GemmaCert

Aanalyzing with GemmaCert

ISRAC
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ISO/IEC 17025

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Analyzing with GemmaCert

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1. Introduction

This paper outlines cannabis analysis using GemmaCert best practices and limitations. This paper does not brief on GemmaCert App use; for that please refer to GemmaCert Quick Reference. Readers are also advised to refer to Customer Portal for troubleshooting instructions.

Limitations are gradually reduced as GemmaCert continues enhancing its database and algorithms. Readers are advised to check Customer Portal for newer document versions.

2. Applicability

GemmaCert applicability is defined along 3 sample characteristics:

- Type
- Maturity
- Moisture

2.1 Sample type

GemmaCert presently analyses 3 sample types:

- Intact flower
- Ground matter (i.e., trim or biomass)
- Raw Extract produced using Ethanol extraction

GemmaCert users have experimented analyzing additional extract types (other than Ethanol-based). Some have reported satisfactory results, e.g., for hashish. GemmaCert appreciates customer creativity and is glad to engage with inquisitive users to explore applicability. However, performance for concentrates produced by other methods is not warranted at this time. GemmaCert continues to validate new extract type compatibility and will update users accordingly.

2.2 Sample maturity

Sample maturity here refers to inflorescence age. Therefore, this characteristic applies to Intact flower and Ground matter only.

GemmaCert presently analyses THC and CBD only. Both cannabinoids are products of CBG conversion, biosynthesized in the first weeks of flowering. GemmaCert does not yet analyse CBG. Therefore, analysis of younger plants is likely to produce erroneous results.

Growers cultivating products under legal THC content regulations may seek appraisal of potential THC content at maturity. To this end GemmaCert recommends starting analyses at the beginning of the flowering stage, usually about 10-14 days after flower induction. Note that at this stage, in which trichomes are developed and cannabinoid synthesis begins, there is a high conversion of CBG into THC and CBD and all concentrations are very low. Therefore, THC reading at this stage may also represent CBG results, causing inaccurate THC appraisal. CBD reading at this stage is accurate.

THC to CBD content ratio depends on plant genetics and varies minimally during plant maturation. Therefore, growers may rely on CBD reading to appraise expected final THC content, provided they know cultivars' THC to CBD ratio.

THC reading inaccuracy occurs only in the early stage of flowering and is resolved a few weeks after flowering and before harvest.

2.3 Sample moisture

High water content obstructs analysis accuracy. Excessive water content of undried material will pollute the device and might result in irreversible damage.

For accurate cannabinoids content results GemmaCert recommends analysing safe-to-store samples, i.e. samples with water content below 12%.

GemmaCert Water Activity analysis alerts on samples which are unsafe to store, susceptible to mold contamination.

GemmaCert is currently validating the Moisture Content analysis feature. Moisture content will allow calculating cannabinoid content in dry weight, along with the presently provided cannabinoid content in total weight.

3. Batch & Representation

3.1 Tools

Customer Portal will be your tool for crop composition analyses. Customer Portal allows analysis of cannabinoid content evolution over time, cannabinoid content variation within crop, drying process and more. To these ends partitioning crop into batches is advised, using any criteria you find useful. These can be location in the lot, maturity, top/ center/ bottom of plant and so forth. Analysed sample association with a batch is first specified during analyses, by typing the batch name when prompted on the app.

However, you may change a sample's batch details later through Customer Portal. Reasons for doing so are reviewed further below. GemmaCert recommends familiarizing with Customer Portal functionality prior to proceeding.

3.2 Dividing crop into batches

Discussion below does not address regulatory obligations. Regulators in various geographies have specified sampling procedures including mandatory samples per lot or weight. GemmaCert users subject to regulatory obligations should refer to these sampling procedures.

