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Influence of the Growth Stage of Hemp (*Cannabis sativa* L.) on Fatty Acid Content, Chemical Composition and Gross Energy

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Abstract: Hemp (*Cannabis sativa* L.) has been studied to determine the Fatty Acid (FA) content, chemical composition and Gross Energy (GE) of the plant during growth. Herbage samples were collected 4 times at progressive morphological stages from the mid vegetative to the early flower stage. The most abundant FA in the plant during these growth stages was α-linolenic acid ($C_{18:3\ n-3}$) and it ranged between 48-54% of the total FA. The FA analyses disclosed quantitative differences between the plant stages. Palmitic acid ($C_{16:0}$), oleic acid ($C_{18:1\ n-9}$) and linoleic acid ($C_{18:2\ n-6}$) decreased with increasing growth stage. There was a lack of γ-linolenic acid ($C_{18:3n-6}$) at all stages of the hemp plant during growth. The dry matter, organic matter and acid detergent lignin content increased with increasing growth stage, while the ash and crude protein decreased from the mid vegetative stage to the shooting and late vegetative stage, respectively. Neutral detergent fibre increased from the first to the third stages and then decreased. GE was higher at the shooting stage than at the other stages.

Key words: *Cannabis sativa*, fatty acid, crude protein, fibrous fraction, gross energy

INTRODUCTION

Hemp (*Cannabis sativa* L.) is an annual, usually dioecious, herbaceous crop that is native to central Asia and which is known to have been grown in China over 4500 years ago (Lewington, 2003). It has been grown agriculturally for many centuries for its fibre and oil. Hempseed contains 20-25% protein, 20-30% carbohydrates, 25-35% oil and 10-15% insoluble fibre and a rich array of minerals (Pate, 1999). The oil extracted from hemp seeds contains Polyunsaturated Fatty Acids (PUFAs), among which these are several essential fatty acids such as linoleic (LA, $C_{18:2\ n-6}$) and α-linolenic acid (LNA, $C_{18:3n-3}$) (Deferne and Pate, 1996; Kriese *et al*., 2004), while the plant organs flowers are richer in oils than the leaves (Lemberkovics *et al*., 1979). The Fatty Acid (FA) composition of the fruit is of great interest, because of their use for nutritive and pharmaceutical purposes (Truþa *et al*., 2002; Leizer *et al*., 2000). Hemp oil reduces the risk of cardiovascular diseases, cancer and age-related macular degeneration (Léger, 2000), is due to the FA and tocopoherol composition of hemp oil (Blade *et al*., 2005). Tocopherols act as antioxidants and prevent the oxidation of unsaturated fatty acids (Kamal-Eldin and Appelqvist, 1996; Yu *et al*., 2005).

Fibre hemp is also, generally grown for its bast fibres, which are used as a raw material for cordage and textile products. For many years, hemp has been a traditional crop in Italy, but at present it has almost disappeared from Italian farmland, mainly because of economic reasons. Recently, in 1997, the Italian Administration launched a programme whit the aim of reintroducing hemp cultivation in the country (Cappelletto *et al*., 2001) due to a demand for non-food crops in farming systems (Struik *et al*., 2000) and a renewed interest in the use of natural fibres for non-woven industrial products.

Although, many researchers have studied the chemical composition of hemp seeds or fruit, no information is available on the changes in the FA and proximate composition of whole plants during the growth cycle. The present work was designed to evaluate the effect of stage of maturity on the FA profile and on the chemical composition and Gross Energy (GE) of hemp during the growth season.

MATERIALS AND METHODS

The research was conducted in the Western Po Valley near Cuneo, Italy. The climatic patterns in North Italy are characterised by high precipitation in April, May and October with very little rainfall in summer and winter. The coldest month is January: the Po valley's mean temperature is between -1 and 1°C, while the hottest month is July with a mean temperature of 22°C. The stands were seeded on 2 June 2005 and no irrigations or fertilisers were applied after sowing because hemp shows high plasticity therefore allowing it to be grown over a wide range of agro-ecological conditions

(Struik *et al.*, 2000) with limited fertiliser requirements (Van der Werf, 1994a). Herbage samples were collected with edging shears (0.1 m cutting width) at 4 progressive morphological stages from mid vegetative to early flower stage, on subplots of 2 m^2 randomly located in 2×12 m^2 plots with 3 replicates cut to a 1-2 cm stubble height. The sampling time ranged from July to August 2005. Sampling was not performed on rainy days and was carried out in the morning, after the disappearance of dew.

