Laboratory Analysis of THC Content in Industrial Hemp Seed

An MRAC supported project

Manitoba Rural Adaptation Council Inc.
508-800 Portage Avenue
Winnipeg, MB.
R3G 0N4

Final Report Prepared By:

Hemp Oil Canada Inc.
PO Box 188
Ste. Agathe, MB.
R0G 1Y0
Shaun Crew, President

Telephone: (204) 275-7616
Email: hempoilcan@escape.ca

March 10, 2000

Laboratory Analysis of THC Content in Industrial Hemp Seed

An MRAC supported project

Project Purpose

The theory was that hemp seeds do not contain THC, but rather that the seed coats are contaminated by the leaf matter surrounding the seed bud. However, this had never been scientifically proven in a laboratory setting. Our project purpose was to determine if hemp seeds do intrinsically contain THC by analyzing the whole seed, seed hulls, nut membrane and hemp nut. This analysis would be conducted on the seed components derived from actual hemp food processes (seed cleaning and dehulling) and by carefully dissecting whole hemp seeds in a laboratory environment. Triplicate subsamples would be analyzed in duplicate for the six hemp seed varieties being tested in this research project. The results of this analysis will ultimately provide the answer as to whether THC-Free status can be realistically be attained by commercial processes presently being researched & developed by Hemp Oil Canada Inc.

Determining the level of THC, if any, in the various compositional parts of a hemp seed would provide important information for,
(1) developing processes to eliminate and/or reduce the THC contaminating the seed,
(2) establish the realistic benchmark that would be attainable and
(3) assisting regulatory bodies in reviewing and establishing the acceptable levels of THC in hemp food products.

The theoretic elimination of THC is thought to be the step necessary to elevate hemp food products into the mainstream and gain the attention of the major food processing companies. The degree to which hemp food markets develop will be directly related to the hemp industry's ability to eliminate or reduce the present trace levels of THC, below that which could be detected in a drug screening test.

Determining the level of THC which can be ingested without failing a drug screening test was not part of this project. However, it should be noted that several research projects are underway in both Canada and the US to answer this question. The results of that outside research & analysis will assist hemp food processors in determining the maximum levels of THC that would be permissible in hemp food products, while at the same time keeping these levels under those allowable by existing government regulations.

Summary

The research work required to complete this project was provided by the applicant, *Hemp Oil Canada Inc.* along with the project partner, *Websar Laboratories Inc.,* of Ste. Anne, Manitoba who provided the analytical services, sample preparation, seed dissection and consultation during the project. Each company provided both direct and "in kind" financial support along with the direct financial support provided by MRAC.

The research work conducted by the project participants determined that:

(1) THC is intrinsically found in all parts of the hemp seed, albeit at far lower levels in the hemp seed nut than that found on the seed coats which receive the highest degree of contamination.
(2) The levels of THC will vary between the different OECD approved industrial hemp seed varieties.
(3) The detected levels of THC will vary between whole hemp seeds, hemp seed hulls, hemp seed nuts, hemp fines and hemp screenings in each individual variety.
(4) The levels of THC in the various components of a hemp seed can be reduced to below 1.0 μg/g (parts per million) when the whole seeds are properly prepared (cleaned) for processing. This applied to both the manually and mechanically separated fractions.
(5) In the majority of the samples analyzed, the THC levels were typically higher in the mechanically cleaned & processed components as compared to the manually dissected seed parts.

Attached to this report is the "final report" of the hemp seed analysis performed by Websar Laboratories Inc., identifying the description of samples analyzed, sample preparation, samples extraction, results & discussion and the conclusions drawn.

In conclusion, it would appear from the results that achieving a "THC Free" status is not achievable in terms of a true zero. However, in terms of a relative zero, properly prepared whole hemp seeds can be cleaned and mechanically processed in such a way that the levels of THC are reduced to below 1.0 μg/g and in a number of cases to below 0.5 μg/g. Health Canada has regulated and limited the content of THC in hemp seed derivatives to 10 μg/g (10 parts per million).

