

Effect of supplementation of garden cress seeds on the amino acid and fatty acid composition of ready to eat traditional foods

TANU JAIN*, KIRAN GROVER AND NAVJOT KAUR GILL

Department of Food and Nutrition, College of Home Science
Punjab Agricultural University, Ludhiana (Punjab) India

ABSTRACT

Garden cress (*Lepidium sativum*, family- Cruciferae) seeds have been known for its medicinal properties. The seeds are anticarcinogenic, antihypertensive, laxative, galactagogue in nature and promote health due to its high nutritional value. The seeds are rich in macro and micronutrients and also contain good amount of fatty acids and amino acids. So, an attempt was made to supplement garden cress seeds in ready to eat traditional foods. Four traditional foods viz., *pinni*, *panjiri*, *laddu* and *burfi* were developed using standard methods which were treated as control and for experimental recipes, 10 percent garden cress seeds were supplemented in the standard methods. Both control and experimental recipes were undergone for amino acid and fatty acid estimation to evaluate the effect of supplementation of seeds. The results showed that supplemented *pinni* contained maximum lysine and tryptophan content while methionine and cystine content were found maximum in control *panjiri*. Supplementation improved unsaturated fatty acids and decreased saturated fatty acid content. Maximum linoleic and linolenic acid was found in garden cress supplemented *pinni*.

Key Words : Amino acids, Fatty acids, Garden cress, Linolenic acid, Lysine, Methionine

INTRODUCTION

Garden cress (*Lepidium sativum*, family-Cruciferae) has been considered as an important nutritional and medicinal plant in India due to its health promoting properties (Mahassni and Al-Reemi, 2013 and Mohite *et al.*, 2012). It is grown in all parts of India and known as “Common cress”, “Land cress”, “Haliv”, “Asalio” or “Chandrasur” in India (Divanji *et al.*, 2012; Kasabe *et al.*, 2012). Seeds of garden cress contain good concentration of protein (22-26 g/100 g), fat (24.5%) (Gokavi *et al.*, 2004), iron (100 mg/100 g) and other nutrients (thiamine, 0.59 mg/100 g; riboflavin, 0.61 mg/100 g; niacin, 14.3 mg/100 g) (Gopalan *et al.*, 2007) which provides 454 Kcal/100 g calories. It acts as memory boosters as it

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contains essential fatty acids. As per Divanji *et al.* (2012), garden cress seeds were loaded with linoleic and arachidic fatty acids. Since they contain phytochemicals that resemble estrogen to some extent, intake of these seeds helps to regulate menstruation and stimulate milk production in lactating mothers. Bhakare *et al.* (1993) reported that linolenic acid constituted major fatty acid in total fat of garden cress seeds. According to Gokavi *et al.* (2004), main saturated fatty acids present were palmitic (8.7 %), stearic acid (3.2 %) and arachidic acid (3.2 %) in garden cress seeds. The essential fatty acids linoleic and linolenic acid comprised 12.1 and 30.2 per cent of the total fat, respectively. Moser *et al.* (2009) also reported that seeds contain 30.60 per cent oleic and 29.3 per cent linolenic acids. Diwaker *et al.* (2010) analyzed fatty acid profile of garden cress seed oil which showed 10.1, 2.9, 22.0, 11.8, 3.4 and 12 per cent for palmitic, stearic, oleic, linoleic, arachidic and eicosanoic acid respectively with highest percentage of linolenic acid (34.0 %). Garden cress seeds were found rich in lysine content (6.26 ± 0.39 g / 100 g protein). Methionine was found to be a limiting amino acid in garden cress seeds which had 0.97 ± 0.02 g per 100 g of protein (Gokavi *et al.*, 2004). Glutamic acid was the most abundant amino acid, leucine and methionine were the highest and the lowest essential amino acids respectively as reported by Divanji *et al.* (2012). Amino acid composition has also been described by Gaafar *et al.* (2013) who reported 1.82 mg tryptophan content per 100 g of protein. So, overall, seeds constitute a good percent of amino acid and fatty acids. Due to its slight bitterness of seeds (Dhiman, 2006) it may be consumed in the form of sweet food preparations. Many studies have been performed on garden cress seeds in recent years. Garden cress supplemented food products have been formulated and analyzed for its proximate composition but no study expressed the amino acids and fatty acid composition of supplemented food products. So, the present study aimed to study the amino acid and fatty acid composition of garden cress supplemented ready to eat traditional foods.