Fresh samples of the whole plants were immediately frozen and subsequently freeze-dried and ground to pass a 1 mm screen. Lipid extraction was performed on freeze-dried samples according to Hara and Radin (1978), while the transesterification of the FAs was carried out according to Christie (1982), with the modifications described by Chouinard *et al.* (1999). The fatty acids were analysed as their methyl esters. The analysis was carried out by gas chromatography, using a Dani GC 1000 DPC (Dani Instruments S.P.A., Cologno Monzese, Italy), equipped with a Supelcowax-10 fused silica capillary column (60 m × 0.32 mm (i.d.), 0.25 μm). The injector and detector ports were set at 245 and 270°C, respectively. The oven temperature program was initially set at 50°C for the first min and then increased at a rate of 15°C min^{-1} to 200°C, where it remained for 20 min and then increased at a rate of 5°C min^{-1} to 230°C, where it remained for the last 3 min. The carrier gas was He. One microlitre was injected with a Dani ALS 1000 auto sampler with a 1:50 split ratio. The peak area was measured using a Dani Data Station DDS 1000, where each peak was identified and quantified according to pure methyl ester standards (Restek Corporation, Bellefonte, PA, USA).

Whole plant samples were immediately dried in a forced-draft oven to constant weight at 65°C to determine the Dry Matter (DM) content and were then air equilibrated, ground in a Cyclotec mill (Tecator, Herndon, VA, USA) to pass a 1 mm screen and stored for later analyses. Dried samples were analysed to determine the total N content (Association of Official Analytical Chemists, 1990), ash by ignition to 550°C, Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF), as described by Van Soest *et al.* (1991) expressed exclusive of residual ash and Acid Detergent Lignin (ADL) determined by solubilization of cellulose with sulphuric acid as described by Robertson and Van Soest (1981). The GE was determined using an adiabatic calorimeter bomb (IKA C7000, Staufen, Germany).

The variability in FA and the herbage chemical composition of the samples harvested at four stages of maturity were analysed by one-way Analysis of Variance (ANOVA) using the Statistical Package for Social Science (v 11.5, SPSS Inc., Chicago, Illinois, USA) to test the effect of the growth stage. When the values of F were significant (i.e., $p<0.001$ and $p<0.01$), the Duncan test was used to detect any differences among the means.

RESULTS AND DISCUSSION

The total FA content in the plant ranged between 2.02 and 2.38 g kg^{-1} DM without any significant differences during the growth, while the FA analyses disclosed quantitative differences for the various plant stages (Table 1). The Palmitic Acid (PA, $C_{16:0}$), Oleic Acid (OA, $C_{18:1\ n-9}$) and LA decreased with increasing growth stage. The LNA was the most abundant FA in the plant during these growth stages and ranged between 48-54% of the total FA. Myristic ($C_{14:0}$), palmitoleic ($C_{16:1}$), stearic ($C_{18:0}$), vaccenic ($C_{18:1\ n-7}$) and stearidonic (SDA, $C_{18:4\ n-3}$) acids were present in small amounts and without significant differences during the growth. The unidentified FAs increased from the mid vegetative to the shooting stage and then decreased. The high amount of LNA, the low percentage of LA, which is about 5 times lower in the plant than the quantity of this FA in the oil and the lack of γ-linolenic acid (GLA, $C_{18:3\ n-6}$) at all stages of development are the main features of the growing plant. The FA profile in the plant during growth (Fig. 1) is generally different from that of the corresponding seed oil, which contains different percentages of unsaturated FA-mainly OA (10-16%), LA (50-60%), LNA (20-25%) and small amounts (2-4%) of GLA (Oomah *et al.*, 2002; Carvalho *et al.*, 2006; Callaway *et al.*, 1996; Mölleken and Theimer, 1997). These are in agreement with literature on other oil seed crops, such as borage (Peiretti *et al.*, 2004a), evening primrose (Peiretti *et al.*, 2004b), *Galega officinalis* (Peiretti and Gai, 2006), false flax (Peiretti and Meineri, 2007), linseed (Peiretti and Meineri, 2008) and chia (Peiretti and Gai, 2008). Mölleken and Theimer (1997) found that the origin of the hemp seed seems to influence FA composition. The FA profile resulted to be different in the various compartements of the fruit, but fruit and seeds of the hemp were very similar in their mean FA composition, while the shells, where lipids like phospholipids or glycolipids are important, differed to a great extent with an increase of 38% of OA and a decrease of 34% of LA (Mölleken and Theimer, 1997).