Attached is the analytical data compiled by Websar Laboratories in support of this project.

Introduction

There has been an ongoing controversy regarding the presence of Δ^9-THC in hemp seed oil and the hemp seed nuts the oil is pressed from. Several studies have shown that consumption of commercially available hemp seed oil results in urine specimens testing positive for Δ^9-THC in the USA (Callaway et al. 1997, Constantino et al. 1997, Struempler et al. 1997), Germany (Meier and Vonesch 1997) and Switzerland (Lehmann et al. 1997). Other studies have reported marijuana-positive urine test results following consumption of hemp seed derived food products such as snack bars (Alt and Reinhart 1998, Fortner et al. 1997) and hempen ale (Gibson et al. 1998). No information was given regarding the procedural details of seed cleaning prior to pressing to recover the commercial oils; however, the hempen ale was prepared from seed which had been washed twice before the brewing process.

The ongoing question concerns the origin of the Δ^9-THC in the hemp seed nuts and hemp seed products. The presence in hemp seed nuts of Δ^9-THC could severely limit the market potential for product derived from the processing of hemp seed nuts. On the other hand, if the nuts are essentially free of Δ^9-THC, there is a significant market for oil pressed from those nuts, oil that can be consumed without the risk of the consumer testing positive for marijuana use.

Objectives of the Study

The objective of the study was to establish the intrinsic level of Δ^9-THC in hemp seed nuts from six (five in the original proposal) varieties of industrial hemp. The intrinsic Δ^9-THC content of the nuts determines the limit below which it is difficult to go for Δ^9-THC in hemp seed oil. The Δ^9-THC content in the hulls of the cleaned seeds indicates the residual Δ^9-THC which oil production processing has to contend with.

Several tasks were undertaken to answer this question. The first task was to determine Δ^9-THC levels in whole seed prior to cleaning to establish base-line levels of Δ^9-THC for each variety. Analysis of mechanically dehulled hemp nut and hulls was also undertaken as was the analysis of manually dehulled hemp nut and the hulls so obtained.

Description of Samples

Seed samples of industrial hemp seed were submitted to Websar Laboratories, Inc. on October 13, 1999 representing the following six hemp varieties: Fedora 19, USO 14, Felina 34, Fin 314, Fasamo and Ferimon 12. Information on each variety is given in Table 1.

A second set of samples, this time of mechanically dehulled seed, was submitted on December 22, 1999:

- Fasamo (hulled hemp seed)
- Fin 314 (hulled hemp seed)
- Mixed French varieties (Ferimon, Fedora, & Felina) (hulled hemp seed)

These hulled samples had not yet been subjected to air washing to remove the hull pieces and the whole seeds from the mechanical dehulling. The hulled seed was manually separated from the mixture in each case to enable analysis of the nut and hulls. All separated material was stored in 15-mL amber bottles with Teflon lined screw-caps.

A third set of samples, this time of fractions from mechanical dehulling, was submitted on January 18, 2000:

- Fin fines
- Fin screenings
- Fin nut
- Fasamo fines
- Fasamo screenings
- Fasamo nut
- Zolo fines
- Zolo screenings
- Zolo nut

The Zolo variety was not one of the original five varieties planned for the study.

Samples of Felina 34, Fedora 19, Ferimon 12, and USO 14 submitted on October 13, 1999 were given to Shaun Crew of Hemp Oil Canada Inc. to enable him to prepare mechanically dehulled nut and hull samples for analysis January 18, 2000. These samples were received for analysis by Websar Laboratories Inc. on January 28, 2000.

Sample Preparation

Whole Seed Analysis
Three replicate samples of whole seed, weighing 10 grams, were taken from each bulk variety sample and separately ground in a coffee mill. Each ground sample was then transferred to an amber storage bottle (with a teflon-lined screw cap) and held at 4^{o} C until analysis could be carried out. The coffee mill was cleaned between each sample.

