Methods for the Detection of Cannabis trace in cooked food

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ABSTRACT

In this study, we aim to develop methods to detect trace Cannabis in cooked food. We compared the use of chemical method and DNA analysis. Firstly, the Cannabis materials were identified by the macroscopic features, presumptive color-testing, and then confirmed by TLC analysis in hexane-dioxane-methanol (7:2:1). This solvent system gave the best resolution for cannabinoid separation in our hands. Thirty-six samples of dried and fresh Cannabis leaves that were boiled in water for 5 min to 8 hr were subjected to TLC and DNA analysis. The results illustrated that all treated samples showed the same TLC fingerprints indicating the presence of (THC), (CBD) and (CBN). However, only the fresh marijuana leaves which were boiled for 5 min showed the 197-bp mitochondrial *trn*L-*trn*F PCR-amplified fragment. Hence, the results suggested that TLC technique is more robust for THC detection in processed Cannabis. In addition, DNA analysis which can be categorized as confirmation method for Cannabis detection seems to be limited when DNA from heat-treated materials were analysed. The experiment suggested that detection of heat-treated Cannabis is possible by TLC. In the future, we aim to test for the presence of Cannabis trace in food, particularly curries and noodle soup which Cannabis is often use as additives.

Key words: Cannabis trace; TLC; DNA

Introduction

Cannabis is the only genus producing cannabinoid compounds (Turner, 1980; Stella, 2005), which are psychoactive substances. Among these cannabinoids the primary psychoactive substance are Δ^9 tetrahydrocannabinol (THC), cannabinol (CBN), cannabidiol (CBD) and the main metabolite of THC, i.e. 11-nor-D9-tetrahydrocannabinol-9-carboxylic acid (THCA) (Uhl and Sachs, 2004; Kojoma *et al*., 2005; Dussy *et al*., 2005). According to Cannabis legislation, the main psychoactive substance, THC, is measured using the application of chemical-based analysis. These are presumptive test, color test or thin layer chromatography (TLC) and confirmative test, Gas chromatography (GC) or High performance liquid chromatography (HPLC).

Cannabis is mostly smoked because this is the easiest way to achieve the desired psychoactive effects (Hall *et al*., 1994). Most users usually smoke it in the hand-rolled cigarretes called *joints*, among other names; some use pipes or water pipes called *bong*s. It is also used to enhance appetite in some tea and food as an additive. However, the technique used to detect Cannabis trace in cooked food has not been reported.

Marijuana DNA marker had been reported by Kojoma et al. (2005) and Linacre and Thorpe (1998). Kojoma et al. (2005) demonstrated that the nuclear THCA synthase gene is specific to the drug type Cannabis. THCA synthase gene is the gene that expresses THCA synthase, which converts into the psychoactive THC when heated. The intergenic spacer between the *trn*L 3′ exon and *trn*F gene in chloroplast had been reported by Linacre and Thorpe (1998). Here, region that is specific to marijuana was identified.

Since marijuana is biological material that contains DNA, so molecular technique by using DNA markers may then be an alternative for identifying Cannabis trace in cooked food. Polymerase chain reaction (PCR) is simple quick and so sensitive that it can be used to analyze small amount or degraded sample by using PCR, it may then be possible to analyze. Therefore, in this study, we aim to develop methods; both chemical method and DNA analysis, to detect trace Cannabis in cooked food.

Materials and Methods

Cannabis materials were obtained from the Office of the Narcotics Control Board (ONCB), Thailand. The general appearance was observed and color test, using Fast Blue B Salt reagent, was used to confirm the Cannabis samples.

Fresh marijuana (100mg) and dried marijuana (20 mg) were separately filled in gauze bags. Total of 36 bags; 18 bags for fresh and 18 bags for dried Cannabis, were prepared. Both fresh and dried, there of each Cannabis samples were cooked in the 40 ml boiling water for 5 minutes, 10 minutes, 1 hour, 2 hours, 4 hours and 8 hours.The boiled Cannabis was filtered using filter papers then Cannabis was collected in an eppendorfs.

Three primer pairs and four solvent systems were compared for DNA and TLC analysis, respectively.The first two primer pairs amplified the intergenic spacer between the *trn*L-*trn*F gene in chloroplast DNA and another primer amplified THCA syntase gene in nuclear DNA. Moreover, Four solvent systems for TLC analysis hexane, dioxane and methanol (7:2:1), petroleum ether and Diethyl ether (8:2), hexane and diethyl ether (8:2), and hexane and dioxane (9:1). The suitable primer pair and solvent systems were then selected to analyse all treated samples.