The total lipid extract of the industrial hemp bast fibers accounted for 0.5% of the total fiber. Free FAs were present in the range from tetradecanoic (C_{14}) to dotriacontanoic (C_{32}) acids, with a strong even-overodd carbon atom predominance. PA and LA were the most abundant followed by OA and stearic ($C_{18:0}$) acid (Gutierrez *et al.*, 2006).

Table 1: Fatty Acid (FA) content and profile of hemp at four morphological stages

Stage (Date of harvest)	Mid vegetative 17/07/2005	Late vegetative 23/07/2005	Shooting 30/07/2005	Early flower 06/08/2005	SEM	Stage effect
FA (g kg^{-1} DM)	2.02±0.26	2.28±0.55	2.17±0.76	2.38±0.32	0.13	ns
Fatty Acid	(g/100 g of FA)					
$C_{14:0}$	0.15±0.03	0.10±0.09	0.08±0.07	0.00±0.00	0.02	ns
$C_{16:0}$	13.03±0.33^{a}	12.67±0.36^{a}	11.47±0.25^{b}	11.28±0.53^{b}	0.25	***
$C_{16:1}$	0.22±0.01	0.24±0.01	0.25±0.02	0.23±0.01	0.01	ns
$C_{18:0}$	1.93±0.56	1.49±0.07	1.39±0.02	1.24±0.07	0.10	ns
$C_{18:1\ n-9}$	3.17±1.14^{a}	2.23±0.23^{ab}	2.15±0.12^{ab}	1.51±0.12^{b}	0.23	*
$C_{18:1\ n-7}$	0.42±0.07	0.52±0.07	0.47±014	0.44±0.13	0.03	ns
$C_{18:2\ n-6}$	16.97±1.28^{a}	14.75±1.46^{b}	12.36±0.90^{c}	11.90±0.90^{c}	0.68	*
$C_{18:3\ n-3}$	47.67±4.93	49.15±2.63	49.83±2.72	53.58±1.01	1.02	ns
$C_{18:4\ n-3}$	0.63±0.05	0.61±0.11	0.68±0.18	0.65±0.02	0.03	ns
Others	15.81±2.51^{a}	18.24±0.63^{ab}	21.32±2.13^{b}	19.2±0.97^{ab}	0.73	*

***Significant response at a 0.001 probability level. *Significant response at a 0.05 probability level. ns: not significant

Table 2: Chemical composition (g kg^{-1} DM) and gross energy (GE) of hemp at four morphological stages

Stage (Date of harvest)	Mid vegetative 17/07/2005	Late vegetative 23/07/2005	Shooting 30/07/2005	Early flower 06/08/2005	SEM	Stage effect
DM (g kg^{-1})	144.6±1.6^{a}	177.1±6.7^{b}	224.9±7.6^{c}	250.3±6.0^{d}	12.50	***
OM	849.1±7.9^{a}	876.5±3.8^{b}	896.0±7.1^{c}	889.5±6.7^{c}	5.60	***
Crude protein	193.4±13.1^{a}	151.6±6.8^{b}	147.8±6.2^{b}	145.0±15.8^{b}	6.60	*
Ash	150.9±7.9^{a}	123.5±3.8^{b}	104.1±7.1^{c}	110.5±6.7^{c}	5.60	***
NDF	444.7±4.5^{a}	478.6±7.8^{b}	484.2±7.9^{b}	465.5±26.2^{ab}	5.80	*
ADF	299.9±13.8	341.8±29.0	342.5±19.7	335.3±49.0	9.30	ns
ADL	70.2±0.4^{a}	71.6±1.8^{a}	74.9±2.3^{b}	77.0±0.7^{b}	0.90	*
GE (MJ/kg DM)	16.47±0.16^{a}	16.70±0.10^{a}	17.17±0.26^{b}	16.67±0.17^{a}	0.09	*