METHODOLOGY

Procurement or garden cress seeds :

Garden cress seeds, used for the preparation of food products, were purchased from local market. The seeds were roasted (at 150°C approximately for 3-5 minutes) in an iron vessel till a prominent aroma of garden cress seeds comes. After cooling, the seeds were ground in grinder. The roasted seed powder was stored at ambient conditions (20°C, 60 % RH) in air tight plastic container.

Preparation of ready to eat traditional foods:

Ready to eat traditional foods were prepared in the Food Laboratory of Food and nutrition Department, College of Home Science, PAU, Ludhiana using the following formulations:

***Pinni* :**

Overnight soaked and ground green gram (60 g, raw weight) was deep fried in ghee (50 g) till it turns golden brown and ground again after cooling. Wheat flour (60 g) was roasted in ghee (10 g) for 8-10 minutes on low flame and mixed all ingredients with jaggery

(75 g) on cooling. The mixture was divided into 5 equal portions by weight and turned it into small balls with the help of palm (Nagi and Mann, 2003).

***Panjiri*:**

Wheat flour (100 g) was roasted with ghee (50 g) on a slow fire for 10 minutes and after cooling the mixture, powdered jaggery (100 g) was added thoroughly (Kaur, 2013).

***Laddu* :**

Mix (whole wheat flour:bengal gram flour :: 1:3) flour (100 g) was roasted with ghee (50 g) on a low flame for 10 minutes and ground sugar (100g) was added after cooling it at room temperature. The mixture was divided into 5 equal portions by weight and turned it into small balls with the help of palm (Pant, 2011).

***Burfi* :**

Bengal gram flour (100 g) was roasted with ghee (100 g) on a slow flame till it turns golden brown and 2 -string sugar syrup (80 g sugar in 80 ml water) was poured into mixture with continuous stirring. While hot, the mixture was spread evenly in an aluminium tray and cut into square shape with the help of sharp edged knife (Bansal, 2013).

Garden cress supplemented traditional foods *viz.*, *pinni*, *panjiri*, *laddu* and *burfi* were developed by supplementing 10 percent of roasted garden cress seed powder with primary ingredient which were found organoleptically acceptable among all treatment (5, 10, 15 % level of supplementation) on the 9 point hedonic scale (Watts *et al.*, 1989) by the panel of 10 judges who were familiar with the major sensory attributes of food products.

Amino acids analysis :

Available lysine:

Lysine was assessed by method of Carpenter (1960) modified by Booth (1971). Only 12 ml of ethyl alcohol containing 0.3 ml of FDNB was added in a conical flask containing 8 ml of 8 per cent NaHCO₃ and 0.5 g of sample which was shaken for 1 hour in a water bath cum shaker at 500 C. After evaporation of ethanol, 24 ml of 8.1 N HCl was added. The contents were refluxed gently for 16 hours. The volume was taken then made to 100 ml and filtered (Stage I). In stage II, 2 ml of filtrate was taken in 3 tubes A, B and C marked at 10 ml. The contents of A and B were extracted with 5 ml of ether twice and ether layers were discarded. The tubes were immersed in hot water to remove residual ether. The volume was made to 10 ml with 1 N HCl. In stage III, in tube C one drop of phenolphthalein was added as an indicator and the contents were titrated against 2N NaOH. The same amount of alkali was added to tube B. Then 2 ml of buffer acetate (pH 8.5) was added. Any precipitates formed were dissolved by adding 0.5 ml of methoxy carbonyl chloride with vigorous shaking for 10 minutes and 0.75 ml of concentrated HCl was added drop wise. Then contents were extracted with ethyl ether twice. The ether layers were discarded. The excess ether was removed by placing the test tube in hot water bath and the volume was made to 10 ml with 1 N HCl after cooling the tube. In stage IV, the extinction coefficients of the contents of tubes A and B were measured at 435 nm. Reading A and reading B was taken as the

extinction due to E-NDP lysine, and was compared with the corresponding values obtained with 2 ml of standard DNP- lysine Solution passed through the procedure from stage 2 onwards, with omission only of the ether washing in stage 2.