GemmaCert may be used also to appraise a particular flower. GemmaCert is more effectively used to characterize a cannabis batch along the supply chain. Batch characterization comprises estimation of batch attributes' average and variance. These attributes presently include Total THC, Total CBD, and Water Activity. Thus, it is worth discussing what constitutes a batch and how many samples need to be analysed to characterize a batch.

Plant genetics of the cultivated variety alone do not determine plant potency. Even when using seeds from the same batch or clones from the same plant, cannabinoid potency may differ. Soil type, light exposure, watering, nutrients, temperature, and other factors could affect your crop potency. Seasonality may cause cannabinoid content differences between cultivars of the same variety.

Dividing your crop into batches properly will help keep track of your crop, and optimize your fertilization, irrigation, illumination, and harvest time.

With GemmaCert one may analyse many more samples than relying on analytical/chemical lab. This allows segmenting a harvest into multiple batches, thus achieving more homogeneous batches and

consequently more representative average appraisal. Yet the logistical effort of maintaining multiple batches and recording their respective results could be burdensome.

At the other extreme, turning entire harvest into a single batch would render batch average values meaningless, unable to predict what a regulator or a business counterpart will find when randomly selecting specimen for analysis.

Neither A nor B in figure above are absolute. B is subject to operators' cost structure, while A is product of crop variability and average accuracy targets. Operators' objective to segment batches which satisfy average accuracy requirements with sustainable effort, i.e. number of batches between A & B in the chart above.

3.3 Considerations

This discourse may not evade statistics any longer, so here it is in brief. Number of samples to estimate average at the required confidence level, appraising population with certain variability is expressed in formula below.

$$n = \left(\frac{(z_{\alpha/2})(\sigma)}{E}\right)^2$$

Where:

- n denotes number of samples
- Z expresses required confidence level, e.g., 1.96 for 95% confidence
- σ denotes standard deviation of the sampled material, e.g. prior experience showing that 95% of analyzed samples are within 12% to 20% THC means σ=2
- E denotes margin of error

Reader need not apply this formula. There are printed and online sources providing number of samples tables, which one may refer to instead. The formula helps explaining the rationale of segmentation into batches. Consider this example:

- Grower desires to appraise crop average at 95% confidence with margin of error equal 1
- Analyses in past seasons have produced THC values of 8% to 24%, likely attributing to differences between bottom, middle and top of plant, i.e., σ=4 (95% of normally distributed samples are with ±2σ)
- Using the formula above, analyzing the entire crop as a single batch would mandate 64 samples.
- Alternatively, grower could attempt segmenting crop into bottom, middle and top of plant batches. That wouldn't be perfect, yet would yield far more homogeneous batches, possibly with σ=2

- With these 3 batches in mind number of samples for each would turn 16, hence 48 samples in total.

The example above shows that in some cases segmenting into more batches provides economies – 48 analyzed samples instead of 64, while achieving equivalent or superior accuracy.

Segmentation into more batches is also driven by another rationale – that of avoiding embarrassment and potentially adverse effects on one's business by regulator or a business counterpart finding specimen far-off the declared average.

Segmentation into batches is a learning process. One should start specifying as many batches as one can handle. Batch could be determined by cultivar variety, location in the lot, height on the plant, cultivation protocol or any combination of these. Some growers using GemmaCert are already identifying their samples by height on the plant. It is a good start yet may not be adequate.

Initial segmentation into batches need not be followed through harvest. Analyses made pre-harvest may show that some batches are similar, hence may be merged. GemmaCert Customer Portal allows batch split and merge by reassociating individual samples with batches.

Batch attributes learned in one season will serve segmentation in next season, particularly where location in the lot comes into play. Thus, one may expect number of analyzed samples decrease from season to season, as experience accumulates. Alternatively, one may exploit experience to boost accuracy, e.g., starting with 90% confidence and improving to 95% in next season.

3.4 Batch analysis in Customer Portal

GemmaCert Customer Portal provides several functions for batch statistical analysis and graphical display. These functions can be used to track batch growth progress, analyze plant maturity, and determine batch homogeneity and assist in planning towards next season.

