

## Evolution of Chemical Composition, Nutritive Value and Fatty Acid Content of Sunflower (*Helianthus annuus* L.) During the Growth Cycle

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**Abstract:** Sunflower (*Helianthus annuus* L.) has been studied to determine the chemical composition, Gross Energy (GE), *in vitro* Organic Matter Digestibility (IVOMD) and Fatty Acid (FA) content of the plant during the growth cycle. Herbage samples were collected five times at progressive morphological stages from the stem extension to the late flowering stage. The evolution of the whole sunflower plant quality during growth was characterised by a progressive increase in the dry matter, neutral detergent fibre and acid detergent fibre contents, while the ash, crude protein and ether extract contents decreased from the stem extension stage to the mid flowering stage and then increased. GE was higher at the mid and late flowering stages than at the other stages. IVOMD decreased with increasing growth stage with a mean decrease of 6 g kg<sup>-1</sup> OM day<sup>-1</sup>. The FA analysis disclosed quantitative differences between the plant stages, which were characterised by a high percentage of Polyunsaturated Fatty Acids (PUFA), that made up from 81-75% of the total FA of the plant during the growth cycle. The FA profiles in the plant were characterised by four dominant FAs: palmitic acid (C<sub>16:0</sub>), linoleic acid (C<sub>18:2 n-6</sub>),  $\alpha$ -linolenic acid (C<sub>18:3 n-3</sub>) and stearidonic acid (C<sub>18:4 n-3</sub>), which ranged from 10.0-12.8, 16.4-21.8, 54.9-44.6 and 6.5-8.8% of the total FA, respectively.

**Key words:** Crude protein, fibrous fraction, gross energy, lipid, organic matter digestibility

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### INTRODUCTION

Sunflower (*Helianthus annuus* L.), which originated in North America, is a short season plant that is potentially useful for seeds, oil and forage crops. Non-dehulled or partly dehulled sunflower meal has been used as a protein supplement for grazing sheep (Coombe *et al.*, 1987), lactating ewes and goats (Economides, 1998), lactating cows (Erasmus *et al.*, 1994) and beef steers (Thomas *et al.*, 1982). Sunflower meal is higher in fiber, has a lower energy value and is lower in lysine, but higher in methionine, than soybean meal. The protein percentage of sunflower meal ranges from 28%, for non-dehulled seeds, to 42% for completely dehulled seeds and it has also been successfully substituted for soybean meal in isonitrogenous diets for swine (Seerley *et al.*, 1974) and poultry feeding (Senkoylu and Dale, 1999).

Although, sunflower is generally planted for seed production, the green sunflower plant is used as silage and a forage source by livestock producers. Sunflower is known to be a drought tolerant crop and because of this property, it can be used as an alternative silage crop in both first and second crop production seasons when

irrigation is a limiting factor (Tan and Tumer, 1996). Sunflower silage or grazing may be the only alternative when seeds do not have sufficient time to mature, because late planted sunflowers may not reach maturity before the first killing frost. The ensiling and nutritional quality of whole crop sunflower depends on the stage of maturity at the harvest time (Tan and Tumer, 1996; Garcia, 2006; Toruk, 2003). Its low Dry Matter (DM) content at maturity stage creates ensiling difficulties and it requires wilting before ensiling. In order to obtain of good quality and high nutritive value silage, the crop should be cut at an appropriate stage of maturity (Goncalves *et al.*, 1999).

The nutritive value of sunflower silage is lower than that of corn silage (McGuffey and Schingoethe, 1980), but it is generally recognized to be adequate for dry cows, steers and low milk producers. Whole plant sunflower silage has slightly more Crude Protein (CP) and considerably more fat than corn silage on a DM basis (Putnam *et al.*, 1990; Gregorie, 2006). Generally, the CP of sunflower decreases and the lignin percentage increases after the flowering stage. The high level of lignin from the fibrous stalk is a disadvantage for sunflower silage since it reduces its nutritive value.

One of the most important factors for the successful utilisation of this crop for forage in dairy systems is the prediction of the harvest date and although information is available on the chemical composition and nutritional quality of sunflower silage, little or no information is available on the changes in the nutritive value and Fatty Acid (FA) content of whole plants during the growth season. The present research was designed to evaluate the effect of maturity stage on the chemical composition, IVOMD, GE and FA content of sunflower during the growth cycle.