The equivalent amount of lysine from the test sample that has reacted with FDNB is calculated, with a suitable correction for losses due to hydrolysis where necessary.

Calculations :

$$\frac{\text{Available lysine}}{100 \text{ g protein}} = \frac{0.85 \times 0.4862 \times \text{dil. factor} \times 100 \times 100 \times \text{conc. of E - DNP lysine HCl.H}_2\text{O}}{\text{Weight of sample taken} \times \text{per cent protein}}$$

Hydrolysis of sample for determination of methionine and cystine :

Extraction of sulphur amino acids was done by hydrolysing the samples in autoclave for 6 hours at 15 lb pressure. After filtration, hydrolyzed samples were used for the determination of methionine (Horn *et al.*, 1946) and cystine (Liddell and Saville, 1959).

Methionine :

Three ml of distilled water, 1 ml of 5 N NaOH and 0.1 ml of freshly prepared 10 per cent sodium nitroprusside was added to 2 ml of the protein hydrosylate filtrate in a test tube. The mixture was shaken for 10 minutes after that 2 ml of 3 per cent glycine solution was added, slowly drop by drop with constant shaking. The absorbance was measured in spectronic-20 spectrophotometer at 540 nm after 10 minutes. Standard and blank were also run similarly. Standard curve was drawn using 200 to 1000 μg / ml of methionine.

Cystine:

Total 200 mg of zinc powder was added in 5.0 ml of protein hydrosylate, and left for 30 minutes and filtered. Two ml of filtrate was taken in 25 ml volumetric flask, 5 ml of solution A was added and set aside for 10 minutes. Then 1 ml of solution B was added and contents were shaken vigorously. After 10 minutes 10 ml of solution C was added rapidly followed by 4 ml of solution D and the colour was read at 550 nm in spectronic-20 after 10 minutes.

Tryptophan:

Estimation of tryptophan was done by Concon, 1975. Total 10 ml of 0.075 N NaOH was added in 100 mg of defatted sample and test tubes were shaken in a mechanical shaker for one hour. The after centrifugation of contents at 12000 rpm for 15 minutes, supernatant was decanted. In 1 ml of protein extract, 3 ml of glacial acetic acid- FeCl_3 solution was added. Then 2 ml of 25.8 N H_2SO_4 was added rapidly and mixed well. Colour was stabilized by incubating the sample at 60°C for 45 minutes. Test tubes were cooled to room temperature in ice water bath and absorbance was read at 545 nm against reagent blank. Standard curve was prepared using 40 - 200 μg of tryptophan and concentration was determined in unknown from the standard curve.

Estimation of fatty acid composition:

Oil was extracted from raw and processed garden cress powder through soxhlet

extraction method and extracted oil samples were analyzed for fatty acid composition by gas chromatography using fatty acid methyl esters (FAME) preparation (Appelqvist, 1968). FAMES were analysed on a gas chromatograph (Varian CP 3800, USA), equipped with a flame ionization detector (FID) and a fused silica capillary column (50m × 0.25 mm i.d.), coated with CP-SIL 88 as the stationary phase. The oven temperature was set at 200°C for 13 min. The injector and FID were at 250°C. A reference standard FAME mix (Supelco Inc.) was analysed under the same operating conditions to determine the peak identity. The samples were analysed for saturated fatty acids (C 16:0 and C 18:0), mono unsaturated fatty acids (C 18:1 and C 20:1) and poly unsaturated fatty acids (C 18:2 and C 18:3). The FAMES were expressed as relative area percentage.