Results, listed in Customer Portal, may be exported to excel for further analysis using tools of user's choice.

Results may be filtered by Batch and analysed via Batch Details form, thereby providing statistical data for both THC and CBD values. Figure 1 below depicts batch statistics display.

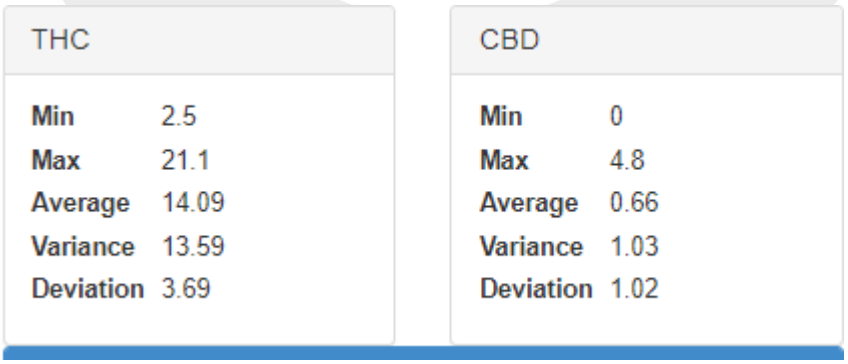

Figure 1 – Batch Statistics in Customer Portal

High variance and deviation in figure 1 above indicate a heterogenous batch, worth segmenting into a few additional batches. Conversely, low variance and deviation indicate homogeneous batches, worth merging.

Batches may be segmented or merged as desired by editing the "Batch" field. To this end it is advisable to type in additional information about the specific sample in the Supplier, Variety & Commentsfields of the app. Details entered in these fields will help segmenting a batch.

Batch details analysis also produces a graphical plot of CBD/THC ratio providing further insight to the batch heterogeneity.

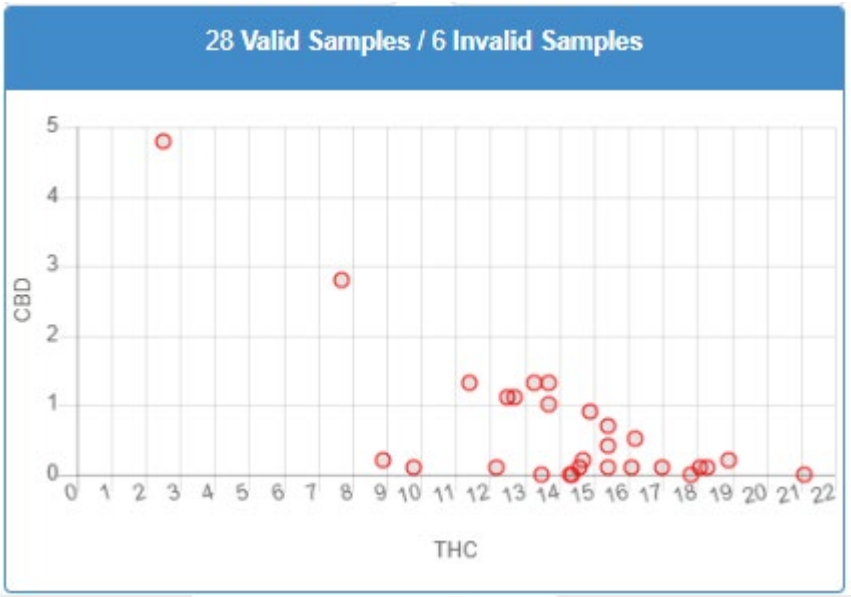

Figure 2 – Batch Composition in Customer Portal

Customer Portal also provides batch Progress Growth Chart that can assist in the analysis of plant maturation; see figure below.

