Effect of Seed Maturity on Seed Physiological Quality, Oil Content and Fatty Acid Composition of Hemp Seed

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ABSTRACT

Hemp (Cannabis sativa L.) is not only a prominent fiber crop but also used for a new value added products. This research was to find out different seed maturities on seed germiability and some chemical compositions. A split plot design was used which four varieties (V50, Mae Sa Mai, Pang Ung and Huay Hoi) were main-plot and 3 different seed maturities. There were 1) 50% brown seed; 2) 80% brown seed and 3) 100% brown seed appear were applied in sub-plot, then seed quality, oil content and fatty acid compositions were determined. The 100-seed weight and the seed quality in each variety were not different. The seed harvested at brown seed appeared 50% has lower mean germination (71%) than the others maturity (brown seed appear 80% and 100% ripens) which was similar to 82%, but AA germinations were 78 and 82%, respectively. However, the interaction between variety and maturity were significantly affected on germination and vigor. Hemp seed harvested from brown seed appear 80% has the highest oil content as 28.88% compared with the others ripeness. The oil composed with unsaturated fatty acid, which was essential for human. Linoleic acid (LA, 18:2 or Omega-6) in var. V50, Pang Ung, Mae Sa Mai and Huay Hoi were 53.79, 52.78, 51.04 and 50.54 g/100 g, respectively while α-linolenic acid (ALA, 18:3 or Omega-3) were 12.79, 10.96, 10.04 and 11.73 g/100 g, respectively. Hemp oil is an alternative high quality of fat, which can be progressively used and developed in Thailand.

Key words: Hemp, Seed maturity, Fatty acid composition

INTRODUCTION

Hemp (*Cannabis sativa* L.) was developed from wild Cannabis plant that originated most probably in Central world over 3000 year ago (Clarke, 1999). These early landraces dispersed to other areas of Asia, Europe and Africa, where they were further domesticated and selected for more specific uses such as fiber, food and for medicinal properties (Renalli, 1999). These uses were not only fibre hemp used for high quality industrial textile, but also seed has been consumed as food and folk medicinal preparation (Oomah et al., 2002). The seed consists of 20-25% protein, 20-30% carbohydrate, 25-35% oil and 10-15% insoluble fiber (Deferne and Pate, 1996). In the highlands of northern Thailand under the Royal Project extension area; hemp have been grown as a fiber crop rather than for value added seed products. Hemp fiber is not only a high quality textile but also the oilseed was used for improve as functional food (foods which provides health benefit) and also as a cosmetic playing a more important role in world market. Quality seeds are needed to increase production.

Hemp is an orthodox seed, which is tolerant to desiccation as higher drying temperatures without adverse effects on seed germination and vigor during the maturity period. Usually an individual plant bears only one kind of flower and the species is accordingly described as dioecious (Ranalli, 1999). The growth pattern is divided into four principal stages: germination and emergence, vegetative phase, flowering and seed formation then senescence. One female has numerous flowers in different developmental stages (Mediavilla et al., 1998), which make it more complicated to determine physiological and harvest maturities. Physiological maturity is described as the highest seed weight, germination and vigour; whereas the seed moisture is high (Tekrony et al., 1979; Copeland and McDonald, 2001). After hemp blooming and fertilization, single seeds mature in three to five weeks and the seed (achenes) turn hard and 50% of the colour of the seed change, then the leaves and stem start to dry (Mediavilla et al., 1998). In addition, moisture content of the seed is an important factor for deciding the harvesting period. According to the studies conducted for industrial hemp in Canada, straight combining was done prior to seed shattering or cut at 85% maturity seed, and normally when the grain contains moisture content at 12 to 20% depending on variety. However, harvesting until the crop ripens and leaf dry down to low moisture content affected drier seed susceptible to crack, resulting in seed deterioration (Mooleki, 2006). Merfield (1999) mentioned in the document of oil seed hemp cultivation in New Zealand that seed harvested when fully ripe affected the viability and showed low nutritive values. On the other hand when harvested at over ripe yield decreased. It should be harvested when the seed coat is hard and the seed coat colour change to like marble pattern and the seed matures until the middle of inflorescence, then dried to down below 12% moisture.