Statistical analysis :

The values were taken in triplicate and data were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) version 16.0. t test was used to obtain the differences between control and garden cress seeds supplemented ready to eat traditional foods. Level of significance was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

Amino acids :

The essential amino acids *viz.*, lysine, methionine, tryptophan and cystine of control and ready to eat traditional foods have been described in the Table 1. The results revealed that supplementation of garden cress had significant impact on the amino acid composition of developed ready to eat traditional foods. Among all products, highest amount of lysine and tryptophan was found in *pinni* (5.65 and 1.02 g/ 100 g protein), methionine and cystine content was found in *panjiri* (2.75 and 1.40 g / 100 g protein).

The lysine content of garden cress seeds supplemented *pinni* (5.65 g / 100 g protein) was found to be increased by 1.07 per cent as compared to control (5.59 g / 100 g protein). The methionine content of supplemented *pinni* (1.94 g / 100 g protein) was found to be decreased than that of control (2.04 g / 100 g protein). This might be due to garden cress which has low methionine content (0.97 g / 100 g protein) than wheat flour (2.81 g / 100 g protein). The content of cystine was also decreased in supplemented *pinni* (1.27 g / 100 g protein) as compared to control *pinni* (1.31 g / 100 g protein). The tryptophan content for control was estimated as 0.97 g per 100 g protein while for supplemented *pinni*, it was observed as 1.02 g per 100 g protein with 5.15 per cent increase.

A significant ($p = 0.05$) increase (9.91 %) in lysine content was observed in case of supplemented *panjiri* (2.55 g / 100 g protein) as compared to control (2.32 g / 100 g protein). The methionine content of control and supplemented *panjiri* was found to be 2.81 and 2.75 g per 100 g protein (Table 1). A decrease of 5.40 per cent in methionine content in supplemented *panjiri* was might be due to low methionine content in garden cress seeds rather than that in wheat flour (2.60 g / 100 g protein). Similarly, cystine content was found to be decreased by 4.10 per cent in supplemented *panjiri* (1.40 g / 100 g protein) as compared to control which showed 1.46 g cystine content per 100 g protein, while tryptophan content of the product was found to be increased in supplemented *panjiri* (1.01 g / 100 g protein)

than that of control (0.88 g / 100 g protein).

The lysine content (4.31 g / 100 g protein) of supplemented *laddu* increased with addition of cress seeds (Table 1). Supplemented *laddu* showed decreased methionine (152.42 g / 100 g protein) and cystine (1.15 g / 100 g protein) content than that of control (2.60 and 1.20 g / 100 g protein). The tryptophan content of supplemented *laddu* (0.93 g / 100 g protein) was also found to be increased by 8.14 per cent than that of control (0.86 g / 100 g protein). Kaur (2011) reported improvement in lysine and methionine but slight decrease in cystine content of *laddu*, developed by using bengal gram leaves which has good amount of methionine and lysine but fair amount of cystine.

In case of *burfi*, 1.32 per cent increase was observed in lysine content of supplemented product (5.37 g / 100 g protein) as compared to control (5.30 g / 100 g protein), while methionine content was found to be decreased in supplemented product (2.10 g / 100 g protein) rather than control (2.23 g / 100 g protein) *burfi*. Similarly, cystine content of *burfi* was also found to be decreased in supplemented product (0.96 g / 100 g protein) with addition of garden cress seeds at 10 per cent level of supplementation (Table 1). On the other hand, an increase of 11.29 per cent in tryptophan content was estimated in supplemented *burfi* (0.69 g / 100 g protein) than that of control (0.62 g / 100 g protein) Garden cress seeds have more lysine but less methionine and cystine content than that of bengal gram flour which was responsible for improvement in lysine and tryptophan content but decrease in methionine and cystine content of supplemented *burfi*.

Garden cress seeds have good amount of lysine and fair amount of tryptophan but limited in methionine and cystine content. Thus value of methionine was decreased in developed ready to eat traditional food products. So, it will be more beneficial to add cress

