Introduction

Essential fatty acids, both ω6 (linoleic acid- LA) and ω3 (α-linolenic acid- LNA), have been part of the diet since the beginning of human life. It appears that human beings evolved consuming a diet that was much lower in saturated fatty acids than is today's diet (Eaton & Konner, 1985). However, over the past 150 years the balance between ω6 and ω3 in our diet has been upset. The current Western diet is very high in ω6 fatty acids (the ratio of ω6 to ω3 fatty acids is 20–30:1 as compared to a desired ratio of 1-2:1). Intake of ω3 fatty acids is much lower today because of the decrease in fish consumption and the industrial production of animal feeds rich in grains containing ω6 fatty acids, leading to production of meat rich in ω6 and poor in ω3 fatty acids (Crawford, 1968). The same is true for cultured fish (Vanvliet & Katan 1990) and eggs (Simopoulos, 1989). Even cultivated vegetables contain less ω3 fatty acids than do plants in the wild (Simopoulos, 2004). Thus, modern agriculture, with its emphasis on production, has decreased the ω3 fatty acid content in many foods. Alternative sources of PUFA are therefore desirable, and the concept of obtaining them from higher plants in commercial and sustainable quantities is particularly attractive. Purslane (*Portulaca oleracea* L.) is the richest source of ω3 PUFAs of any terrestrial green leafy vegetable yet examined (Simopoulos et al., 1992, Ezekwe et al, 1999, Palaniswamy, 2001; Hites et al., 2004), with an extremely good ratio of ω6 to ω3 fatty acids as well as antioxidants, such as α-tocopherol, ascorbic acid, β-carotene and gluthathione, minerals, vitamins and proteins (Simopoulos, 2004). Purslane is a heat- and drought-tolerant plant, and is an important vegetable crop in southern Europe and Asia. It is eaten fresh, cooked or dried and interest in cultivating it as a food crop has increased all over the world in recent years since its identification as a rich source of ω3 PUFAs and antioxidants (Su et al., 2004, Simopoulos, 2008). Moreover, purslane is promising for providing both, novel biologically active substances and essential compounds for human nutrition. The increased interest in the potential health benefits associated with the consumption of long-chain ω-3 fatty acids has led to the sale of supplements and fortified foods containing these fatty acids. In these contexts the identification of functional foods, like purslane, that could be suitable for human consumption it is very important.

Materials and Methods

Plant Material

Twenty one days-old seedlings of purslane cultivars of "Golden leave" (GL) and "Green leaves" (GR) were transplanted into a closed hydroponic system in the greenhouse. Nitrogen at 200 μg ml^{-1} was providing as NO_3^- and NH_4^+ forms to yield NO_3: NH_4^+ ratio of 1:1. Five saline treatments were applied: 20, 40, 60, 90, and 120 mmol NaCl; the control had only the base nutritive solution. Eight plants of each cultivar were randomly placed in each tank, fulfilling a total 8 plants for each treatment replication. The nutrient solutions in the hydroponic system were aerated for 15 minutes every 2 hours using a time-controlled air bubbler. The solution pH was monitorized and maintained at 6.6-6.8 by adding 0.5 M HCl or NaOH as needed. Two plants were randomly selected and harvest from each treatment replication after 15 and 30 days of saline treatment exposure. Samples of those plants were stored and used in determination of total oxalic acid and fatty acids composition.

Total oxalic acid quantification

Total oxalic acid concentration was determined in the leaves of purslane plants submitted to 15 and 30 days of saline treatment exposure. All determinations were

conducted in four replications, by the procedures described by Savage *et al* (2000). The oxalic acid peak was identified comparing with the retention time of standard organic acids. All blank and standard solutions were filtered throw a 0.45 mm cellulose acetate membrane syringe filter prior to analysis.

Fatty acids determination

Fatty acid composition was determined in leaves of purslane plants exposed to saline treatments for 30 days. A sample of 50 g of fresh tissue leaves was dehydrated in a incubator at 60 ºC for 48 h. The dehydrated tissue was macerated and stored in 15 ml vials at room temperature, protected from light. An aliquot of 1 g of each sample was used to determine fatty acid composition, according with the procedures described by Liu, *et al.* (2000). After hexane extraction, the samples were vortex-mixed, centrifuged, and the upper phase was collected prior to GC analysis. Samples (1µl) were injected via an auto sampler onto a fused-silica capillary column (Supelco; Omegawax 250; 30 m x 0.25 mm I.D., 30 µm film thickness) in a HP 6890 gas chromatograph (GC-MS) system fitted with a flame ionization detector and eluted with helium (He) at 44.0 ± 1 ml/min, with a split ratio of 1:17. The injector and detector were heated to 250 ºC. The column was temperature programmed from 130 ºC (hold 1 min) to 180 ºC at 25 ºC/min, and then to 230 ºC (old 7 min) at 2.5 ºC/min. Fatty acid methyl esters were identified by comparing GC retention times with those of a mixture of standard fatty acids methyl esters (FAMEs) Mix C14-C22, (Supelco). Fatty acids were quantified using peak areas integration against internal standard.