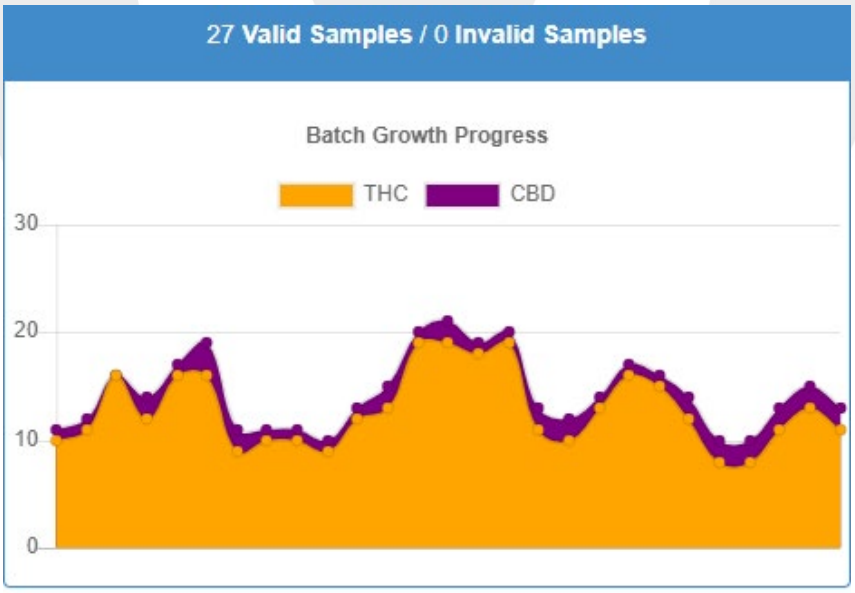

Figure 3 – Batch Evolution in Customer Portal

Figure above is an example of incorrect batch segmentation. Evidently, such increase and subsequent decrease of cannabinoid content can be explained only by analysing very different samples as one batch. Growth chart feature can help you better know your crop, it will optimize your harvest time to reach your plant's maximal potency. If you grow the same variety repeatedly, it can help you detect and isolate factors influencing your crop, while learning toimprove the conditions on your next harvest.

4. Sample analysis

4.1 Timing & Sampling

Start analyzing flowers in the first two weeks after flowering to improve your knowledge about cannabinoid accumulation, both accumulation rate and THC to CBD ratio.
In these early stages sample 3 plants out of 100, 3 flowers per plant - top, middle, and bottom of the plant. Sampling from different parts of the plant is crucial as there could be great differences in potency between upper and lower parts of the plant (1). Identify top, middle, and bottom flowers as distinct batches.
Sample repeatedly same plant/group of plants (same area/treatment/genetics) from the beginning of flowering to follow crop maturation and represent it accurately in growth charts generated at Customer Portal.

As analysis applies only to safe-to-store matter (12% moisture or lower), we refer you to our wet flower testing protocol (https://gemmacert.com/wp-content/uploads/2020/09/GemmaCert_Wet_Flower_Analysis_Protocol-1.pdf) or article 4.1.

a. After drying the flowers, mix them and grind together to get a general idea about your crop. In the calibration screen on your GemmaCert application (figure 4), enter the batch number.
b. Customer portal "growth progress chart" feature, shows you graphically your defined batch's cannabinoid accumulation (https://prod.gemmacert.com/CustomerPortal). Example is shown in figure 3.