Table 1 : Amino acid content of ready to eat traditional foods on DM basis (g /100 g protein)				
Traditional foods	Lysine	Methionine	Cystine	Tryptophan
<i>Pinni</i>				
Control	5.59 ± 0.07	2.04 ± 0.09	1.31 ± 0.07	0.97 ± 0.04
Experimental	5.65 ± 0.04	1.94 ± 0.04	1.27 ± 0.06	1.02 ± 0.02
t value	0.94	2.10	1.10	01.69
<i>Panjiri</i>				
Control	2.32 ± 0.11	2.81 ± 0.07	1.46 ± 0.13	0.88 ± 0.07
Experimental	2.55 ± 0.20	2.75 ± 0.06	1.40 ± 0.05	1.01 ± 0.08
t value	2.31*	1.27	1.07	2.08
<i>Laddu</i>				
Control	4.12 ± 0.02	2.60 ± 0.11	1.20 ± 0.06	0.86 ± 0.08
Experimental	4.31 ± 0.05	2.42 ± 0.06	1.15 ± 0.02	0.93 ± 0.03
t value	0.82	1.87	1.07	0.45
<i>Burfi</i>				
Control	5.30 ± 0.10	2.23 ± 0.04	0.99 ± 0.02	0.62 ± 0.01
Experimental	5.37 ± 0.04	2.10 ± 0.05	0.96 ± 0.05	0.69 ± 0.02
t value	0.76	1.14	0.97	0.54

Values are mean ± SD *Significant at 5% level **Significant at 1% level
Experimental (Acceptable level for *pinni*, *panjiri*, *laddu* and *burfi* at 10 % level of garden cress supplementation)

seeds to those products which show poor lysine and tryptophan content but good amount of methionine, so that good amino acid scores can be attained. It was also observed that preparations made up of wheat flour had less proteins which instinctively decreased value of amino acids also. So addition of cress seeds in cereals to improve protein quantity as well as quality will be more advantageous.

Fatty acid composition :

Fatty acid composition of ready to eat traditional foods is presented in the Table 2. Saturated fatty acid (palmitic and stearic), mono unsaturated fatty acids (oleic acid and ecosanoic acid) and poly unsaturated acids (linoleic acid and linolenic acid) have been estimated in traditional food products, which showed improvement of the essential fatty acids and fall of saturated fatty acids content in supplemented foods than that of control.

Overall, highest palmitic acid content was found in control *pinni* (36.3 %) while minimum was found in supplemented laddu (33.17 %). Maximum *desi* ghee was used in preparation of *pinni*, which is a rich source of palmitic acid. Mirghani *et al* (2010) also observed high value of palmitic acid (C16:0) in homemade animal fat.

The percentage of palmitic acid decreased significantly ($p = 0.05$) from 36.3 to 32.7 per cent with supplementation of garden cress seeds at acceptable level of 10 per cent in *pinni* (Table 2). The stearic (C18:0) (11.6 %) and oleic acid (C18:1) (29.2 %) were also found to be decreased slightly in supplemented *pinni* (11.03 % stearic acid and 28.17 % oleic acid), while linoleic (C18:2) (5.67 %) and ecosanoic (C20:1) (1.43 %) acids were significantly ($p = 0.01$) improved with supplementation of seeds in *pinni* as compared to control (3.57 and 0.57 %). Linolenic acid (C18:3) increased from 1.23 to 2.70 % in supplemented *pinni*.

Table 2 : Fatty acid composition of ready to eat traditional foods						
Traditional foods	C16:0 (%)	C18:0 (%)	C18:1 (%)	C18:2 (%)	C18:3(%)	C20:1 (%)
<i>Pinni</i>						
Control	36.3±2.72	11.60±1.00	29.20±3.60	3.57±0.35	1.23±0.78	0.57±0.06
Experimental	32.7±2.59	11.03±0.95	28.17±2.51	5.67±0.58	2.70±0.20	1.43±0.15
t value	2.27*	1.32	1.05	6.51**	1.74	10.99**
<i>Panjiri</i>						
Control	36.13±2.90	11.93±0.95	28.30±2.20	4.17±0.31	0.47±0.06	0.43±0.05
Experimental	34.73±3.20	11.40±1.05	27.53±2.50	4.67±0.42	1.73±0.31	0.80±0.10
t value	1.18	1.32	1.04	2.28**	2.67*	6.64**
<i>Laddu</i>						
Control	34.70±2.91	11.43±0.96	30.83±2.57	5.00±0.46	0.53±0.06	0.90±0.10
Experimental	33.17±2.71	10.90±0.90	26.87±2.24	5.23±0.40	1.13±0.35	2.40±0.89
t value	1.28	1.31	2.65*	1.27	3.66*	3.64*
<i>Burfi</i>						
Control	35.77±2.65	12.10±0.92	27.47±2.05	4.87±0.35	0.63±0.16	0.43±0.04
Experimental	34.37±3.30	11.17±1.06	27.80±2.70	5.00±0.50	1.63±0.15	0.43±0.05
t value	1.19	1.74	0.86	1.03	8.03**	0.74

Values are mean ± SD *Significant at 5% level **Significant at 1% level.

