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# LEAF YIELD AND FATTY ACID COMPOSITION OF PURSLANE (*PORTULACA OLERACEA* L.) AT DIFFERENT GROWTH STAGES

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**Abstract:** Purslane is a nutritious vegetable crop rich in the polyunsaturated essential fatty acids (PUEFA), which are essential for normal human growth, health promotion and disease prevention. Fatty acid concentrations at different stages of harvest (6, 10 and 14 true-leaf stages) were examined in a cultivated variety. Fourteen-leaf stage of growth was found to be ideal for harvest because at this stage the leaf area, leaf weight and the PUEFA concentrations per gram leaf dry weight (DW) were higher than at the six and ten true-leaf stages of growth. The α-linolenic acid to linoleic acid ratio was also the highest at fourteen-leaf stage.

**Key words:** a-linolenic acid, essential fatty acids, linoleic acid, polyunsaturated fatty acids, purslane

## INTRODUCTION

Purslane (*Portulaca oleracea* L., Portulacaceae) is an important vegetable crop in Southern Europe, Mediterranean countries, and Asia (Rosse, 1955; Carleton, 1967). It is palatable and has a mild flavour. The tender stems and leaves can be eaten raw, cooked, or pickled. The leaves can also be frozen or dried and stored in jars for several years. The lipids present in this plant are rich in the polyunsaturated fatty acids, linoleic acid (LA) and a-linolenic acid (LNA). While both the LA and LNA are essential for normal growth, health and disease resistance in man, they belong to two different families of fatty acids - the LA to the omega-6 family and the LNA to the omega-3 family. Because of the distinctly different properties of these two groups of fatty acids and the prostaglandins (special group of hormones) derived from them, the ratio of these fatty acid families in the human diet is important (Gudbjarnason *et al.*, 1991; Simopoulos, 1999a). Simopoulos (1999a&b) brings to our attention the differences in properties of the omega-6 and omega-3 fatly acids and the importance of achieving a dietary balance in the range of 1:1, compared to the existing ratio of 20:1 in the modem diet. While most animal sources of the PUEFAs (except fish) have high concentrations of LA, they have very low concentrations of LNA and thus a low LNA/LA ratio (Simopoulos, 1986). It is important to identify food sources that have a higher ratio of LNA to LA, which is more desirable in the human diet.

It is reported that 100 g of fresh purslane leaves contains about 300-400 mg of LNA, 12.2 mg of α-tocopherol, 26.6 mg of ascorbic acid, 1.9 mg of β-carotene, and 14.8 mg of glutathione (Simopoulos *e1 al.*, 1986; Omara-Alwala *et al.*, 1991). In a series of studies, Palaniswamy *et al.* (1997, 1998, 2000) found that photosynthetic photon flux as well as nitrate to ammonium ratios in hydroponics enhanced the LNA to LA ratio in purslane leaves. At the University of Connecticut, USA during 1995, a pot experiment was conducted to study the pattern of growth, leaf production and fatty acid (FA) accumulation and the LNA to LA ratio in purslane.

## MATERIALS AND METHODS

*Variety:* Seeds of a green leaved purslane (Valley Seed Service, Fresno, Calif.) variety was used in this study.

*Season:* The experiment was conducted under greenhouse conditions (~18-20°C) during January-April, 1995.

*Pot culture:* Seeds were sown on 29 January 1995 in 288-count plug trays filled with commercial medium Metro 510 (O.M. Scotts, Marysville, Ohio). The plug trays were kept in the greenhouse at a temperature of ~18-20°C. The seedlings were irrigated as needed with tap water and thinned periodically to retain only one to two seedlings in each plug. The seedlings (21 day old) were transplanted on 19 February 1995 into 500 cm" square pots containing the same Metro 510. Plants were fertilized with nitrogen at 25 $\mu g.\ ml^{-1}$ for the first week and then 50 $\mu g\ ml^{-1}$ for the next week, followed by 100 $\mu g\ ml^{-1}$ until harvest using a 20 : 4.36 : 16.6 NPK water-soluble fertilizer in the irrigation water. The leaves were harvested at six, ten and fourteen true-leaf stages (at 35, 49 and 60 days after sowingrespectively).

