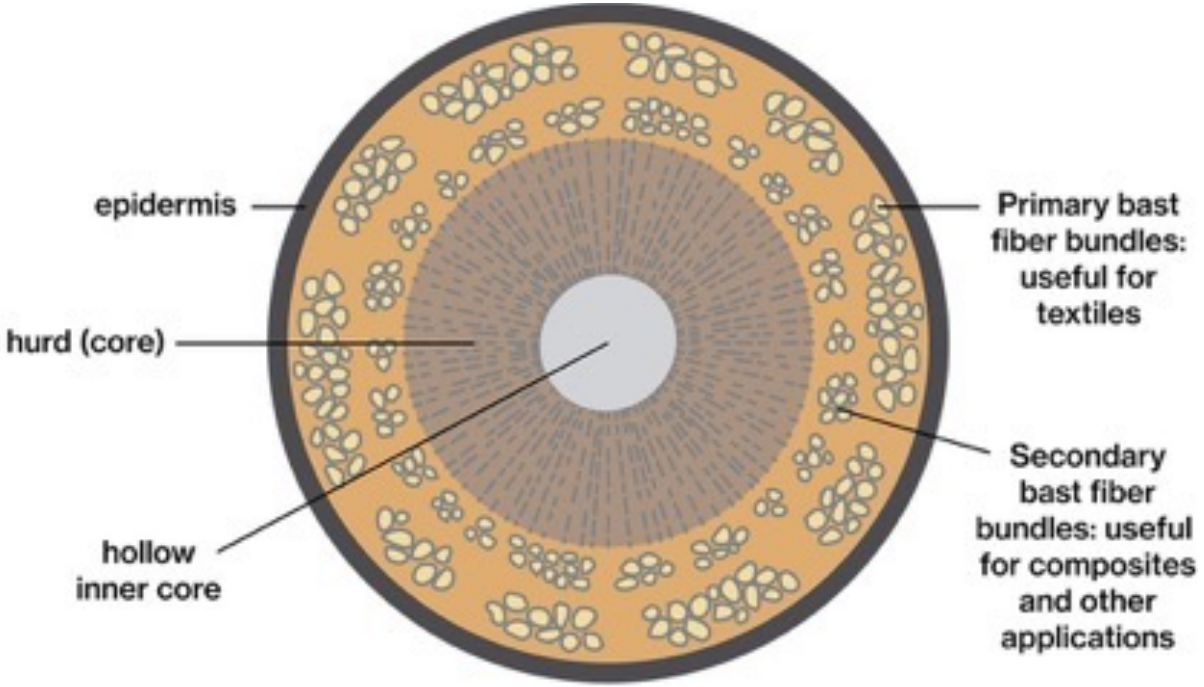
- Supercritical CO_2
  Flow rate: 50 g/min
  Co-solvent: 5% EtOH
  Temp.: 60 °C, Runtime: 30min



LIGNOCELLULOSIC WASTE

Hemp Fibers

- Traditional processing requires a decorticator to separate bast/hurd
- Minimal information is available on fibers of high-cannabinoid varieties