In general, hemp prefers growing a mild climate and high humidity as well as temperature between 14-27 ºC. In Thailand, hemp has been grown in highland by hill tribes and used as raw material to make their apparel or used on special occasions. After the Thai Royal Project was interested in developing industrial hemp as an economic crop to support the hill tribes, quality seed was needed, and seed types were derived from segregation of the local landrace. They originate from the same fields of the Royal Project. Cultivated seed are usually sown in early March to May, and harvested in the end of November. The process of harvest usually done by traditional method and most of hemp seed germination is usually low if harvested too early, whereas the processes for maturity are not well understood. This experiment was to find out harvesting time of seed for high seed physiological quality, oil content and fatty acid composition of hemp.

MATERIALS AND METHODS

Plant material and experimental design

Hemp was planted in 2010 at the Royal Agricultural Station Ang Khang (19°54'27.37"N), the Royal Project Development Center Pang Ung (18°46'57.10"N), Mae Hae (18°47'25.97"N) and Mae Sa Mai (18°53'3.73"N) where elevations rang from 1410, 1345, 1200 and 990 meters above mean sea level. The experiment was to find out the suitable of hemp seed maturity for harvest. A split plot design was used with four varieties (V50, Mae Sa Mai, Pang Ung and Huay Hoi). They were planted in each location with a main-plot, then 3 different seed maturities [i.e. 1) brown seed appear 50%, yellow leaf emerge; 2) brown seed appear 80%, yellow leaf descend and 3) brown seed appear 100%, leaf dried out] were applied in sub-plot. Since the three harvests were harvested repeatedly from the sample plot in three blocks, repeated measures analysis of the data was done by following:

Physiological parameters

Moisture content: Moisture content was tested by gravimetric method as high constant temperature oven method at 105ºC for 20 hr (ISTA, 2006).

Thousand seed weight: seed were sampling and count for 100 seeds with 4 replications and weight in units of a gram.

Seed germination: Four replications of 100 seeds were germinated between moistened papers and incubated for 7 days at 20ºC (ISTA, 2006).

Vigour test: Accelerated aging (AA) test were applied from Delouche (1996). Two hundred seed samples were conducted at 41 ºC for 72 hr using the inner chamber method; the seed were tested for germination and calculated as percentage.

Tetrazolium test: Presoaked 100 seeds were cut in the half, and then incubated in 0.1% tetrazolium solution for 3-4 hr at 41ºC in darkness. After that the red staining was evaluated and calculated as a percent of the total number of completely stain seed (ISTA, 2006).

Biochemical parameters

Determination of oil content: Ground hemp seed samples of 5 g were extracted in Soxhlet extractor containing petroleum ether. The results were reported as percentage of total oil (AOAC, 1990).

Determination of fatty acid composition: Fatty acid composition was determined according to the method of AOAC by gas liquid chromatography (AOAC, 2005).

Data analysis

Data of hemp seed quality were subjected to analysis of variance which means from each treatment were separated using least significant difference (LSD) test at the probability level of 0.05.

RESULTS

Moisture content

Moisture content of all hemp seed progressively decreased from early to late maturity time (Table 1). However the moisture at harvested times are differ in each variety originated from each location, which V50 was the highest followed by Mae Sa Mai, Pang Ung and Huay Hoi, respectively. The seed harvested at 50% turned brown in each variety showed the highest moisture content.

Table 1. Physiological quality of 4 *Cannabis sativa* genotypes at different maturities.