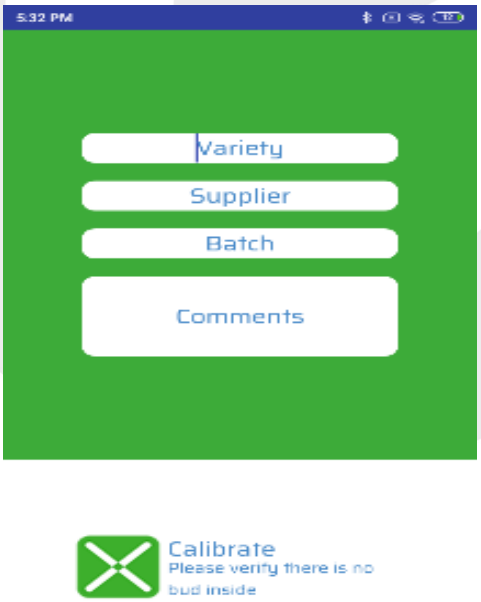

Figure 4. Calibration screen on the GemmaCert application

4.2 Sample Preparation

Drying

Cannabinoid content analysis applies to safe-to-store matter only, i.e., 12% moisture or below. Drying recommendations differ between pre-harvest and post-harvest sample preparation procedures.
Pre-harvest procedure mandates a rapid drying cycle in support of optimized harvest timing. Whereas proper curing is recommended for post-harvest sample preparation. Both procedures will produce samples dried to moisture under 12%, but the process of proper curing will keep your terpenes and cannabinoids intact longer and will prevent the aggregation of excessive undesired compounds, such as sugars. The goal of proper curing is to get the flowers into long term preservation mode, keeping vital terpenes and cannabinoids.

A whole plant dries within 24 hours to about 15% moisture when spread evenly to a depth of approximately 6 inches (15 cm) at 105˚F (40˚C). If hung to dry at 86˚F (30˚C), 15% moisture is achieved in 36 hours. Fresh flowers, stored in paper bags at 70˚F (21˚C) and 40% humidity, will reach about 11% moisture in 5 days.

Therefore, we recommend a semi-air-drying protocol for reliable and quick results:

1. Cut flower buds into a few chunks (do not shred!) and place or wrap in a paper bag.
2. Place the paper bag in a relatively warm place with a temperature of about 86˚F (about 30˚C).
3. If an incubator is unavailable, place the paper bag next to a heater on low temperature or even on top of low heat dissipating appliance such as a TV.
4. The buds should be dry in 24 hours and ready for potency analysis by GemmaCert.

Warnings:

1. Avoid exposing the flowers during the drying process to temperatures which exceed 150F° (65˚C), as it may cause decarboxylation of cannabinoid acids and lead to an inaccurate measurement.
2. Avoid humidity of the surrounding area exceeding 50%, as it may harm the drying process.

Pre-harvest drying protocol detail is available at https://gemmacert.com/wp-content/uploads/2020/09/GemmaCert_Wet_Flower_Analysis_Protocol-1.pdf.

Trimming

Leaves should be trimmed as much as possible from the stem prior to GemmaCert analysis. Trimming the flower prior to analyzing assures placing sensors closer to the analyzed flower, resulting in higher quality spectra and consequently more accurate results. To avoid sensor lens pollution and potential damage of the analyzed flower GemmaCert strives to avoid sensor being in contact with the flower. Therefore, any protruding leaves prevent moving the sensor closer.

Grinding

GemmaCert does not recommend use of electrical grinders, as these are likely to break trichomes, spreading their sticky contents on grinder walls. The likely outcome is reduced cannabinoid content readings.

Grind flowers manually with a simple grinder like Volcano grinder by Storz & Bickel.

Figure 5 – Manual Grinders

When grinding the flower make sure to remove branches and seeds, as these may cause erroneous results.

Do not grind into powder, three rotations with the grinder are adequate.

Note that aging commences following grinding, resulting in cannabinoids decomposition. Therefore, analysis shortly after grinding is recommended.

5. Analysis

5.1 Environment

Place the device on a stable horizontal surface with no environmental interference. Device sensitivity to vibrations mandates placing the device with no other equipment nearby. Device proximity to compressors, air-conditioners and other vibrating machinery must be avoided. Device placed on table or counter standing on a wooden floor may experience vibrations produced by steps on the floor.