Results and Discussion

According to Fast Blue B salt test, the result showed a red-orange color stain (figure 1), which can indicate the presence of cannabinoid in Cannabis materials. Moreover, the solvent system of hexane-dioxane-methanol (7:2:1) (figure 2) and primer G and H, Cannabis-sapecific marker (figure 3), gave the best results for Cannabis identification using TLC and DNA analysis, respectively.

The results illustrated that all treated samples showed the same TLC fingerprints indicating the presence of Δ^9 tetrahydrocannabinol (THC), cannabinol (CBN), cannabidiol (CBD) (Figure 4). However, only the fresh marijuana leaves which were boiled for 5 min showed the 197-bp mitochondrial *trn*L-*trn*F PCR-amplified fragment (Figure 5).

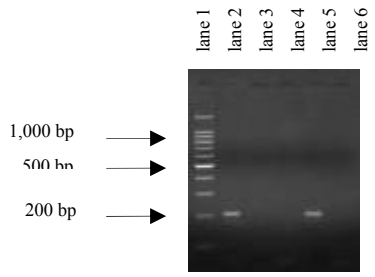
a) b)

Figure 1: Presumptive Test for marijuana. Figure a), red color indicated the presence of Cannabinoids. As shown in figure b), negative control reaction.

a b c d

a= hexane: dioxane: methanol (7:2:1) system
b= petroleum ether: diethyl ether (8:2) system
c= hexane: diethyl ether (8:2) system
d= hexane and dioxane (9:1).

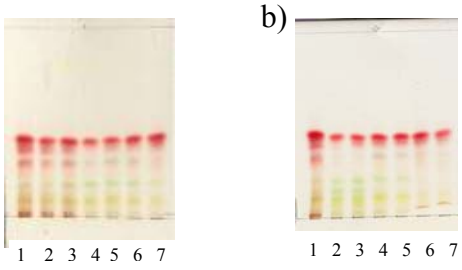
Figure 2: Cannabinoids separated on silicagel GF_{254} TLC plate and four solvent systems were compared for cannabiniod separation.



Lane	Detailed	Lane	Detailed
1	100 bp DNA Ladder	6	*Solanum torvum* Sw.
2	*Cannabis saiva* L.	7	*Chionanthus parkinsonii* (Hutch) Bennet. & Raizada
3	*Papaver somniferum* L.		
4	*Mitragyna speciosa* (Roxb.) Korth.	8	*Ipomoea aquatica* Forssk.
		9	*Vigna radiata* (L.) R. Wilczek.
5	*Nicotiana tabacum* L.	10	No DNA (Negative control)

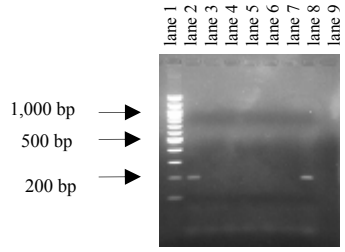
Figure 3: A 2% (w/v) agarose gel depicting PCR products amplified with primer G and H and DNA extracted from *Cannabis sativa* L. (lane2) and the other plants (lane 3-9).

a) b)



Lane	Detailed
1	Untreated Cannabis
2	Fresh/dried Cannabis boiled for 5 min.
3	Fresh/dried Cannabis boiled for 10min.
4	Fresh/dried Cannabis boiled for 1 hr.
5	Fresh/dried Cannabis boiled for 2 hr.
6	Fresh/dried Cannabis boiled for 4 hr.
7	Fresh/dried Cannabis boiled for 8 hr.

Figure 4: Cannabinoids extracted from boiled fresh (a) and dried Cannabis (b), separated by TLC using silicagel GF_{254} and hexane: dioxane: methanol (7:2:1) as solvent system.



Lane	Detailed
1	100 bp DNA Ladder
2	Fresh Cannabis boiled for 5 min.
3	Fresh Cannabis boiled for 10 min.
4	Fresh Cannabis boiled for 1 hr.
5	Fresh Cannabis boiled for 2 hr.
6	Fresh Cannabis boiled for 4 hr.
7	Fresh Cannabis boiled for 8 hr.
8	untreated Cannabis (Positive control)
9	no DNA (Negative control)

Figure 4: A 2% (w/v) agarose gel depicting PCR products amplified from DNA extracted from fresh Cannabis leaves boiled in water for 5 min – 8 h (b), using the G and H primers.

Conclusion and Suggestion

The results suggested that TLC technique is more robust for THC detection in processed Cannabis. In addition, DNA analysis which can be categorized as confirmation method for Cannabis detection seems to be limited when DNA from heat-treated materials were analysed. The experiment suggested that detection of heat-treated Cannabis is possible by TLC. In the future, we aim to test for the presence of Cannabis trace in food, particularly curries and noodle soup which Cannabis is often use as additives.

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