Variety	Maturity	Physiological quality				
		Moisture content (%)	Seed weight (g)	Germination (%)	Vigour (%)	TZ-test (%)
V50	1) 50% brown seed appear, yellow leaf emerge	24.8	2.92	63 e	65 f	70 c
	2) 80% brown seed appear, yellow leaf descend	19.6	2.79	74 cd	78 cd	77 b
	3) 100% brown seed appear, leaf dried out	16.9	2.91	81 ab	79 cd	83 a
Mae Sa Mai	1) 50% brown seed appear, yellow leaf emerge	22.6	3.47	71 d	58 g	69 c
	2) 80% brown seed appear, yellow leaf descend	17.2	3.33	80 bc	71 e	75 bc
	3) 100% brown seed appear, leaf dried out	13.5	3.17	79 bc	76 d	70 c
Pang Ung	1) 50% brown seed appear, yellow leaf emerge	18.5	2.72	72 d	76 d	79 b
	2) 80% brown seed appear, yellow leaf descend	15.4	2.6	81 ab	77 cd	82 a
	3) 100% brown seed appear, leaf dried out	13.8	2.72	86 a	88 a	83 a

Table 1. Continued.

Variety	Maturity	Physiological quality				
		Moisture content (%)	Seed weight (g)	Germination (%)	Vigour (%)	TZ-test (%)
Huay Hoi	1) 50% brown seed appear, yellow leaf emerge	15.6	3.20	78 bc	82 bc	76 b
	2) 80% brown seed appear, yellow leaf descend	14.0	3.26	83 ab	86 ab	77b
	3) 100% brown seed appear, leaf dried out	12.7	3.31	83 ab	86 ab	78 b

Means followed by the same letter within a column are not significantly different at 0.05% level.

Thousand seed weight

The 100-seed weight in each variety was different (Table 1). Mae Sa Mai and Huay Hoi were similar having heavier weight than V50 and Pang Ung. Moreover, three different seed maturity stages also affected seed weight, which harvesting at 50% brown seed showed highest means of seed weight (3.08 g).

Seed germination

Seed germination of hemp in each variety was not different, but harvesting seed at different maturity significantly affected the germination (Table 1). The seed harvested at 50% brown seed appeared had a lower mean germination (71%) than the others (80% brown seed appear and 100% ripen), which were similar as 82%. However, the interaction between variety and maturity was also significantly affected seed germination. The late harvest (brown seed appeared 100%) was promptly for getting better germination in each variety.

Seed vigour

Accelerated aging (AA) test before germination resulted in further reduction of seed germination, which depended on maturity and variety. Hemp harvested at brown seed 50% exhibited lower mean of germination than the other maturity (brown seed 80% and 100% ripen) for which AA germinations were 78 and 82%, respectively (Table 1). This result suggested that harvesting at early maturity composed of small seed because of un-ripened seed especially at the uppermost inflorescence. A slight increase in AA was observed for seed var. Pang Ung and Huay Hoi harvested at brown seed appeared 100%. On the other hand, AA greatly decreased in var. Mae Sa Mai especially harvested at 50% brown seed and yellow leaf emergence.

Tetrazolium test

The tetrazolium test is widely recognized as an accurate means of estimating seed viability and vigour, which is interpreted according to the topographical staining pattern of the embryo and the intensity of the coloration. The result found that maturity and hemp variety significantly affected on high seed vigor percent. Hemp var. V50 and Mae Sa Mai harvested when turned brown 50% showed lower percent of seed stained as high viability than the others (Table 1). In both brown seed appear 80% and 100% ripens harvests, the significant increased in high viability only occurred in var. V50 and Pang Ung. This result suggested that early harvest includes a mixture of seed at different stages of development and immature seed.

Oil content

The mean of oil content (%w/w) of hemp seed each maturity was not different (Table 2). Significant differences in oil content were observed among the variety while Huay Hoi has the highest (29.62%) followed by Pang Ung, V50 and Mae Sa Mai. Oil content increased as seed matured for var. V50. It was noted that the seed in earlier stages (brown seed appear 50%) of development had lesser oil content than late mature (brown seed appeared 80%) which also found in var. Mae Sa Mai and Huay Hoi.

Table 2. Oil seed contents of 4 *Cannabis sativa* genotypes in different maturities.