**MATERIALS AND METHODS**

**Plant material and environmental conditions:** The study was conducted in the Western Po Valley near Cuneo, Italy (latitude 44°N, longitude 7°E). The stands were seeded on 20 May 2006 and no irrigations or fertilisers were applied after sowing. Herbage samples were collected with edging shears (0.1 m cutting width) at five progressive morphological stages from stem extension to the late flowering stage, on subplots of 2 m<sup>2</sup> randomly located in 3×10 m<sup>2</sup> plots with three replicates cut to a 1-2 cm stubble height. The sampling time ranged from June-July 2006. Sampling was not performed on rainy days and was carried out in the morning, only after the disappearance of dew.

**Chemical analysis:** Whole plant samples were immediately dried in a forced-draft oven to constant weight at 65°C in order to determine the 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 (AOAC, 1990), ash by ignition to 550°C, Ether Extract (EE) using the Soxhlet method (AOAC, 1990), Neutral Detergent Fibre (NDF) without sodium sulfite and α-amylase and Acid Detergent Fibre (ADF), as described by Van Soest *et al.* (1991) expressed exclusive of residual ash and lignin 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) while, the organic matter digestibility was determined according to the two-stage rumen fluid technique (Tilley and Terry, 1963).

**Fatty acid analysis:** Fresh samples of the whole plants were immediately frozen and then subsequently freeze-dried and ground to pass a 1 mm screen. Lipid extraction was performed on the 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 FA methyl esters were then determined by gas chromatography according to Peiretti and Meineri (2006).

**Statistical analysis:** The variability in FA and the herbage chemical composition of the samples harvested at five 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.

**RESULTS AND DISCUSSION**

**Crop quality:** The evolution of whole sunflower plant quality at the five different stages of development is reported in Table 1 and it is characterised by a progressive increase in the DM, NDF and ADF content, while, the ash, CP and ether extract decreased from the stem extension stage to the mid flowering stage and then increased at the late flowering stage. Lignin was lower at the early flowering stage than at the other stages. GE was higher at the mid and late flowering stages than at the other stages.

Due to the extremely low DM content (8.1% at the stem extension stage and 10.2% at the late flowering stage) sunflowers should be wilted for ensiling or grazed as an alternative.

In two successive years, Demarquilly and Andrieu (1972) studied green sunflower plants during their growth cycle between the head formation and kernel maturity

**Table 1: Chemical composition (percentage of DM) and Gross Energy (GE) of *Helianthus annuus* at five morphological stages**

Stages	Stem extension 25/06/2006*	Visible bud 01/07/2006*	Early flowering 08/07/2006*	Mid flowering 15/07/2006*	Late flowering 21/07/2006*	SEM	Stage effect
DM (%FM)	8.06	8.52	8.38	9.79	10.2	0.25	**
OM	82.2	83.2	83.2	89.4	85.6	0.78	**
Ash	17.8	16.8	16.8	10.6	14.4	0.78	**
Crude protein	16.2	14.0	13.7	7.38	7.63	0.97	*
Ether extract	3.28	2.65	2.09	1.51	1.88	0.17	*
NDF	34.7	37.9	38.2	42.2	44.9	0.97	*
ADF	29.8	33.0	34.5	39.8	42.6	1.3	*
Lignin	10.5	9.07	8.49	9.86	10.7	0.28	***
GE (MJ kg <sup>-1</sup> DM)	15.5	15.6	15.1	16.1	16.0	0.11	**

\*Date of harvest. \*Significant response at a 0.001 probability level. \*\*Significant response at a 0.01 probability level. \*\*\*Significant response at a 0.05 probability level

stage and reported that the DM content increased from 12-15 and from 10-25%, respectively and it slightly increased until the milk kernel stage and then increased rapidly due to the high dehydration of the leaves and water losses of the grains. The relative DM contributions from the different component parts of the plant developed regularly: it increased from 0 to 55-60% in the head and from 0 to 35-40% in the kernel.