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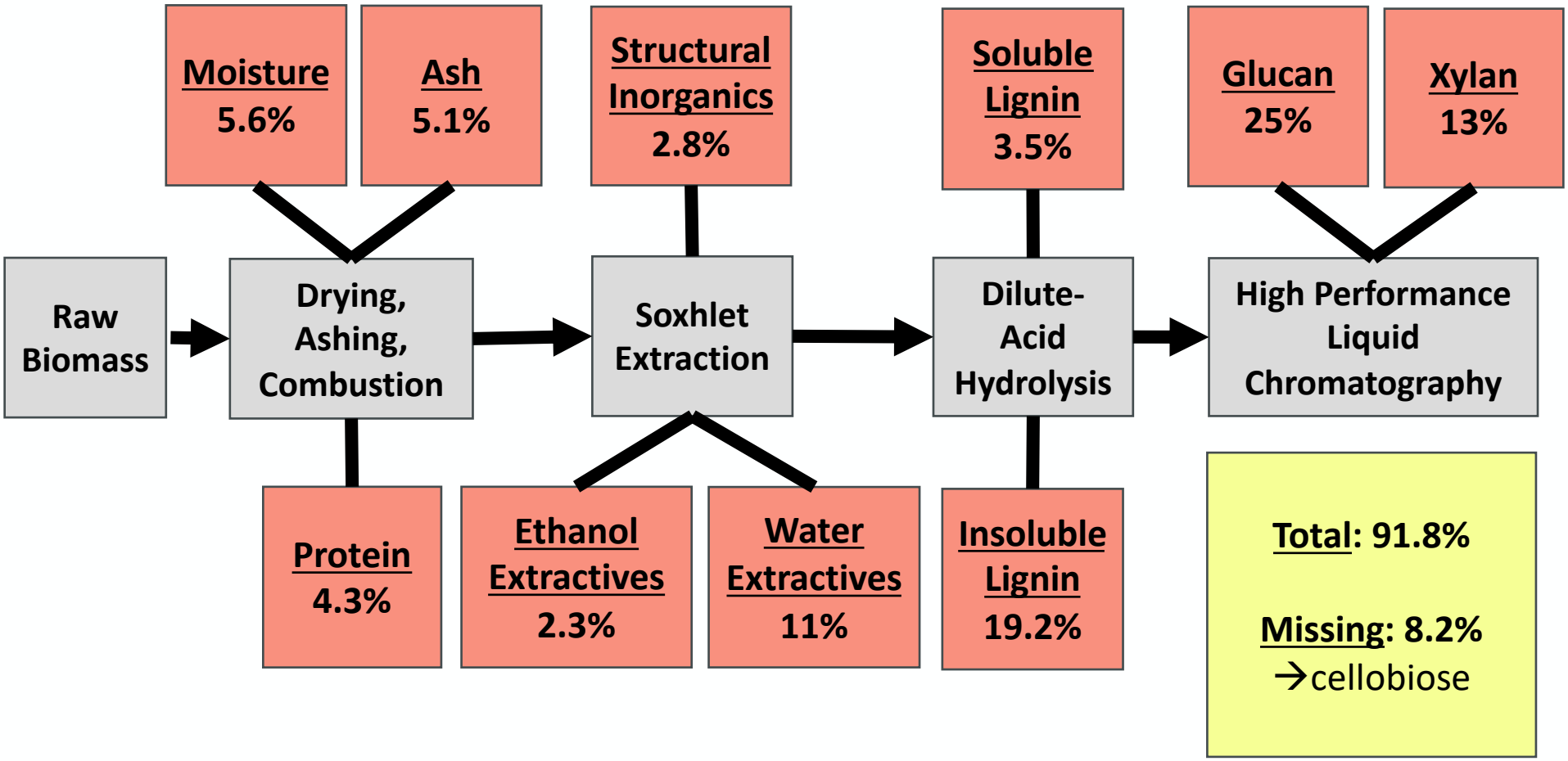
Comparison to Grain & Fiber Type Hemp

Reported Average Values for Structural Components in Untreated Hemp Fiber Samples				
Fiber Sample	*Glucan [%]*	*Xylan [%]*	*Lignin [%]*	*Reference*
Industrial hemp (Futura 75; < 1mm particle size)	36.5	17.0	21.9	Das et al., 2017
Industrial hemp (11 cultivars)	43.81-51.14	11.63-14.2	15.35-29.35	Das et al., 2020
Industrial hemp (Felina 32; conventional cultivation)	39.8	14.4	15.0	Kuglarz et al., 2014
Industrial hemp (Felina 32; organic)	42	14.8	13.2	
Industrial hemp (Fedora 17)	46.4	20.1	15.0	Kuglarz et al., 2016
Industrial hemp (unspecified variety; hurds only; 40-60 mesh sizes)	42.37	19.2	17.5	Moxley et al., 2008
Industrial hemp (unspecified variety; powered; bast)	57.5	1.6	16.2	Singh et al., 2018
Industrial hemp (unspecified variety; powdered; shives)	42.9	19.9	23.9	
Industrial hemp (4 varieties)	33.56-44.52	10.62-15.48	17.92-21.48	Viswanathan et al., 2020
CBD hemp (ACDC x Cherry Wine)	**32.63**	**12.90**	**16.98**	
Industrial hemp (4 varieties)	40.12-42.71	12.53-16.56	14.56-17.79	Zhao et al., 2020a
Industrial hemp (Tygra)	40.66	13.25	15.74	Zhao et al., 2020b

How similar are fibers from high-CBD types to fibers from fiber/grain types?