Maturity	Variety				Average
	V50	Mae Sa Mai	Pang Ung	Huay Hoi	
	Oil content (%) of seed mass				
1) 50% brown seed appear, yellow leaf emerge	27.47	27.69	30.03	29.78	28.74
2) 80% brown seed appear, yellow leaf descend	28.78	28.63	27.99	30.12	28.88
3) 100% brown seed appear, leaf dried out	29.6	27.85	28.55	28.97	28.74
Average	28.62b	28.06c	28.86b	29.62 a	

Fatty acid composition

The oil from mature seed harvested at brown seed appeared 80% composed with unsaturated fatty acid, which were essential for human. The fatty acid patterns of hemp 4 varieties are similarity as shown in Table 3. Hemp seed also contain saturated fatty acid ranged from 11-13%, which was palmitic acid (7-8%). In addition, unsaturated fatty acids were the major fatty acid (more than 82-84%). Among these, fatty acid with more double bonds or polyunsaturated fatty acids (PUFAs) illustrated more than 60%. Only two out of the four essential were in hemp seed, which was linoleic acid and α-linolenic acid. Linoleic acid (LA, 18:2 or Omega-6) in var. V50, Pang Ung, Mae Sa Mai and Huay Hoi were 53.79, 52.78, 51.04 and 50.54 g/100 g, respectively and α-linolenic acid (ALA, 18:3 or Omega-3) were 12.79, 10.96, 10.04 and 11.73 g/100 g, while only a few seed oil having. A small amount monounsaturated fatty acid (MUFAs) also detected as oleic acid (18:1 or Omega-9) about 16-20.5%.

Table 3. Fatty acid patterns of the oil extracted from the seed of 4 *Cannabis sativa* varieties harvested at 80% brown seed appear and yellow leaf descend.

Fatty acid	Variety			
	V50	Mae Sa Mai	Pang Ung	Huay Hoi
	(g/100g)			
8:0 (Caprylic acid)	0.01	0.01	0.02	0.02
14:0 (Myristic acid)	0.05	0.05	0.04	0.04
15:0 (Pentadecanoic acid)	0.02	0.02	0.02	0.02
16:0 (Palmitic acid)	7.24	8.14	7.48	7.31
16:1n7 (Palmitoleic acid)	0.10	0.10	0.11	0.10
17:0 (Heptadecanoic acid)	0.05	0.05	0.05	0.05
18:0 (Stearic acid)	3.21	3.25	2.73	3.58
18:1n9c (Oleic acid)	10.58	14.23	11.07	13.25
18:2n6 (Linoleic acid)	53.79	51.04	52.78	50.54
18:3n6 (γ-Linolenic acid)	0.62	0.58	1.05	0.57
18:3n3 (α-Linolenic acid)	12.79	10.04	10.96	11.73
20:0 (Arachidic acid)	0.80	0.56	0.81	0.74
20:1n11 (Eicosenoic acid)	5.32	6.16	7.42	6.71

Table 3. Continued.

Fatty acid	Variety			
	V50	Mae Sa Mai	Pang Ung	Huay Hoi
	(g/100g)			
20:2 (Eicosadienoic acid)	0.07	0.06	0.07	0.06
21:0 (Heneicosanoic acid)	0.03	0.02	0.02	0.02
22:0 (Behenic acid)	0.32	0.35	0.34	0.28
22:1n9 (Erucic acid)	0.03	0.03	0.03	0.03
23:0 (Tricosanoic acid)	0.04	0.04	0.04	0.03
24:0 (Lignoceric acid)	0.15	0.19	0.17	0.17
24:1n9 (Nervonic acid)	0.03	0.03	0.03	0.00
Saturated fatty acid	11.92	12.98	11.72	12.26
Monounsaturated fatty acid	16.06	20.55	18.66	20.09
Polyunsaturated fatty acid	67.27	61.72	64.86	62.90
Unsaturated fatty acid	83.33	82.27	83.52	82.99