Experimental (Acceptable level for *pinni*, *panjiri*, *laddu* and *burfi* at 10 % level of garden cress supplementation)

Similar pattern was also observed in case of *panjiri*, in which palmitic (34.73 %), stearic (11.4 %) and oleic acid (27.53 %) were found to be low, while linoleic (4.67 %), linolenic (1.73 %) and ecosanoic acid (0.80 %) were found to be improved in supplemented *panjiri* than that of control (Table 2). A significant difference ($p = 0.05$) was found between control and supplemented *panjiri* for linoleic, linolenic and ecosanoic acid.

Control *laddu* contained 34.7 per cent palmitic acid while in supplemented *laddu*, it was decreased to 33.17 per cent due to supplementation of garden cress seeds (Table 2). The stearic (10.90 %) and oleic acid (26.87 %) were also reported to be slightly decreased in supplemented *laddu* (11.43 and 30.83 %), while linoleic acid increased from 5.00 to 5.23 %. The linolenic (1.13 %) and ecosanoic (2.40 %) acids were improved significantly ($p = 0.05$) with addition of seeds in supplemented *laddu* as compared to control (0.53 and 0.90 %).

Saturated fatty acids were found to be decreased in supplemented *burfi* (34.37 % palmitic and 11.17 % stearic acid) as compared to control which had 35.77 and 12.1 per cent of palmitic and stearic acid (Table 2). A non significant increase in oleic acid content of supplemented *burfi* (27.80 %) as compared to control (27.47 %) was observed. The linoleic (5.00 %) and linolenic acid (1.63 %) was found to be increased in supplemented *burfi* as compared to control (4.87 and 0.63 %) with significant ($p = 0.01$) difference for linolenic acid, while ecosanoic acid showed no difference between the two (0.43 % control and 0.43 % supplemented *burfi*).

Garden cress is rich in linolenic acid. Thus, it increased almost half to double in amount in all food preparation with addition of cress seeds. Value addition with incorporation of garden cress seeds (*Lepidium sativum*), greengram *dhal* (*Phaseolus aureus* Roxb.) or linseed (*Linum usitatissimum*) increased 3 fatty acids 116 mg per 100 g in the value added flakes (Kotagi and Chimmad 2011). Being rich in unsaturated fatty acids, garden cress seeds provide a good option to balance the polyunsaturated : saturated fatty acid ratio as well as to increase linolenic acid content in diet through food products.

Addition of garden cress seeds increased the percentage of unsaturated fatty acids especially linolenic acid in ready to eat traditional foods which was found to be highest in *panjiri* followed by *burfi*. The percentage of linoleic and oleic acid were also found to be increased, but saturated fatty acids (palmitic and stearic acid) decreased proportionately in supplemented products. Over all it may be concluded that ready to eat traditional foods supplemented with garden cress seeds were found with good fatty acid composition.

Conclusion:

Garden cress is an oilseed crop and its seeds have good composition of fatty acids including essential fatty acids. The seeds contain good amount of amino acids too. So the ready to eat traditional foods viz., *panjiri*, *pinni*, *laddu* and *burfi* were also found with good amino acids and fatty acid composition than control products. Supplemented *pinni* contained maximum lysine and tryptophan content while methionine and cystine content were found maximum in control *panjiri* due to low content of sulphur amino acids in garden cress seeds. Supplementation improved unsaturated fatty acids and decreased saturated fatty acid content. Among all traditional foods, maximum linoleic and linolenic acid was

found in garden cress supplemented *pinni*.

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