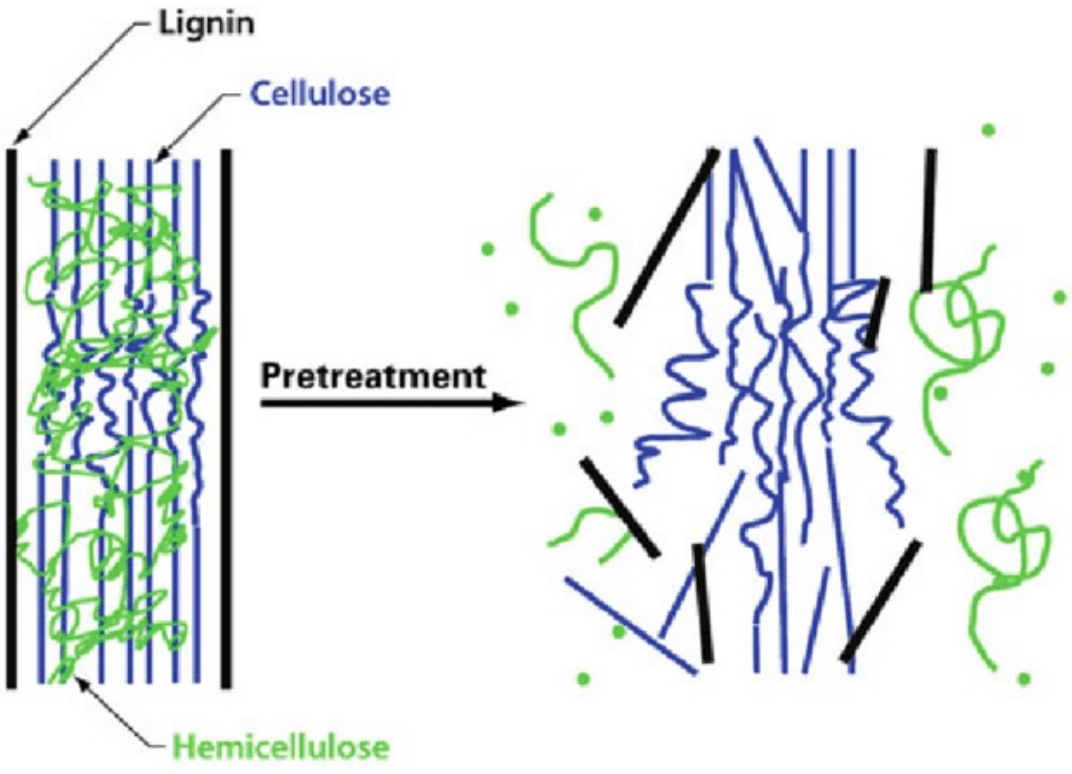
Fiber Mass Balance (w/w%)



Structural Components

- Dilute-acid assisted hydrolysis
  - 72% H_2SO_4, 30 °C water bath for 1 h
  - 4% H_2SO_4, autoclave, 121 °C for 1 h