5.2 Sample Identification

GemmaCert recommends practicing a consistent sample identification and description procedure. To this end smartphone app allows optional entry of Supplier, Variety, Batch and Comments (Figure 4). GemmaCert does not mandate any specific syntax or notation of these fields; users are free to choose any.

Users practicing repeated analyses of same sample, comparison between GemmaCert and some lab results or serving analyses to other parties will do best implementing unique sample identification, independent of FlowerID maintained by GemmaCert. Comments field could serve sample identification.

GemmaCert recommends placing every flower in a dedicated container and attaching a barcode bearing that flower identification to the container. Installing barcode reader software on the smartphone will allow error-free feeding sample identification into the app.

Batch identification is prerequisite to any statistical analysis using Customer Portal. Supplier and Variety fields may serve input for aftermath sample association with a Batch. Growers may use Supplier field for any other purpose, e.g., to identify lot.

GemmaCert analysis does not use any of these fields. They are there entirely to serve users in any customized manner they favour.

5.3 Analysis Repetitions

Averaging across repeated analyses of a sample will produce superior accuracy. Users pursuing superior accuracy need to conduct five analysis repetitions at least, rotating the analyzed flower between repetitions.

Spectrometer embedded in GemmaCert device illuminates samples to measure their light absorbance and subsequently derive composition attributes from measured absorbance. This illumination does not decarboxylate the samples and does not produce any other irreversible effects. Illumination does warm-up the samples slightly and this temperature increase affects spectra.

Therefore, conducting repeated analyses of the same sample, as recommended above, **one must let the sample cool for about 10 minutes between consecutive analyses.**

6. Results Interpretation

6.1 Results

GemmaCert presently produces Total THC, Total CBD, and Water Activity results. These totals represent expected THC & CBD contents upon consumption, i.e. in fully decarboxylated product. Results assume 0.877 conversion ratio from the acid from of THCA and CBDA into the active form of THCΔ9 and CBD, respectively. Accordingly, totals are calculated as percentages of total weight using formula below.

%Total THC =0.877× (%THCA) + (%Δ9THC)

%Total CBD=0.877× (%CBDA) + (%CBD)

Note that some cannabis products on the market misstate totals as plain summation, disregarding conversion ratio.

GemmaCert results rely on Reference data produced by HPLC analyses. To this end GemmaCert maintains an in-house ISO-certified analytical lab.

Note that GemmaCert results are percentages of total weight whereas HPLC results are percentages of dry weight, details in section below.

GemmaCert is presently validating Moisture model. Upon completion percentage of dry weight will become available as well.

6.2 Dry weight versus Total weight

Labs serving the cannabis industry mostly use HPLC, producing results as percentages of dry weight. GemmaCert produces results as percentages of total weight. Total weight has value in commercial transactions, where buyer is interested in THC and CBD content in the material procured. Dry weight would be of interest as well, providing base for comparison with lab results.

GemmaCert will soon deliver both. In the meantime, rule of thumb for comparing dry and total weight results could be useful.

Moisture content of properly dried cannabis flowers ranges from 4% to 12%. Below 4% cannabis flowers are too fragile, breaking into small pieces. Above 12% cannabis flowers eventually develop mold. For the sake of this discussion let us assume moisture content at the median value of 8%. Under this assumption GemmaCert results should be divided by 0.92 for comparison with lab results produced by HPLC.

Users in possession of moisture analyzers can apply more accurate scaling for comparison of GemmaCert results with dry weight results. Moisture analyzers destroy analyzed samples; devices implementing LOD (Loss On Drying) and implementing Karl Fischer method do not differ in this respect – both make analyzed samples not available for any further analysis. Consequently, users willing to apply the scaling will measure moisture of one sample and apply it to another sample. Caution must be practiced here – the two flowers may not contain identical moisture. Flowers kept for many days in same closed container, not exposed to sunlight or any heat source, may be assumed to contain practically identical moisture. Flowers dried using rapid procedure and analyzed shortly after drying may differ in moisture content substantially.

