Stage of Harvest and Polyunsaturated Essential Fatty Acid Concentrations in Purslane (*Portulaca oleraceae*) Leaves

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Purslane is a nutritious vegetable crop rich in the polyunsaturated essential fatty acids (PUEFA) α-linolenic acid (LNA) and linoleic acid (LA), which are essential for normal human growth, health promotion, and disease prevention. Total lipids and fatty acid concentrations at three stages of harvest (6-, 10-, and 14-true-leaf stages) were examined in a cultivated variety of purslane (*Portulaca oleraceae* L. var. *sativa*). The 14-true-leaf stage of growth was found to be ideal for harvest because at this stage the leaf area, shoot fresh weight, shoot dry weight, and PUEFA concentrations per gram of leaf fresh weight were higher ($P \leq 0.05$) than at the 6- and 10-true-leaf stages of growth. The LNA to LA ratio was also highest at the 14-true-leaf stage.

Keywords: *Plant growth; α-linolenic acid; linoleic acid; omega-3 fatty acids*

INTRODUCTION

Purslane (*Portulaca oleraceae*; Portulacaceae) is an important vegetable crop in southern Europe, Mediterranean countries, and Asia (*1*, *2*). It is palatable and has a mild flavor. The tender stems and leaves can be eaten raw, cooked, or pickled. Interest in cultivating purslane as a food crop in the United States has been stimulated since its identification as a rich source of omega-3 fatty acids (ω3FA) and antioxidants (*3*, *4*). One hundred grams of fresh purslane leaves (about one serving) can supply up to 300−400 mg of α-linolenic acid (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 (*4*).

The lipids present in purslane are rich in the polyunsaturated essential fatty acids (PUEFA) linoleic acid (LA) and α-linolenic acid (LNA). Although both LA and LNA are essential for normal growth, health promotion, and disease resistance in man, they belong to two different families−LNA to the ω3 family and LA to the ω6 family. Because of the distinctly different properties of these two groups of fatty acids and the prostaglandins derived from them, the ratio of these fatty acid families in the human diet is important (*5*−*7*). Numerous investigators have described the essentiality of ω3FAs in human health, the role of ω3FAs in health promotion and disease prevention, the need for a higher ω3FA/ω6FA ratio in the diet, and the importance of achieving a dietary balance in the range of 1:1−2 compared to the ratio of 1:20−30 that appears to be typical of the modern Western diet (*4*, *6*−*9*).

Although most animal sources of PUEFAs (except fish) have high concentrations of LA, they have very low concentrations of LNA and thus a low LNA/LA ratio (*10*). Because plant leaves are richer in LNA than LA, they provide a more desirable LNA/LA ratio than most animal meats. Identifying newer sources and enriching or optimizing the ω3FAs in known plant sources offer us ways of increasing the availability of ω3FAs in the food supply.

Purslane has been studied in detail as a prolific weed (*11*), but very little is known about its production as a food crop and the effects of cultural conditions on its nutritional value (total lipids and PUEFA concentrations). More recently, Mohamed and Hussein (*12*) analyzed the chemical composition of purslane whole plants, leaves, stems, and roots harvested at different developmental stages but did not examine the essential fatty acid concentrations.

Previous studies (*4*, *13*) demonstrated that cultural conditions can influence the ω3FAs concentrations in purslane leaves. However, the harvest stages in these studies were based on the number of days from transplantation and do not specifically characterize the developmental stage of the plant at harvest. Indeed, the physiological stage of development of plants at a given number of days after sowing or transplanting can vary greatly depending on the environmental and cultural conditions and hence may in fact refer to plants at different stages of development.

Therefore, we grew purslane in the greenhouse, harvested the leaves at 6-, 10-, and 14-true-leaf stages, and analyzed the leaves for total lipids and PUEFA concentrations. This is a part of a larger study to identify and characterize the environmental conditions that can optimize PUEFA in purslane leaves. The objective of our study was to determine the PUEFA concentrations in purslane leaves at 6-, 10-, and 14-true-leaf stages and examine the ω3FA/ω6FA ratios in these stages.

MATERIALS AND METHODS

Plant Materials. The experiment was conducted under greenhouse conditions (∼18−20 °C) during January−April of 1995. Seeds of a cultivated green-leafed purslane (*Portulaca oleracea* L. var. *sativa*) (Valley Seed Service, Fresno, CA) were

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sown in 1 cm × 1 cm cell size seedling trays filled with commercial medium Metro 510 (O. M. Scotts, Marysville, OH). The seedling trays were kept in the greenhouse at a temperature of ~18−20 °C, irrigated as needed with tap water, and thinned periodically to retain only one or two seedlings in each cell. The seedlings (21 days old) were transplanted into 500 cm^3 square pots containing the same Metro 510. Plants were fertilized three times a week with nitrogen at 100 μg/mL until harvest using a 20 N−4.36 P−16.6 K water-soluble fertilizer in the irrigation water. The terminal three nodes of shoots were harvested at 6-, 10-, and 14-true-leaf stages, and the leaves and stems were frozen separately (−59 °C) until chemical analysis. At each harvest the shoot fresh weight (FW), shoot dry weight (DW), and leaf area were determined. The leaf area was determined using a planimeter (model LI 3100, LI-COR Inc., Lincoln, NE).