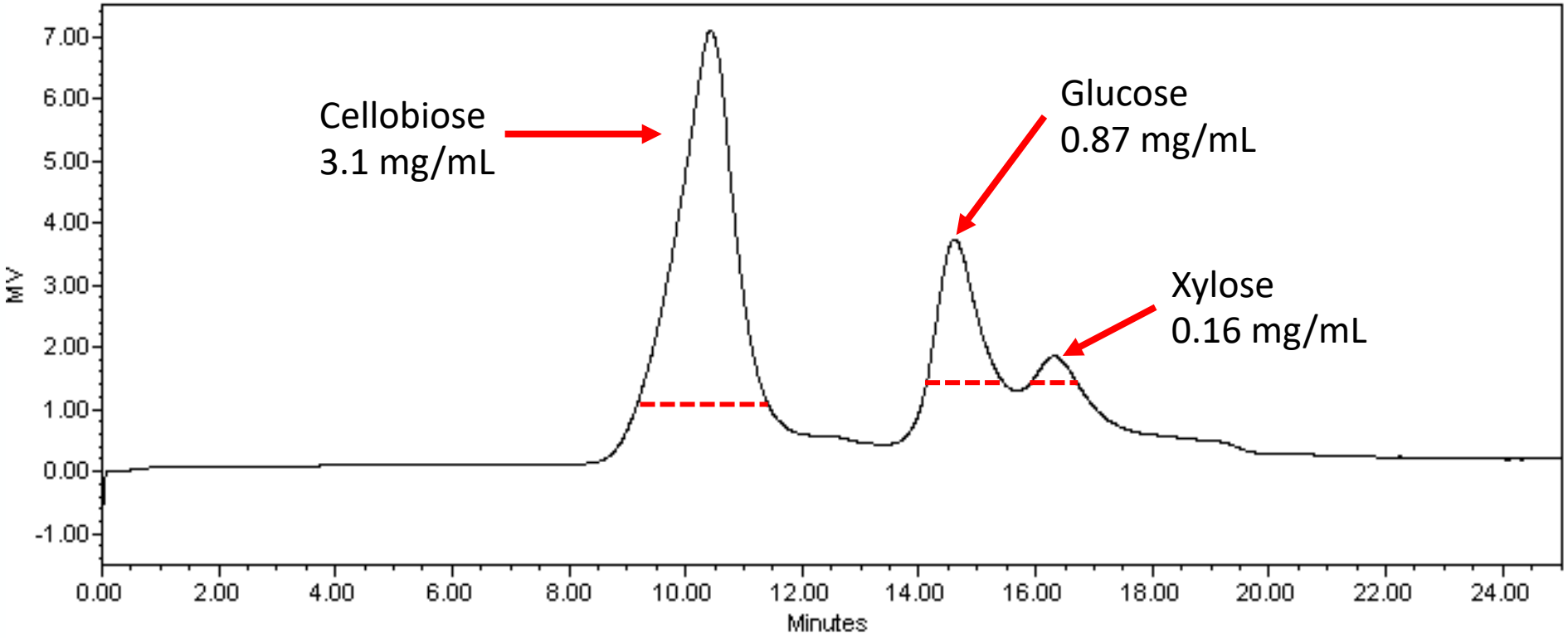
- Determine lignin from acid-soluble/insoluble fractions and total sugars (glucose + xylose) in hydrolysis liquor



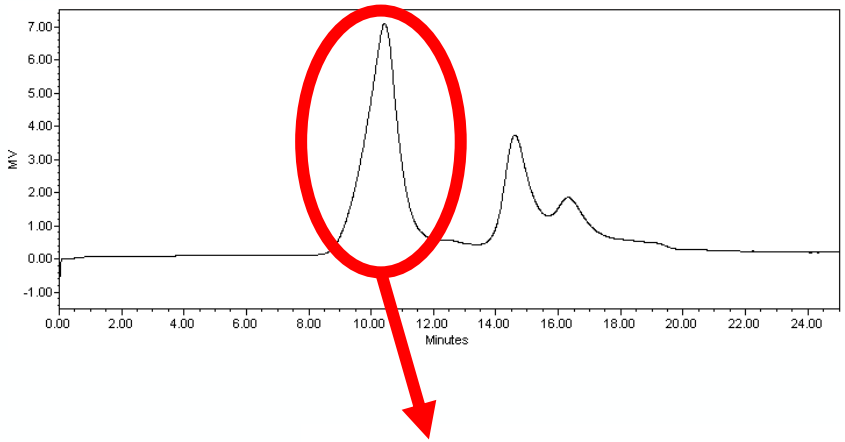
Ji, X., Huang, H., Nie, Z., Qu, L., Xu, Q., & Tsao, G.T. (2012). Fuels and Chemicals from Hemicellulose Sugars. *Advances in biochemical engineering/biotechnology. 128,* 199-224. https://doi.org/10.1007/10_2011_124