Manual Dissection Fractions (nut and hulls)
Individual seeds were removed from the lot and viewed under a 3x magnifier. Only nuts that were undamaged were used. The seed was opened using a scalpel along the midpoint of the two halves and the seed was cut right through. The nut was removed using tweezers and placed in a screw capped amber bottle (see above). The hulls were placed in separate amber bottles. Enough seed was dissected to allow for triplicate sample lots. Duplicate samples from each lot were analyzed for each variety. All utensils used for manipulation of the seed, nut, and hulls were rinsed in toluene after each use on individual seeds. It was impracticable to remove the seed membrane from each seed; however, at the time of sampling for analysis, any membrane material that could be removed was manually removed before extraction. The samples were stored at 4^{o} C until analysis. Each sample was ground in a coffee mill prior to analysis.

Mechanically Dehulled Seed fractions (nut, hulls, fines, and screenings)

These samples were prepared, extracted, and analyzed as whole seed samples.

Sample Extraction and Analysis

A 200 mg subsample from each sample of the replicate set for each variety was weighed out for analysis in duplicate using the protocol for analysis of Δ^9-THC in hemp oil and seed cake as developed by Websar Laboratories, Inc. Fresh solutions of 5% ethanolic potassium hydroxide were prepared for the saponification step. Final extract volume was 1mL. Δ^9-THC-d_3 was used as the internal standard. Analysis was carried out using a Varian Saturn 2000 GC/MS calibrated using against a 10 µg/g Δ^9-THC calibration standard. Instrument performance must be within specification using control standards at both 4 µg/g and 30 µg/g before analysis can proceed. Injection volume was 1 µL. Duplicate injections were made for each analysis.

Results and Discussion

Whole Seed

Results of the analyses of whole seed used in the study are presented in Table 2.

Triplicate subsamples were analyzed in duplicate for each of the six hemp seed varieties submitted for the study. Each of these extracts, in turn, was analyzed using duplicate injections, resulting in averaged instrument responses for six replicate samples per variety.

Some variation in Δ^9-THC levels among replicates was noticed, especially in the case of Ferimon 12. This variation would be consistent with Δ^9-THC contamination of the outer seed coating through dust where dust deposit would not be expected to be uniformly dispersed throughout the seed sample.

During the manual dissection, it was observed that there was a hard waxy coating on the hulls of the seeds which crazed when the dissection was being initiated. The waxy coating was removed with the hulls and was analyzed as part of the hulls fraction.

Manual Dissection

Results of the analyses of manually dissected seeds are given in Table 3.

Triplicate subsamples were analyzed in duplicate for each of the six hemp seed varieties submitted for the study. Each of these extracts, in turn, was analyzed using duplicate injections, resulting in averaged instrument responses for six replicate samples per variety.

Levels of Δ^9-THC in the dissected out nut from the six varieties were in general low, i.e., well below 1 µg/g (ppm). The lowest level was in the Fedora 19 and the lowest variability was also seen here. The relatively high level reported for Fin 314 nut must be viewed with the knowledge that the variation is high. Hulls in general show higher levels of Δ^9-THC than the nut, except for USO 14, where the levels are essentially equivalent.