Experimental Design and Data Analysis. Plants were arranged in a randomized complete block design with six replications. There were 12 plants in each treatment. Data were analyzed using the SAS General Linear Models procedure (*14*).

Fatty Acids Extraction. The lipids were extracted according to the dry column method (*15*). Three to five grams of plant tissue was ground in 25 mL of dichloromethane (DCM)/methanol [9:1 (v/v)] with a homogenizer (model Power Gen. 125, Fisher Scientific) for 30 s. The homogenate was mixed with 4 g of anhydrous sodium sulfate (a drying agent) and passed through a 22 mm × 30 cm glass column (fitted at the bottom with a coarse fritted disk and stopcock), packed with 1:9 (w/w) calcium phosphate/Celite. The column was flushed with 150 mL of DCM/methanol [9:1 (v/v)] for complete extraction of lipids from the homogenized plant tissue. The filtrate was collected into 250 mL round-bottom flasks and completely vacuum-dried in a rotary evaporator. The dried lipid fraction was dissolved in 1 mL of DCM and transferred to 2 mL glass screw-cap vials, flushed with nitrogen, and refrigerated.

Transesterification Procedure. The transesterification procedure given by Lepage and Roy (*16*) was followed to methylate the fatty acids. A 100 μL aliquot of the extracted lipid was transferred into a test tube. Two milliliters of the internal standard [100 μg/mL of heptadecanoic acid (17:0) in methanol/hexane [4:1 (v/v)]] was added. Next, 200 μL of acetyl chloride was added drop by drop the test tube was shaken after the addition of each drop. The test tubes were then sealed 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 and mixed well, and the tubes were centrifuged at 5000 rpm for 10 min. The hydrophobic upper layer was transferred into small vials. The collected lipid layer was dried under nitrogen completely, dissolved in 50 μL of DCM, and injected into a gas chromatograph (Varian 6000, Palo Alto, CA).

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 i.d. × 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, respectively. The carrier gas (nitrogen) flow rate was 1 mL/min. The column and ion temperatures were 200 and 240 °C, respectively. A column temperature gradient from 190 °C (held for 1 min) to 235 °C (held for 1 min) at 2 °C/min was used. The analysis time was ~30 min. The peak areas were determined with an integrator (Hewlett-Packard 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.

RESULTS

The shoot fresh weight (FW), shoot dry weight (DW), and leaf area increased with increasing number of true leaves at harvest (Table 1). The FW, DW, and leaf area were highest at the 14-true-leaf stage and lowest at the 6-true-leaf stage.

Table 1. Plant Growth Characteristics of a Cultivated Variety of Purslane at Three Stages of Harvest

stage of harvest (no. of true leaves)	shoot FW[a]	shoot DW[a]	leaf area[a] (cm^2)
6	1.2 ± 0.1 c	0.1 ± 0.01 c	16.9 ± 1.2 c
10	6.1 ± 0.6 b	0.3 ± 0.03 b	70.3 ± 6.8 b
14	20.2 ± 0.6 a	0.9 ± 0.1 a	197.3 ± 8.4 a

[a] Data are means of six replications. Mean separation within columns by LSD ($P \leq 0.05$).

The fatty acid species identified from the leaf extracts were palmitic, stearic, oleic, linoleic, and α-linolenic acids (Table-2). The total fatty acids (calculated in this study as the sum of palmitic, stearic, oleic, linoleic, and α-linolenic acids) as well as the individual fatty acids concentrations in purslane leaves varied with the stage of plant development. At the 6- and 14-true-leaf stages, PUEFA concentrations in purslane leaves were 30−52% higher than in leaves harvested at the 10-true-leaf stage.

Although the total fatty acids and the PUEFA concentrations at the 6- and 14-true-leaf stages did not differ significantly from each other, the leaves harvested at the 14-true-leaf stage had 29% lower stearic acid (a saturated fatty acid) and oleic acid (a monounsaturated fatty acid) (Table 2). The ω3FA/ω6FA ratio was highest at the 14-true-leaf stage and lowest at the 6-true-leaf stage (Table 2).

DISCUSSION

It is evident from our study that fatty acid concentrations in purslane leaves vary with the number of true leaves at the time of harvest. Whereas others have shown that purslane is an excellent source of ω3FAs (*4*, *9*, *13*), our study indicates that the stage of development at harvest can affect the nutritional value of the harvested crop. Our results show that although purslane leaves harvested at the 14-true-leaf stage had PUEFA concentrations similar to those of the leaves harvested at the 6-true-leaf stage, they were of greater nutritional value because of their higher ω3FA/ω6FA ratio as compared with the leaves harvested at the 6-true-leaf stage (Table 1). Leaves harvested at the 14-true-leaf stage also had lower concentrations of saturated and monounsaturated fatty acids compared to the leaves harvested at the 6-true-leaf stage. Additionally, the leaf yield and hence the PUEFA yield per plant were greater in the plants at the 14-true-leaf stage, because of greater FW, DW, and leaf area (Tables 1 and 2) compared with the plants at the 6- and 10-true-leaf stages.