Structural Sugar Profile

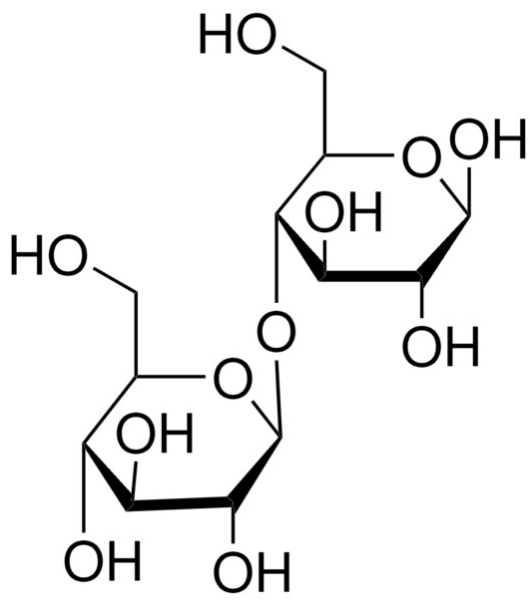
- High performance liquid chromatography (HPLC):
  - Shodex sugar column
  - Water mobile phase
  - Refractive index (RI)



Hydrolysis Troubleshooting



- Cellobiose → disaccharide formed by the condensation of two glucose molecules



- Indicative of incomplete hydrolysis
  - Hydrolysis time?
  - Sulfuric acid concentration?

Structural Sugar Content (mg/mL)

- ***ONGOING***

Location	Variety	Treatment	Cellobiose	Glucose	Xylose
Leyendecker	Sweetened	Standard	3.22	0.95	0.46
		Water-stress	-	-	-
	Wife	Standard	3.20	0.84	0.58
		Water-stress	3.75	0.89	0.44
Alcalde	Sweetened	Standard	3.16	0.76	0.47
		Organic	-	-	-
	Wife	Standard	2.98	0.93	0.24
		Organic	-	-	-

- Glucose concentration data from use of this method is less reliable when cellobiose content is > 3 mg/mL

CONCLUSIONS & CONSIDERATIONS

Can fibers from high-CBD hemp be used alongside fibers from industrial types as feedstock for bio-based chemicals?