6.3 Water Activity

Significance

Water activity, common notation "Aw", represents the amount of "free" water available to microorganisms – fungi, mold, and bacteria. These microorganisms can thrive at water activity levels higher than 0.65 Aw (fungi, mold) and 0.7 Aw (bacteria). Water activity can be used as an indicator for microbiological stability and a low water activity value indicates a potentially long shelf life at ambient temperature. Fresh-cut flowers feature water activity of about 0.95 Aw, but the water activity needs to be below 0.65 Aw to prevent most fungal growth. This Aw value should be reached within 2 days after harvest to avoid the risk of postharvest fungal growth and subsequent mycotoxin formation.

Several states, such as Oregon recommend water activity test instead of specific mold identification tests. Oregon currently relies on the water activity measurement to monitor mold and other microbial contaminants on cured flower.

Intact flower vs. ground matter

GemmaCert recommends analyzing intact flowers for water activity. Grinding increases surface area, turning the ground matter more susceptible to moisture exchange with the environment. Ground matter will dry much faster, potentially obstructing analysis accuracy. Avoiding sample destruction is another advantage of intact flower analysis.

Business impact

Water activity results interpretation:

- Values between 0.55 and 0.65 are safe to store.
- Below 0.55 could result in loss of potency. Trichomes become fragile and break down, causing loss of cannabinoids and terpenes.

- Above 0.65 bear high risk of fungal and bacterial contaminations, with health risk for the consumer.

6.4 Comparing GemmaCert and HPLC/lab results

Labs usually analyse ground matter. Therefore, comparison with GemmaCert analyses conducted in Ground mode as well, following the procedure below is recommended:

I. Pick buds from top, middle and bottom of the plant, such that you will have about 2 grams of chaff after grinding it. It should only include bud and no leaf or stem.
II. Grind the buds and MIX the ground matter WELL. Use a simple plastic grinder, as instructed under 'Sample preparation' above.
III. VERY IMPORTANT – Mix well right before taking from the chaff from wherever the pile is and inserting it into the ground accessory. Trichomes, producing the smallest grain in ground matter, will trickle to the bottom of the pile through the gaps between larger grain produced by the green material. Mixing well will avoid under-representation of the trichomes in analyzed sample.
IV. Fill about 0.2 grams of ground matter into ground accessory; that should cover the glass entirely with a thin layer.
V. Analyze the sample. On analysis completion empty the ground accessory without returning the material to the original pile.
VI. Perform the above 3 times. Conduct repetitions within a short period to avoid ground matter changes due to environmental exposure. Results may differ between repetitions, but if conducted accurately, the results should be close. Contact support in case you have any doubt.
VII. Average results of the 3 repetitions serve results for comparison with lab analysis.
VIII. Add analyzed matter to the pile and dispatch everything to the lab for comparison reference analysis.

7. Avoiding excessive THC in Hemp

Hemp composition is sensitive to its cultivation conditions, resulting in unexpected composition changes which may turn legal product into illegal one. Procuring seeds from a certified supplier alone may not warrant hemp legality. Plant stress such as heat/cold, drought/flooding and lack of nutrients can cause an undesired increase in the synthesis of cannabinoids. Therefore, along with controlled cultivation conditions, GemmaCert recommends repeated analyses of the crop.

Analysis timing

Start analyzing at the first week of flowering to study the rate of cannabinoid accumulation as it changes in different varieties and seasonally. Usually, the accumulation rate increases, sometimes doubles, in the last few weeks before harvest. Hemp plants have relatively low THC to CBD ratio compared with marijuana (2).

A few studies suggest that THC and CBD are derived from a common precursor, CBG (3 and figure 6), and that THC to CBD ratio might be controlled by a single gene affecting cannabinoid biosynthesis (4). This is another reason to begin analyzing as early as the first week of flowering as this THC to CBD ratio is determined genetically, and it does not change through flower development.