Results and Discussion

Total oxalic acid content of leaves of purslane exposed to saline stress solutions

Total oxalic acid (TOA) concentrations (means) from all samples are summarized in **Table 1**, affected by the respective standard error values. The results presented on **Table 1** suggest that for both cultivars a decrease on total oxalic acid concentration occurs when the salt stress concentration increase in the hydroponic solution. The decrease of oxalic acid accumulation on the leaves can be associated to a competitive accumulation between oxalic acid with chloride ion, since the chloride ion it has been observed in previous studies (Teixeira & Carvalho, 2009) that this plant accumulates chloride ions in the leaves when submitted to saline stress. Those results could be very interesting in nutritional point of view hence TOA must be reduced in human diets.

Table 1. Total oxalic acid concentration in *P. oleracea* leaves exposed to 15 and 30 days of saline stress

[NaCl] mmol	[oxalic acid] mg/g DW			
	Golden Leaf (GL)		Green Leaf (GR)	
	15 days	30 days	15 days	30 days
0	79.94 ± 4.900	113.78 ± 4.980	63.58 ± 3.976	111.86 ± 4.316
20	66.29 ± 1.487	79.56 ± 2.805	65.56 ± 5.925	117.88 ± 7.075
40	49.75 ± 2.606	64.64 ± 6.327	60.59 ± 4.403	92.80 ± 3.137
60	36.57 ± 2.799	62.30 ± 4.068	44.73 ± 2.596	73.64 ± 8.665
90	29.08 ± 4.076	55.22 ± 4.373	37.09 ± 2.669	43.78 ± 1.707
120	26.73 ± 4.323	32.72 ± 4.607	21.43 ± 0.469	41.47 ± 2.575

Data are means±S.E. (n=4).

Fatty acid content acid content of leaves of purslane exposed to saline stress solutions

The FAMEs identified in purslane leaves were palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), araquidonic

acid (C20:0), and behemic acid (C22:0). The results obtain in this analysis are summarized in **Table 2**. In general, the total amount of fatty acids did not significantly change with increasing saline stress but slightly increased with the increase of NaCl concentration until 40 mmol of NaCl, decreasing for higher concentration of salt in both purslanes.

In GL and GR cultivars, submitted to 30 days of saline treatment, the main fatty acids detected were C16:0, C18:3 and C18:2. Such high amounts of palmitic acid, has it was found in the *P. oleracea* leaves of control plants is unusual, previous studies reported an proportion of 17% of this fatty acid in the total FAMEs detected (Liu *et al*., 2000). Palmitic acid, the most abundant fatty acid in the human diet, causes oxidative DNA damage, DNA strand breakage, necrosis and apoptosis in human cells in vitro, but when consumed with others fatty acids, like PUFAs, is unlikely to have any significant impact on human health. The amount of unsaturated fatty acids (UFAs) was also a little bit lower than expected, although the proportions between UFAs and SFAs were maintained (**Table 3**); it has been reported in other studies (Palaniswany *et al.* 2001, Liu *et al*. 2000 & Ezekwe *et al.*, 1999) that the linolenic acid content in *P. oleracea* leaves was circa 50% of total fatty acids and linoleic acid content 3- 4 times lower. The lipids present in purslane are rich in the PUFAs linoleic acid and linolenic acid. Although both fatty acids are essential for normal growth, health promotion, and disease resistance in man, they belong to two different families, C18:3 to the ω3 family and C18:2 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 acids families in the human diet is important (Palaniswamy et al., 2001).

Table 2. Fatty acid composition of *P. oleracea* leaves of plants submitted to 30 days of saline treatment exposure.