Our results are consistent with those of Omara-Alwala et al. (*13*), who reported significant differences in fatty acid concentrations and in ω3FA/ω6FA ratios in purslane leaves at “different ages” after planting. However, they also reported the presence of other ω3FAs—eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA)—in purslane leaves. In our study we did not detect EPA, DPA, and DHA, which is in agreement with the study by Liu et al. (*9*), who noted that in fact Omara-Alwala et al. (*13*) did not confirm the presence of EPA, DPA, and DHA by MS.

Mohamed and Hussein (*12*) reported significant increases in total solids and protein in purslane harvested at three growth stages during plant development. They

Table 2. Fatty Acid Concentrations of Purslane Harvested at Three Stages of Growth[a]

stage of harvest (no. of true leaves)	fatty acid composition (mg/100 g of leaf FW)						
	palmitic acid	stearic acid	oleic acid	LA	LNA	total	LNA/LA
6	33.2 ± 4.4 a	4.1 ± 0.7 a	10.2 ± 1.5 a	34.0 ± 5.2 a	132.8 ± 22.0 a	214.2 ± 33.3 a	3.9 ± 0.2 c
10	18.3 ± 2.3 b	2.2 ± 0.3 b	4.7 ± 0.2 b	15.5 ± 1.0 b	69.7 ± 3.8 b	110.4 ± 7.1 b	4.5 ± 0.1 b
14	25.7 ± 2.2 ab	2.9 ± 0.2 b	7.2 ± 0.7 b	24.0 ± 2.2 ab	124.7 ± 11.3 a	184.6 ± 16.4 a	5.2 ± 0.03 a

[a] Results represent mean values ($n = 10$) of samples extracted and analyzed in duplicates. Mean separation by LSD ($P \leq 0.05$). Means within columns followed by the same letter are not significantly different.

observed that purslane leaves had the highest amount of protein in the third growth stage (59 days after emergence) and significantly higher soluble carbohydrates in growth stage 1 (30 days after emergence) and growth stage 2 (49 days after emergence). Fatty acid concentrations were not reported in this study. In our study, plants were harvested 35, 49, and 60 days after sowing or at intervals comparable to the harvest days described by Mohamed and Hussein. On the basis of Mohamed and Hussein's data, one could speculate that a higher amount of protein at the 14-true-leaf stage supported the greater accumulation of higher PUEFA concentrations, and perhaps the lower soluble carbohydrates at this stage decreased the saturated and monounsaturated fatty acids concentrations.

Lipids are important structural constituents of membranes involved in maintaining cell and organelle integrity and composition, energy transduction, carbon and energy storage, and regulation of plant development (*17*). The decrease in concentration of essential fatty acids at the 10-true-leaf stage compared to the 6-true-leaf stage is perhaps due to higher photosynthetic and growth rates, which may utilize the fatty acids synthesized in the chloroplast and/or used to repair the cell membrane damage during greater oxidative processes such as photosynthesis and active growth.

LNA in purslane has been differentially quantified by several authors (in mg/100 g of FW)—10−29 (*13*), 300−400 (*4*), 97−160 (*9*), and 18−30 (*18*). The LNA concentration in our study ranged from 70 to 133 mg/100 g of FW, a range comparable to that reported by Liu et al. (*9*), which is ~3−5 times lower than that reported by Simopoulos et al. (*4*). This difference in concentrations reported by others (*4*, *13*, *18*) may be due to differences in plant cultivars, sample material, sampling procedures, and/or analytical methods including sample preparation procedures and chromatographic conditions.

Previously Palaniswamy et al. (*19*−*21*) reported that PUEFA concentrations in purslane increased as much as 2−3-fold under specific nutritional or environmental conditions. The results of our study indicate that the optimization of PUEFA concentrations in purslane also requires harvesting plants at a suitable stage of development.

The current trend in crop production is "phytochemically oriented plant cultivation", aspiring to a specific phytochemical composition of the harvested crop (*22*), and offers a viable solution to increasing phytoceuticals and nutraceuticals in the food supply and dietary intake for health promotion and disease prevention. Our study specifically identified the developmental stage of harvest as an important factor influencing the concentrations of PUEFA in purslane leaves that will be a useful pointer for growers to consider while manipulating the nutritional value of the harvested produce. However, a more detailed study is required to determine the variation of fatty acids in purslane throughout its ontogeny and to suggest more specific "best production practices" for the optimization of PUEFA in purslane.

SAFETY

Polyunsaturated fatty acids are highly susceptible to oxidation by air. At every step of plant extraction, sample preparation, and storage, precautionary measures should be taken to protect them from atmospheric exposure.

ABBREVIATIONS USED

DCM, dichloromethane; DW, dry weight; FW, fresh weight; LNA, α-linolenic acid; LA, linoleic acid; PUEFA, polyunsaturated essential fatty acids.

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