CBD content, much higher than THC in hemp, can serve a marker of THC accumulation. When CBD content reaches 4.3-4.5%, THC levels will likely approach 0.2%. Thus, at this point, you should schedule the regulators test.

We note here that at this early stage of flowering the presence of CBG could be detected by GemmaCert as THC. Therefore, in early stages of flowering, our advice is to disregard THC result. An update solving this issue by enabling CBG analysis is currently being developed.

Number of samples

Regulators may have specified sampling procedures. Since GemmaCert offers you much faster and cheaper results, you may exceed regulators' demands to be on the safe side. Analyze a few plants from each lot, especially if you notice any visible change. Sample flowers from the top 3-5 inches of the plant, as the top of the plant has greater potential of testing hot.

GemmaCert is your daily decision support tool, so your hemp would not exceed 0.3% THC. GemmaCert is also your tool for cultivation improvement in next seasons. User-friendly customer portal gives you tools to save your results, divide them into batches according to lot/plant age/date or any other chosen criteria.

Biosynthesis of cannabinoids (3)

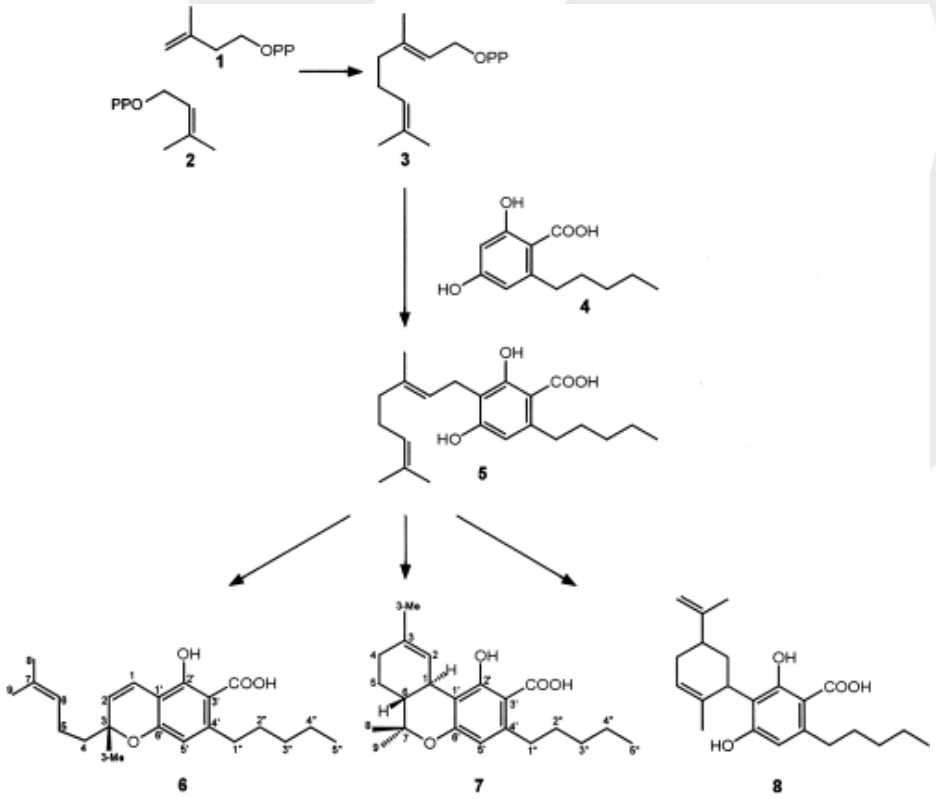

Figure 6. Biosynthesis of cannabichromenic acid (6), tetrahydrocannabinolic acid (7) and cannabidiolic acid (8)

References

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3 https://febs.onlinelibrary.wiley.com/doi/full/10.1046/j.1432-1327.2001.02030.x

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