		[Fatty Acids] mg FAME/g DW							
[NaCl] mmol	Cultivar	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C22:0	TOTAL
0	GL	19.13 ± 3.546	1.32 ± 0.072	1.53 ± 0.163	5.07 ± 0.572	16.13 ± 1.411	0.49 ± 0.045	0.39 ± 0.071	45.57 ± 2.667
	GR	27.37 ± 0.097	1.59 ± 0.332	1.59 ± 0.253	5.31 ± 0.255	16.24 ± 0.783	0.45 ± 0.150	0.39 ± 0.108	54.46 ± 0.275
20	GL	21.89 ± 5.136	1.19 ± 0.114	1.56 ± 0.041	5.49 ± 0.286	16.70 ± 0.847	0.49 ± 0.098	0.65 ± 0.045	49.54 ± 4.130
	GR	26.45 ± 1.451	1.23 ± 0.095	1.23 ± 0.057	4.66 ± 0.476	16.39 ± 0.460	0.49 ± 0.011	0.41 ± 0.156	52.52 ± 2.004
40	GL	24.37 ± 2.500	1.18 ± 0.129	1.53 ± 0.149	6.08 ± 0.549	16.96 ± 0.740	0.53 ± 0.041	0.69 ± 0.033	52.95 ± 3.095
	GR	26.87 ± 0.578	1.20 ± 0.106	1.20 ± 0.136	4.68 ± 0.398	16.43 ± 0.841	0.49 ± 0.072	0.48 ± 0.071	52.68 ± 1.573
60	GL	24.39 ± 0.952	1.04 ± 0.077	1.38 ± 0.074	4.93 ± 0.271	15.18 ± 0.770	0.63 ± 0.098	0.79 ± 0.077	49.73 ± 1.341
	GR	24.15 ± 0.191	1.11 ± 0.046	1.11 ± 0.097	4.55 ± 0.273	16.07 ± 1.464	0.51 ± 0.058	0.75 ± 0.040	49.50 ± 1.619
90	GL	24.58 ± 3.223	1.02 ± 0.263	0.95 ± 0.282	4.65 ± 0.159	14.48 ± 0.686	0.71 ± 0.062	0.81 ± 0.071	48.14 ± 2.336
	GR	23.94 ± 0.790	1.11 ± 0.203	1.11 ± 0.080	4.52 ± 0.328	14.54 ± 0.204	0.78 ± 0.042	0.77 ± 0.117	47.98 ± 2.684
120	GL	24.76 ± 1.776	0.90 ± 0.080	1.01 ± 0.266	4.44 ± 0.658	11.86 ± 0.970	0.71 ± 0.032	0.88 ± 0.026	45.56 ± 2.051
	GR	17.50 ± 1.031	1.06 ± 0.013	1.06 ± 0.080	4.03 ± 0.059	13.06 ± 2.217	0.85 ± 0.048	0.95 ± 0.118	39.72 ± 2.684

Data are means±S.E. (n=4).

The ratio of ω6/ ω3 and total unsaturated to total saturated fatty acids ranged from 0.31 to 0.33 and 4.71 to 3.03 respectively for GL and GR cultivars in control plants (**Table 3**). Those values did not significantly differ as a result of increasing saline stress in hydroponic solutions. In nutritional point of view, a lower ratio of ω6/ ω3 fatty acids is more desirable, once, is better in reducing the risk of many of the chronic diseases of high prevalence in Western societies, as well as, in the developing countries (Simopoulos, 2008). Both purslane leaves have a higher amount of ω3 FA family, corresponding to a very good ratio ω6/ ω3. Relatively large reserves of C18:2 in body fat would tend to slow down the formation of long chain ω3 fatty acids from C18:3. Therefore, the role of C18:3 in human nutrition become important in terms of long term dietary intake.

Our study specifically identified the saline stress as an important factor influencing the concentrations of TOA and the concentrations of UFA but not SFA in purslane leaves that will be a useful pointer for growers to consider while manipulating the nutritional value of the harvested produce. In summary, both cultivars did not differ significantly in their fatty acids content and both are very good source of PUFAs manly linoleic and linolenic acid with about 3 times more ω3 FA family relatively to ω6 FA family. Sever saline stress slightly decrease PUFAs and MUFAs concentration but did not affect SFA. Growing cultivated purslane with saline stress until a maximum of 40 mmol of salt concentration may improve their fatty acid profile. This study could contribute to the assumption that human consumption of purslane will contribute to ω3 PUFA intake, with consequential benefit to health.

Table 3. Unsaturated/ Saturated fatty acids and ω 6/ ω 3 ratio of *P. oleracea* leaves of plants submitted to 30 days of saline treatment exposure.

[NaCl] mmol	Cultivar	Unsaturated/ Saturated	ω 6 fatty acids	ω 3 fatty acids	ω 6/ ω 3
0	GL	4.71	5.07	16.13	0.31
	GR	3.03	5.31	16.24	0.33
20	GL	2.86	5.49	16.70	0.33
	GR	3.41	4.66	16.39	0.28
40	GL	2.34	6.08	16.96	0.36
	GR	2.94	4.68	16.43	0.28
60	GL	1.70	4.93	15.18	0.32
	GR	2.12	4.55	16.07	0.28
90	GL	1.39	4.65	14.48	0.32
	GR	1.26	4.52	14.54	0.31
120	GL	1.24	4.44	11.86	0.37
	GR	1.21	4.03	13.06	0.31

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