

RESEARCH NOTES

The Changes in Proximate Composition, Antioxidant Activity and Fatty Acid Profile of Germinating Safflower (*Carthamus tinctorius*) Seed

A. Shirvani¹, M. Jafari², S. A. H. Goli^{1*}, N. Soltani Tehrani³, and M. Rahimmalek⁴

ABSTRACT

In this work, the effect of three-day germination on proximate composition, total phenolic compounds, fatty acid profile, vitamin C content, radical scavenging activity, malondialdehyde content and reducing power of safflower seed was investigated. Apart from ash, other parameters including oil, protein and crude fiber contents decreased significantly during germination. Phenolics content increased gradually from 4.72 to 9.51 mg g⁻¹. Vitamin C sharply increased at the beginning of germination (4.22 to 7.04 mg 100 g⁻¹ after 1-day germination) and after that no significant difference was observed. Germination improved the quality of safflower oil by increasing the unsaturated and decreasing the saturated fatty acids (about 5%). Results indicated that germination could be a suitable bioprocess to improve chemical composition and nutritional value of safflower seed.

Keywords: Antioxidant activity, Germination, Malondialdehyde, Oil, Phenolic.

INTRODUCTION

Safflower (*Carthamus tinctorius* Linn) is a deep-rooted crop that belongs to the Cynareae tribe, subfamily of *Tubulifloreae* and *Asteraceae* family. This genus is an Eastern Mediterranean genus with about 25 species (Mokhtari *et al.*, 2013). Safflower has been cultivated since ancient times for its flowers and oil rich seeds (Tonguc *et al.*, 2012). This plant has been used as herbal medicine in Korea for the promotion of bone formation and in the treatment of

osteoporosis and rheumatism (Koyama *et al.*, 2006). It is found that safflower oil has the highest quantity of linoleic acid as well as tocopherol among other edible oils (Mokhtari *et al.*, 2013).

Germination or sprouting is a critical period in plant life in which the nutrient composition and certain functional properties of seeds might be improved. During sprouting, complex starch and proteins break down to simple units and the content of some components with antioxidant activity would be increased

¹ Department of Food Science and Technology, College of Agriculture, Isfahan University of Technology, Isfahan 84156 83111, Islamic Republic of Iran.

² Department of Food Science and Technology, College of Agriculture, Shahrekord Branch, Islamic Azad University, Shahrekord, Islamic Republic of Iran.

³ Department of Food Science and Technology, College of Agriculture, University of Zabol, Zabol, Islamic Republic of Iran.

⁴ Department of Plant Breeding, College of Agriculture, Isfahan University of Technology, Isfahan 8415683111, Islamic Republic of Iran.

* Corresponding author; e-mail: amirgoli@cc.iut.ac.ir



(Chen *et al.*, 2016; Kumari *et al.*, 2015). Tonguc *et al.* (2012) studied the seed composition of two safflower cultivars during germination and early seedling growth and observed that significant changes took place in the oil, protein and sugar metabolism. Sadeghi *et al.* (2011) showed that the seed size was significantly effective on germination percent, germination speed, coleoptiles fresh weight, radicle dry weight and 1000 seeds weight.

Since there is no comprehensive research on the changes in safflower seed composition during germination, the objective of this study was to evaluate the variation in proximate composition, vitamin C, phenolic content, reducing power and fatty acids profile of safflower seeds.

MATERIALS AND METHODS

Materials

Safflower seeds were purchased from Pakan Bazr Co. (Isfahan, Iran). All solvents and chemicals were of analytical grade and obtained from Merck (Darmstadt, Germany). 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and pure fatty acids as standards including myristic, palmitic, stearic, oleic, linoleic and linolenic acids were purchased from Sigma Chemical Co. (St Louis, MO).

Germination

After cleaning, safflower seeds were soaked in 15% sodium hypochlorite solution for 10 minutes at room temperature in the dark. Afterwards, the seeds were washed with distilled water until they reached to neutral pH. Then, the seeds were soaked in deionized water for 90 minutes. The imbibed seeds were drained and placed in germinator at 22°C and 75% relative humidity for 3 days without light. The seeds were hydrated manually by occasionally spraying sterile water. Finally, the un-germinated and

germinated seeds were dried, milled and then stored in 18°C for chemical analysis. Moreover, some properties such as germination percentage, raw seed, sprout weight and length of shoots were determined every 24 h during germination.

Proximate Analysis

For proximate analysis, the seeds were ground into powder using coffee grinder. The content of moisture, total ash, crude fat, protein and crude fibre of samples were measured according to the Association of Official Analytical Chemists (AOAC, 2002).