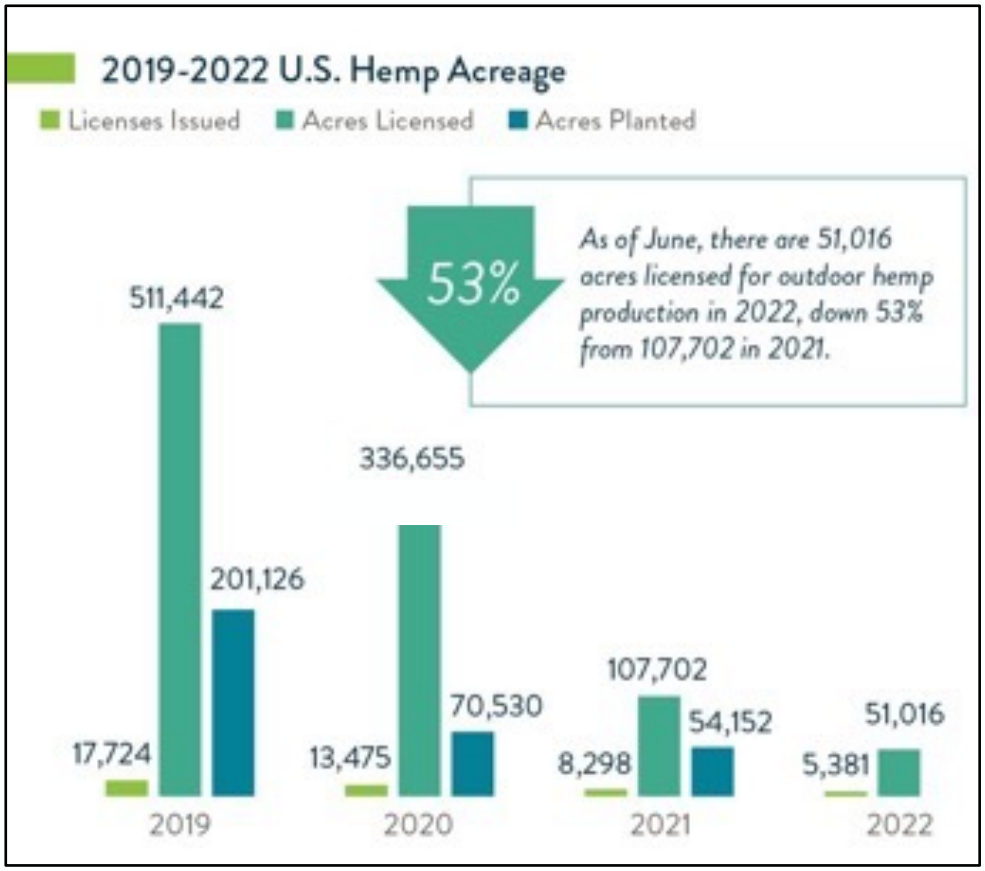
Conclusions

- Values in most compositional categories fall within ranges reported for other hemp types (ash, extractives, protein, total lignin)
- Estimated glucan content is half of expected amount based on literature
- Large presence of cellobiose likely explains decreased glucan content
- Feedstock may need more rigorous hydrolysis conditions

Why does this matter?

Hemp's Future in New Mexico

- Industrial hemp production slow to develop
  - Difficulty growing grain/fiber varieties at low latitudes
  - Limited access to processing
- Many hemp growers have switched to recreational production



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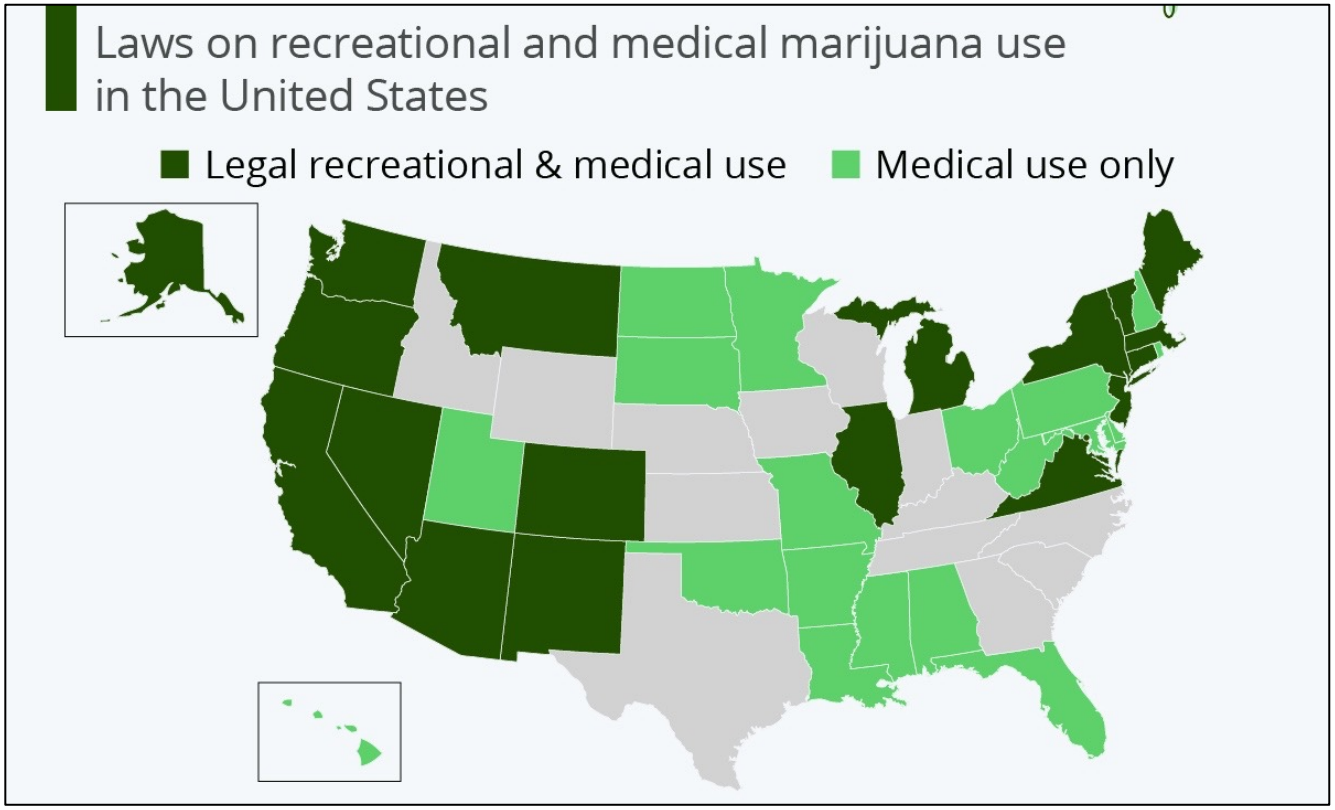
Expansion of Cannabis Legalization