Mechanically Dehulled Seed

Results for mechanically dehulled seed show (these results replace those for the combined French varieties in the Final Report) that

a) the **mechanically dehulled nuts** appear to contain somewhat more Δ^9-THC than do the manually dehulled nuts, except in the case of Fin 314. The differences are statistically different, however, only in the case of Fedora and Fasamo.
b) the levels in the **hulls** are higher than those in the mechanically dehulled nuts in the case of Fedora, Felina 34, and Fin 314, and are essentially equivalent to those in the mechanically dehulled nuts for USO 14, Ferimon 12, and Fasamo.
c) the levels in the **fines** are higher than in the hulls for Felina 34, essentially the same for USO 14, Fin 314, and Ferimon 12, and lower for Fedora, and Fasamo.
d) the levels in the **screenings** are higher than in the fines for Fedora, Felina 34, Fin 314, Fasamo, and Ferimon 12, and essentially the same for USO 14.
e) Hulls in all cases contain less Δ^9-THC thane the screenings.
f) Fines in all cases (except Felina 34 where the fines are higher) contain essentially the same levels of Δ^9-THC as the mechanically dehulled nuts.

The results are also shown in the six figures (one for each variety) at the end of the report.

The mechanically dehulled Zolo nut contained 0.125 ± 0.05 μg/g; the fines contained 0.25 ± 0.13 μg/g, and the screenings contained 0.27 ± 0.11 μg/g.

Conclusion

The results show that it will be feasible to prepare hemp seed products with levels of Δ^9-THC below 1.0 μg/g, and for some varieties, well below 1.0 μg/g with the exception of Ferimon 12. The fines from the mechanical dehulling process also fit this category for Fedora, Fin 314, and Fasamo; the fines for USO 14, Felina 34, and Ferimon 12 do not. For each variety, the screenings also do not.

Table 1. Industrial Hemp Seed Submitted for the Dissection Study.

Variety	Moisture Content (%)	Comments
Fedora 19	8.1	Seed from the 1998 crop year. Commercially cleaned.
USO 14[a]	7.2	Seed from the 1998 crop year. Cleaned by hand sieving.
Felina 34	7.4	Seed from the 1999 crop year. Cleaned by hand sieving.
Fin 314	11.8	Seed from the 1999 crop year. Cleaned by hand sieving.
Fasamo	10.3	Seed from the 1999 crop year. Commercially cleaned.
Ferimon 12	18.8	Seed from the 1999 crop year. Cleaned by hand sieving. Seed was dried at room temperature for 10 days to reduce moisture content. New moisture content not determined as yet.

[a] USO 14 was not one of the original five varieties planned for the study

Table 2. Δ^9-THC in Whole Seed before Dissection.

Variety	Sub-Sample Size (grams)	Sample Size Analyzed (mg)	Δ^9-THC Range (μg/g)	Δ^9-THC Level (μg/g)
Fedora 19	10.0	200	2.19 – 2.96	2.53
USO 14	10.0	200	0.40 – 0.66	0.54
Felina 34	10.0	200	2.15 – 2.84	2.55
Fin 314	10.0	200	2.17 – 3.05	2.57
Fasamo	10.0	200	2.19 – 2.79	2.46
Ferimon 12	10.0	200	2.62 – 4.66	3.57

Table 3. Δ^9-THC in Seed Fractions (values in μg/g)

VARIETY	NUT (man)	NUT (mech)	HULLS (man)	FINES (mech)	SCR'N'GS (mech)
Fedora 19	0.11 ± 0.02[a]	0.46 ± 0.28	1.42 ± 0.21	0.61 ± 0.3	2.21 ± 0.41
USO 14	0.23 ± 0.09	0.57 ± 0.42	0.15 ± 0.08	1.02 ± 0.68	1.15 ± 0.43
Felina 34	0.24 ± 0.17	0.38 ± 0.14	0.88 ± 0.12	1.82 ± 0.18	2.67 ± 0.53
Fin 314	0.65 ± 0.39	0.41 ± 0.18	0.92 ± 0.13	0.58 ± 0.32	1.84 ± 0.30
Fasamo	0.12 ± 0.10	0.43 ± 0.17	0.62 ± 0.22	0.27 ± 0.06	1.36 ± 0.32
Ferimon 12	0.60 ± 0.31	1.18 ± 0.48	1.35 ± 0.51	2.13 ± 0.46	3.19 ± 0.42

[a] mean ± standard deviation