*Statistical methods:* The pots were arranged in randomized complete block design in six replications. In each replication, there were six pots for every treatment. Totally there were 36 pots in the experiment. For each stage, the concentrations of palmitic acid, stearic acid, oleic acid, linoleic acid, α-linolenic acid, total fatty acids and the ratio of LNA to LA were determined. The relationship between the leaf area and fresh weight and leaf area and dry weight were studied by correlation analysis.

*Growth measurements:* At each harvest the dry weight (DW), fresh weight (FW) and the leaf area were determined for two plants that were randomly chosen for this purpose.

*Leaf area measurements:* The leaf area was determined using a planimeter (Model LI 3100, LI-COR Inc. Lincoln, NE). The terminal three nodes of shoots of the remaining four plants in each treatment were harvested at six, ten, and fourteen true-leaf stage and the leaves and stems were frozen separately (-59°C) until chemical analysis.

*Fatty acid analysis*

*(a) Extraction of lipids*: The lipids were extracted by the dry column method as described by Maxwell *et al.* (1980). Three to five g of plant tissue was ground in 25 ml dichloromethane (DCM): methanol (9:1) for 30 s using a homogenizer (Model - Power Gen. 125, Fisher Scientific). The homogenate was mixed with 4 g of anhydrous sodium sulfate) and passed through a 22 mm x 30 cm glass column (fitted at bottom with coarse fritted disc and stop-cock), packed with 2g 1: 9 calcium phosphate : Celite. The column was eluted with 150 ml DCM : methanol (9:1), for complete extraction of lipids. The eluate was collected in a 250 ml round-bottom flask and the solvent was removed in a rotary vacuum evaporator. The crude lipid was dissolved in 1 ml DCM and transferred to 2 ml glass screw cap vials, flushed with nitrogen and refrigerated.

*(b) Derivatistion*: The trans-esterification procedure given by Lepage and Roy (1986) was followed to methylate the fatty acids. A 100μl aliquot of the extracted lipid was transferred into a test tube. Two ml of the internal standard (100 $\mu g\ ml^{-1}$ of heptadecanoic acid in methanol : hexane 4:1) was added. Next, 200 μl acetyl chloride was added drop by drop, shaking the test tube after the addition of each drop. The test tubes were then sealed tightly with Teflon thread seal tape and kept in a heating module for 1 h at 100°C. The test tubes were allowed to cool, 5 ml of 6% $K_2CO_3$ was slowly added with a dispenser, mixed well and centrifuged at 5000 rpm for 10 min. The hydrophobic upper layer was transferred into small vials. The collected lipid layer was dried under a stream of nitrogen, dissolved in 50 μl DCM and injected into a gas chromatograph (Varian 6000, Palo Alto, CA).

*(c) Chromatographic conditions:* For the separation and analysis of the fatty acid methyl esters, a capillary column (Supelcowax, 10 stationary phase, 1 μm film thickness, 0.53 mm ID x 30 m; Supelco, Bellefonte, PA) was used. The gas chromatograph was fitted with a flame ionization detector. Air and hydrogen flows at the detector end were 300 and 30 ml $min^{-1}$ respectively. The carrier gas (nitrogen) flow rate was 1 ml $min^{-1}$. The column and the detector temperatures were at 200 and 240°C respectively. The column temperature was held at 190°C (for 1 min) and then increased to 235°C at the rate of 2°C $min^{-1}$ and held at that temperature for 1 min. The analysis time was ~30 min. The peak areas were determined with an integrator (HP 3395, Wilmington, DE), and identified by comparison of retention times with a standard (polyunsaturated fatty acid [PUFA-2], Matreya, Inc. Catalog No. 1081) separated under similar chromatographic conditions. The concentrations of the essential fatty acids were quantified based on the peak area of the compound relative to the peak area of the known quantity of the internal standard used per gram dry weight of the leaf.

## RESULTS AND DISCUSSION

Data on the mean fresh and dry weights of leaves and leaf area per plant at six, ten and fourteen true-leaf stages of purslane are presented in Table 1. The weight of leaves showed a steady increase with progress of time up to the 14 true-leaf and the effects were statistically significant. A substantial fresh leaf yield of 20.2 g per plant was obtained at the 14-leaf stage and this was about three times more than that at the 10-leaf stage and 17 times more than that at the six leaf

stage. As is expected, dry matter yield of leaves also showed the same trend. A similar trend was observed in case of leaf area of the plant. Significant positive correlation was observed between leaf dry weight and leaf area (r = 0.9842**) attributing the increase in leaf weight to increase in leaf area. The results showed that among the three growth stages investigated, the fourteen true-leaf stage was the ideal stage for obtaining the maximum leaf yield in purslane.