- NM Cannabis Control Division has issued 851 active producer/ micro-producer licenses *(as of 8/27/23)*



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Buchlolz, K. (2022). The state of marijuana legalization in the U.S. *Statista*. https://www.statista.com/chart/6681/the-states-where-its-legal-to-smoke-marijuana/

Potential Economic Value-Added

- How much residual fiber material is available from high-cannabinoid production?

Given: 520,105 plants (state count as of 8/27/23), NM Cannabis Control Division)

Assuming: Average flower yield of 1.5 lb as 60% of total plant weight

Biomass Availability:
40% of 2.5 lb gives 1 lb fiber per plant
1 lb/plant * 520k plants = 520k lb fiber

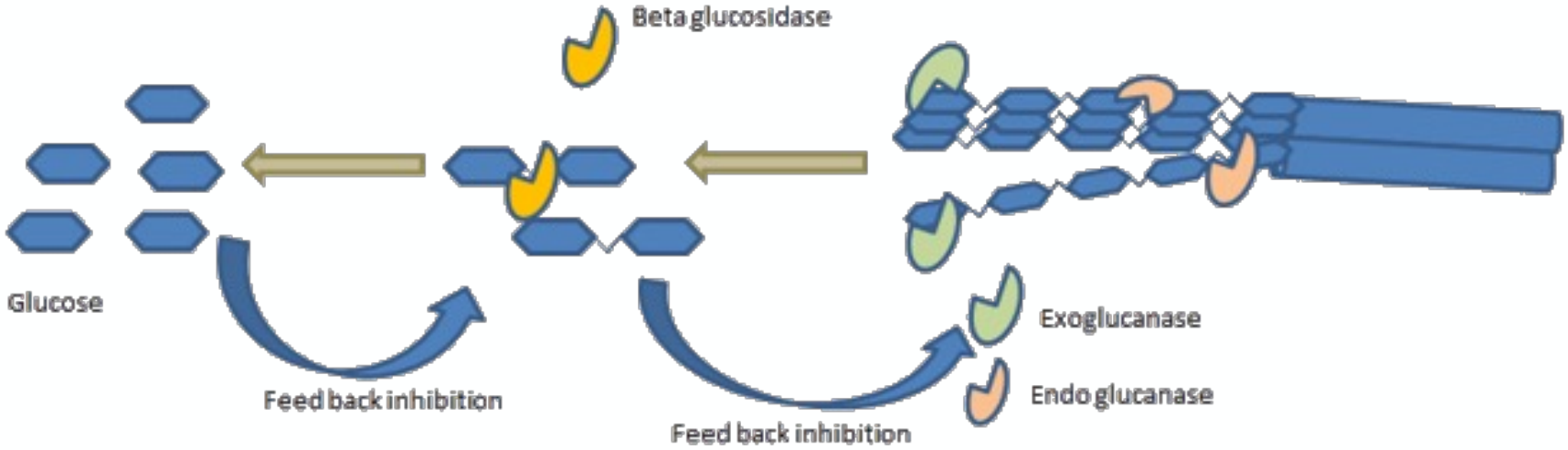
→ Estimate ~500k lb of fiber waste available annually
→ What is the value of biomass for bioconversion?