The ideal DM content for ensiling sunflowers is between 30 and 40%, since low DM silages can cause an undesirable fermentation and excessive effluent seepage from the silo. Tan and Tumer (1996) ensiled sunflower at several stages of maturity and concluded that the final flowering stage was the best for silage making. Goncalves *et al.* (1999) evaluated different harvest times of four sunflower genotypes and found that the best harvest time for ensiling varied according to the genotype and was between 30 and 51 days after flowering. Demarquilly and Andrieu (1972) reported that the best sunflower silages were obtained by harvesting the plants when the kernels were well developed, probably because of their relatively high soluble carbohydrates contents (14%). Murphy (1978) evaluated sunflowers for use as a new crop in cool climates with short growing seasons in high inter mountainous areas, such as Central Oregon and showed that sunflowers have adapted to high inter mountainous conditions and that forage yields would be sufficient to warrant their use. The best use of sunflower silage is for livestock and for highly producing dairy cows. The forage quality could be improved by supplementing it with other forage alternatives such as corn silage, haylage, or hay. Demirel *et al.* (2008) found that high quality silages could be obtained from the green herbage of corn, sunflower alone or a sunflower-corn mixture and that a 50% sunflower-corn mixture ratio could produce very desirable results in terms of silage quality. They also found that the partial replacement of corn silage with sunflower silage did not affect the milk, fat or protein yield (Leite *et al.*, 2006).

The ash content of whole sunflower plants ranged from 17.8 and 10.6% during growth. These results are in agreement with Demarquilly and Andrieu (1972), who found values that ranged from 16 and 10%.

Sheaffer *et al.* (1977) determined the yield and chemical composition of whole oilseed plant and confectionery sunflowers. The sunflowers contained 45-56% ADF and 14-17% lignin.

***In vitro* organic matter digestibility:** The IVOMD decreased with increasing growth stage, with a mean decrease of 6 g kg<sup>-1</sup> OM day<sup>-1</sup> (Fig. 1); this decrease was approximately double that found in flax during the spring growth cycle (Peiretti and Meineri, 2008). This is due to the higher decrease in CP content during sunflower

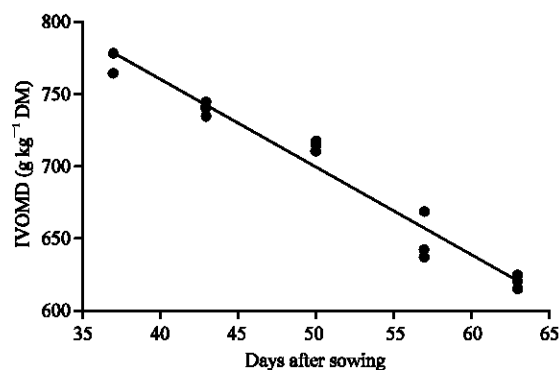


Fig. 1: *In vitro* OM Digestibility (IVOMD,  $-6.05 \text{ day}^{-1} + 1001$ ;  $r^2 = 0.97$ ) during the growth cycle of *Helianthus annuus*

maturation than in flax. However, Demarquilly and Andrieu (1972), measuring the digestibility of fresh or ensiled whole sunflowers in sheep, found that IVOMD was only slightly related to the CP or crude fiber content during the growth cycle. These authors found that the digestibility of the organic matter, which was about 75-77% at the time of heading, remained almost constant till the beginning of flowering, after which it decreased (0.35-0.40 points per day) over 5-6 weeks and then, from the dough kernel stage, stabilized again between 60 and 65%, according to the variety, while at the same time the crude fibre contents increased (on an average from 17-19% to about 28-30%), whereas the CP content decreased (from 16-18 to about 12%).

Valdez *et al.* (1988) found that the nutrient digestibility of intercropped silage was not adversely affected by the increased content of Ether Extract (EE) and the percentage of EE digestibility was greatest for corn-sunflower and sunflower silage, compared to the digestibility of corn silage.

Sheaffer *et al.* (1977) found that the *in vitro* Dry Matter Digestibility (IVDMD) of whole plant oilseed and confectionery sunflowers ranged from 63-69% and concluded that oilseed varieties had higher feeding value than confectionery varieties. Vandersall (1976) reported that dairy cows fed confectionery sunflower silage performed in a similar way to cows fed corn silage plus alfalfa hay.