***Significant response at a 0.001 probability level. *Significant response at a 0.05 probability level. ns not significant

The chemical composition and GE of the whole hemp plant are reported in Table 2 for 4 different growth stages. The DM, Organic Matter (OM) and ADL content increased with increasing growth stage, while the ash and crude protein decreased from the mid vegetative stage to the shooting and to the late vegetative stage, respectively. NDF increased from the first to the third stage and then decreased, while ADL increased in the 2 last stages, which differed from the two vegetative stages studied, while ADF did not differ during growth. The increase in some fibrous fractions with increased stage of maturity is due to the progressive translocation of the soluble cell contents from the leaves and stems to the fruit. Among the structural carbohydrates, hemicellulose and cellulose (calculated as the difference between NDF and ADF and between ADF and ADL, respectively) ranged between 130 and 145 g kg^{-1} DM and between 230 and 270 g kg^{-1} DM, respectively.

The GE was higher at the shooting stage than the other stages: this difference was primarily due to variations in the ash content. Similar trends have been observed in other oilseed plants, such as borage (Peiretti *et al*., 2004a), evening primrose (Peiretti *et al*., 2004b) and chia (Peiretti and Gai, 2006).

To the best of the authors' knowledge, few studies exist regarding the composition of hemp plants during growth. Mediavilla *et al*. (2001) described how the fibre yield and the number of primary and secondary fibre cells depend on the growth stage of the plant, while Van der Werf *et al*. (1994b, 1995) demonstrated the effect of

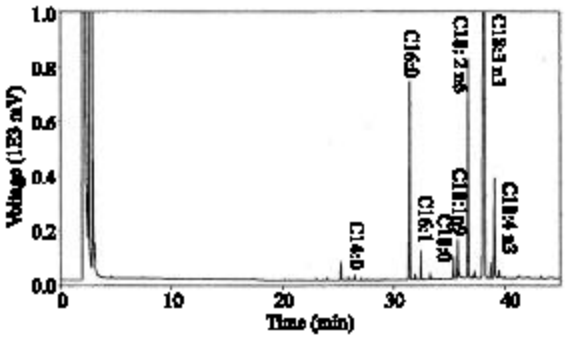

Fig. 1: Typical GC-Chromatogram of the identified fatty acid of a *Cannabis sativa* plant at mid vegetative stage ($C_{14:0}$ = myristic acid, $C_{16:0}$ = palmitic acid, $C_{16:1}$ = palmitoleic acid, $C_{18:0}$ = stearic acid, $C_{18:1\ n-9}$ = oleic acid, $C_{18:2\ n-6}$ = linoleic acid, $C_{18:3\ n-3}$ = α-linoleic acid, $C_{18:4\ n-3}$ = stearidonic acid)

daylength on yield and quality of fibre hemp and the effect of temperature on leaf appearance and canopy establishment in fibre hemp.

It is therefore, important to find the optimal harvest time, i.e., the growth stage to obtain mature fibres of optimized quality with the smallest amount of ADL possible (Mediavilla *et al*., 2001). Keller *et al*. (2001) showed there was no difference in the hemp bark, during the vegetative growth stage, between the ADL content in the bottom and the top parts, whereas the ADL content in the middle part decreased. With the start of flower formation, the ADL content increased more than during

the vegetative stage. The ADL content in the top part increased more than the content of the other 2 parts. During the change from vegetative to generative stage, the top part of the bark showed the highest ADL content, followed by the bottom and the middle part. The ADL content of the female plants increased more than the ADL content of the male plants. They concluded that the optimal harvest time is at the beginning of seed maturity, which is approximately 3-4 weeks after 'technical maturity'. In our experiment, the ADL content was higher than those found by Keller *et al.* (2001), because we took in account the whole plant instead of just the bark of the plant.

CONCLUSION

The FA profile and chemical composition of hemp are closely connected to the development of the plant. The composition of unsaturated fatty acids in the hemp consists of a high percentage of LNA, which represents half of the total FA. There was a lack of the GLA at all stages during the hemp growth, thus the whole plant, unlike the seeds, is not a source of GLA. Further studies, could be conducted on hemp collected from a wide geographical range and in different years to determine the DM yield and nutritional quality in order to have more complete information about this plant and its potential uses.

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