New Mexico Cannabis Control Division. (2023). https://qimw5q0w5j.execute-api.us-west-2.amazonaws.com/prod/plants.html

Next Steps: More Hydrolysis

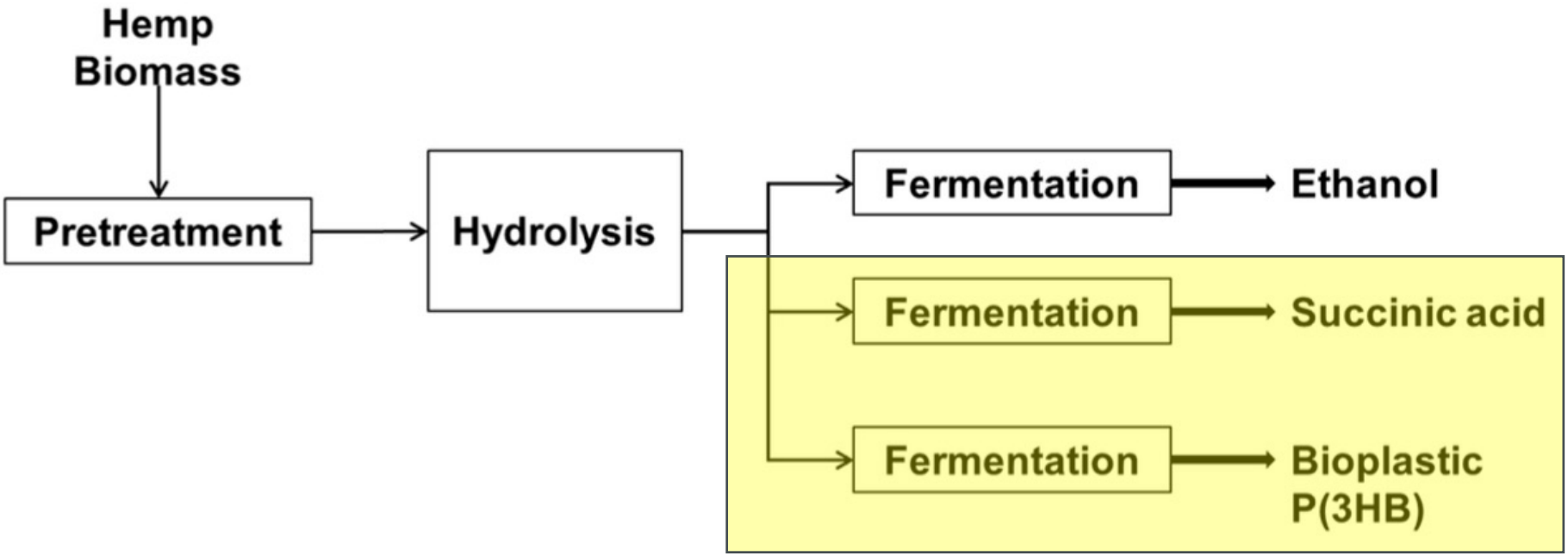
- Investigate effect of different pretreatment conditions on extent of hydrolysis

- Subject pretreated material to enzymatic hydrolysis; identify most effective conditions



Enzymatic Hydrolysis. (n.d.) Celignis Analytical. https://www.celignis.com/enzymes.php

Next Steps: Fermentation Pathways



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Thank you!

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