**Fatty acid profile:** The FA analyses disclosed quantitative differences between the plant stages (Table 2) and they were characterised by a high percentage of Polyunsaturated Fatty Acids (PUFA), which made up from 81-75% of the total FA of the plant during the growth cycle. The FA profiles in the plant were characterised by four dominant Fas: palmitic acid (C<sub>16:0</sub>), linoleic acid (C<sub>18:2 n-6</sub>),  $\alpha$ -linolenic acid (ALA, C<sub>18:3 n-3</sub>) and stearidonic

Table 2: Fatty acid composition (percentage of total FA) of *Helianthus annuus* at five morphological stages

Stages	Stem extension 25/06/2006 <sup>a</sup>	Visible bud 01/07/2006 <sup>a</sup>	Early flowering 08/07/2006 <sup>a</sup>	Mid flowering 15/07/2006 <sup>a</sup>	Late flowering 21/07/2006 <sup>a</sup>	SEM	Stage effect
C <sub>14</sub>	0.268	0.153	0.183	0.190	0.225	0.014	**
C <sub>16</sub>	10.0	10.2	11.2	12.8	12.2	0.32	*
C <sub>18</sub>	0.998	0.903	0.923	1.08	1.16	0.039	NS
C <sub>18:1 n-9</sub>	1.74	1.66	1.73	2.13	1.92	0.070	NS
C <sub>18:1 n-7</sub>	0.270	0.253	0.323	0.390	0.370	0.016	**
C <sub>18:2 n-6</sub>	16.7	16.4	19.8	21.8	17.4	0.59	*
C <sub>18:3 n-6</sub>	1.16	0.510	1.30	1.65	1.51	0.19	NS
C <sub>18:3 n-3</sub>	54.9	55.9	48.2	44.6	48.1	1.3	*
C <sub>18:4 n-3</sub>	8.19	7.60	7.37	6.50	8.77	0.24	***
Others	5.75	6.43	8.93	8.86	8.39	0.48	***

<sup>a</sup>Date of harvest. <sup>b</sup>Not significant. \*Significant response at a 0.001 probability level. \*\*Significant response at a 0.01 probability level. \*\*\*Significant response at a 0.05 probability level

acid (SDA, C<sub>18:4 n-3</sub>), which ranged from 10.0-12.8, 16.4-21.8, 54.9-44.6 and 6.5-8.8% of the total FA, respectively.

ALA was the most abundant FA, as was also reported for evening primrose (Peiretti *et al.*, 2004a), false flax (Peiretti and Meineri, 2006), galega (Peiretti and Gai, 2006) and chia (Peiretti and Gai, 2009), over the entire studied growth cycle.

SDA was present in the whole plant at all growth stages and with similar percentages to those found in evening primrose (Peiretti *et al.*, 2004a) but lower percentages than those found in borage (Peiretti *et al.*, 2004b) or in flax (Peiretti and Meineri, 2008).

Izquierdo *et al.* (2002) found that an increment in the night temperature during fruit filling affected the FA composition in field grown plants and this effect was greater during the early stages of fruit filling. In general, the oleic acid percentage was higher with higher night temperatures and was not related to the minimum daily temperature. The largest variation in oleic acid percentage was observed in the traditional hybrid and the lowest in the high oleic hybrids. The higher oleic acid percentage was associated with an increase in night temperature early during fruit filling. The variation in the oleic acid percentage was related to the variation in the linoleic acid percentage in all the sunflower oil experiments, depending on the hybrid and the phenological stage. The effect of temperature during the dark period on FA composition indicates that light, or a metabolite associated with the day-night cycle, could affect the activity of the enzymes involved in the FA synthesis.

### CONCLUSION

The nutrient contents of the green sunflower plant depends on its stage of maturity and in order to obtain an optimal compromise between yield and nutritional value, the forage crops should be harvested at the early flowering stage, since the fibrous fractions and CP contents decrease and the nutritional quality deteriorates when cutting is delayed.

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