Table 1. Leaf yield and leaf area of purslane at different stages of growth

| Stage of growth (True-leaf stage) | Plant characteristics | | |
|---|---|---|---|
| | Fresh weight, g | Dry weight, g | Leaf area, $cm^2$ |
| 6 | 1.197 [a] | 0.0683 [a] | 16.85 [a] |
| 10 | 6.1380 [b] | 0.2500 [b] | 70.25 [b] |
| 14 | 20.1867 [c] | 0.9233 [c] | 197.30 [c] |
| SEm | 0.5138 | 0.0528 | 6.83 |
| LSD (P≤0.05) | 1.3166 | 0.1352 | 17.5025 |
| CV (%) | 13.7 | 31.2 | 17.4 |

Any two means followed by the same letter are not statistically different

The fatty acid profile of lipids in purslane at six, 10, and 14 true-leaf stages is given in Table 2. The levels of the five fatty acids studied namely palmitic acid, stearic acid, oleic acid, linoleic acid, α-linolenic acid, and also the total fatty acids and the LNA : LA ratio, exhibited significant differences with advancement of growth. Mohamed and Hussein (1994) who studied the nutritional aspects in purslane at three crop growth stages reported significant increases in total solids and protein in purslane harvested at three growth stages during plant development. They observed that while purslane leaves had the highest amount of protein in the third growth stage (59 days after emergence), soluble carbohydrate was significantly higher in growth stage 1 (30 days after emergence) and stage 2 (49 days after emergence). Since the observation stages of Mohammad and Hussein (1994) correspond to those in this study, the differences in fatty acids concentrations reported in this study may be explained in light of their observation that higher soluble carbohydrates at the six-leaf stage and higher protein at the 14 true leaf stage probably provided the substrate for the production of high PUEFA in the leaves. It can also be speculated that the decrease in the fatty acid concentrations at 10-leaf stage may be due to a decreased fatty acids synthesis or greater use of the fatty acids synthesized for growth and development at that stage. Higher fresh and dry

Table 2. Fatty acid composition of purslane lipids at different stages of growth

| Stage of harvest (true-leaf stage) | Fatty acids composition, mg $g^{-1}$ (dry weight) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Palmitic acid | Stearic acid | Oleic acid | Linoleic acid (LA) | α-Linolenic acid (LNA) | Total FAs | LNA/LA ratio |
| 6 | 5.9067 [a] | 0.733 [a] | 1.8150 [a] | 5.9750 [a] | 23.1983 [a] | 37.6283 [a] | 3.8967 [c] |
| 10 | 4.4717 [b] | 0.495 [b] | 1.2883 [b] | 3.9200 [b] | 17.8967 [b] | 28.0717 [b] | 4.6133 [b] |
| 14 | 5.7283 [a] | 0.655 [a] | 1.5983 [a] | 5.2783 [a] | 27.1883 [a] | 40.4483 [a] | 5.1383 [a] |
| SEm | 0.3627 | 0.0346 | 0.0990 | 0.2911 | 2.4494 | 2.1575 | 0.0825 |
| LSD (P≤0.05) | 1.1430 | 0.1091 | 0.3121 | 0.9171 | 4.9799 | 6.7986 | 0.2599 |
| CV(%) | 16.5 | 13.5 | 15.5 | 14.1 | 17.0 | 14.9 | 4.4 |

Any two means followed by the same letter arc not statistically different

weights as well as greater leaf area may also be due to higher protein at 14-leaf stage. No difference was observed among fatty acids content in six and 14-leaf stages. These results show that harvesting purslane crop at 14-leaf stage of development not only produced a crop of greater

yield, but also a crop of greater nutritional value with higher concentrations of PUEFA and a higher ratio of LNA to LA which is more desirable in human diet. This is in agreement with Palaniswamy *et al.* (1998) who reported that the i'atty acids content could be increased by adopting proper cultural practices and environmental conditions in purslane crop production.

The results of these studies provide evidence to the fact that the cultivation practices in crop production such as the harvest stage can significantly influence the nutritional value of purslane leaves used for consumption. However, a more complete study is required to study the PUEFA concentrations in purslane leaves after the 14-leaf stage and throughout the ontogeny of the plant growth and development.

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