DISCUSSION

Hemp seed moisture decreased from early to late maturity time due to field drying condition. The difference in initial and final moisture content between var. V50 and Huay Hoi occurred as a result of a much cooler in Royal Agricultural Station Ang Khang where temperature was 12-14ºC and 86% RH during harvesting time (late November to early December, 2010). Cooler weather and high humidity did not allow for seed to dry down after physiological maturity was reached. High seed moisture at harvest was affected from mixture of seeds of different maturities due to numerous flowers in different developmental stages (Mediavilla et al., 1998). Late harvesting time and weather conditions in Royal Project Development Center Mae Hae allowed var. Hauy Hoi for drying of the seed on any position of the inflorescent of plant. However, at turned brown 50%, seed moisture content in var. V50 was still high (24.8%) for harvest. This also resulted in low initial viability as the germination and vigour. Nevertheless, even though the dry weight of seed harvested at turned brown 50% was similar to the others maturities, low initial viability as the germination and vigour were observed. The result suggests that harvesting at brown seed appeared 50% does not reached physiological maturity, which leads to obtain high cost for drying and further poor storage (Copeland and McDonald, 2001). Under field condition, the moisture content of seed harvested at brown seed appeared 80% slightly decreased while the germination greatly increased from the previous harvest and similar to 100% brown seed especially in var. Mae Sa Mai and Huay Hoi. However, the vigour of both varieties were different and the vigour of var. Mae Sa Mai was lower than Huay Hoi due to the complex inflorescence and field conditions allowed to contamination of diseases to the seed. In addition, most of the late maturity or seed completely brown showed the low moisture content (12-13%), reflecting the susceptible to cracking and shattering (Mooleki, 2006) and risky for yield loss from animal pest.

The mean of oil content (% w/w) of hemp seed in each mature stage was not difference (Table 2). Mediavilla et al. (1998) recommended that one to three weeks before seed maturity or 50% of seed hard is the best time for production of essential oil. Oil content was significantly differences between varieties while Huay Hoi has the highest (29.62%) and Mae Sa Mai was the lowest (28.06%). This result is in general agreement with prior report of Kriese et al. (2004) who found hemp seed oil content of 51 genotypes ranged from 26.25 to 37.5%. In addition, the fatty acids composition varied throughout hemp genotypes, which were noted that it was largely affected by environmental factors. The result of the present analysis the fatty acid composition of hemp seed oil native to Thailand comprised primarily of unsaturated fatty acid (more than 82-84 g/100g), which were essential for human especially PUFAs illustrated more than 60 g/100g i.e. linoleic in var. V50, Pang Ung, Mae Sa Mai and Huay Hoi were 53.79, 52.78, 51.04 and 50.54 g/100g, respectively as well as α-linolenic acid were 12.79, 10.96, 10.04 and 11.73 g/100g. These results are parallel with the report of Deferne and Pate (1996) and Callaway and Laakkonen (1996). However, The variability of fatty acids concentration results from the natural variation of individual samples of hemp including processing and further storage methods as well as the age of the seed (Leizer et al., 2000). High quality of hemp oil var. V50 revealed that this genotype may be a suitable parents for further breeding program for improving hemp oil quality. Moreover, hemp contain high level of essential fatty acids and PUFAs that can not made by humans, thus linoleic acid (LA, 18:2 or Omega-6) used α-linolenic acid (ALA, 18:3 or Omega-3) certainly can be used to enhance human health and development (Simopoulos, 1991; Callaway, 2004). In addition, the nutrition values were demonstrated health benefits, including the effect on lowing of cholesterol and high blood pressure (Oomah et al., 2002).

CONCLUSION

Regarding to seed quality, the data presented that the moister content of hemp seed harvested at 80% brown seed was decreased under field condition as well as the germination greatly increased similar to 100% brown seed harvesting. However, the seed susceptible to crack and shatter and possibly causes yield loss from animal pest. The oil content was not different between various maturities and hemp oil is an alternative high quality of fat, which can be progressively researched and developed for functional foods and pharmaceuticals in Thailand.

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