Extraction and Determination of Phenolic Compounds

The phenolic extraction was conducted according to the method described by Zielinska-Dawidziak and Singer (2012). Total phenolic content (TPC) of the extracts was determined by a spectrophotometric method according to Singleton *et al.* (1995). The results were expressed as mg of gallic acid equivalents (GAE) 100 g⁻¹ of sample (dry basis).

Reducing Power Assay

The reducing power assay was determined according to the method of Oyaizu (1986).

Radical Scavenging Activity

The antioxidant activity of the extracts was measured by the DPPH radical scavenging method (De Ancos *et al.*, 2002). The result was expressed as percentage of DPPH inhibition and calculated according to the following equation:

$$\% \text{ Inhibition of DPPH} = (\text{Abs control} - \text{Abs sample} / \text{Abs control}) \times 100$$

Where, *Abs control* is the absorbance of the DPPH solution without the extracts.

Malondialdehyde Content

Malondialdehyde (MDA) content was determined using thiobarbituric acid (TBA) reagent according to Cai *et al.* (2011). Lipid peroxidation was expressed as MDA content in mole per gram fresh weight, by using an extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$ according to the following formula:

$$\text{Mol ml}^{-1} \text{ MDA equivalents} = \frac{(\text{Abs } 532 - \text{Abs } 600) / 155000}{1} \times 10^6$$

Determination of Vitamin C

Ascorbic acid content was measured using 2,6 dichlorophenol indophenol (DIP) and titration method (Omran *et al.*, 2013). The measurement was expressed as milligram of vitamin C per 100 grams of the sample.

Fatty Acids Profile

The fatty acid methyl esters (FAMES) were prepared using the method described by Goli *et al.* (2008). One hundred microliters of sodium methoxide (0.5M) was added to 50 μL of the sample in 1 mL *n*-hexane. The mixture was shaken vigorously for 15 minutes and allowed to stand and separate. Hexane phase was removed and 1 μL was injected to gas chromatograph (GC) at a split ratio of 20:1. The gas chromatographic analysis of FAME was performed on an Agilent 6890N gas chromatograph equipped with a Flame Ionization Detector (FID). The column used

was a HP-88 (100 m, 0.25 mm i.d., and 0.2 μm film thickness). The temperature program consisted of increasing the temperature first from 150 to 210°C at a rate of $5^\circ \text{C min}^{-1}$ and holding for 8 min, then increasing to 240°C at a rate of $5^\circ \text{C min}^{-1}$ and holding for 6 minutes. Temperatures of injector and detector were 230 and 250°C, respectively. Ultra high-purity nitrogen was used as the carrier gas.

Statistical Analysis

Results were shown as the mean \pm SD of three separate determinations. The data were statistically analyzed by ANOVA program in Statistix 8 software. The means evaluation was done using least significant difference (LSD) test at a confidence level of 95% (Mead and Curnow, 1983).

RESULTS AND DISCUSSION

The germination characteristics of safflower seed are shown in Table 1. At the end of the period, about 89% of seeds were germinated and the greatest shoot length (21.06 mm), sprout weight (0.024 g) and seed weight (0.071 g) were recorded.

Proximate Analysis

Table 2 indicates the changes in seed composition through germination. As germination progressed, crude protein, fat and fiber content was gradually decreased

Table 1. Safflower germinated seed characteristics during three-day germination.^a

Germination (Day)	Germination rate (%)	Shoot length (mm)	Seed weight (g)	Sprout weight (g)
1	89.08 ^a ±1.02	8.29 ^c ±0.36	0.051 ^c ±0.0	0.01 ^b ±0.00
2	87.42 ^a ±0.45	14.20 ^b ±0.19	0.057 ^b ±0.0	0.011 ^b ±0.00
3	89.32 ^a ±0.78	21.06 ^a ±0.61	0.071 ^a ±0.0	0.024 ^a ±0.00

^a Results are mean of three determinations \pm SD. Values with different letters in each column represent significant difference ($P < 0.05$).

**Table 2.** Chemical component, Total Phenolic Content (TPC), DPPH inhibition and reducing power of safflower seed during germination.^a

Germination (Day)	Moisture (%)	Crude protein (%)	Fat (%)	Crude fibre (%)	Ash (%)	TPC (mg g ⁻¹ db) ^b	DPPH inhibition (%)	Reducing power
0	4.46 ^a ±0.26	19.49 ^a ±0.42	29.67 ^a ±0.00	31.82 ^a ±1.71	2.95±0.08	4.72 ^a ±0.12	50.00 ^b ±0.0	0.44 ^b ±0.01
1	46.41 ^c ±0.64	8.12 ^b ±2.03	11.57 ^b ±0.56	20.50 ^b ±0.40	2.88±0.21	7.21 ^c ±0.22	54.54 ^c ±0.0	0.69 ^c ±0.01
2	51.05 ^b ±0.26	9.05 ^b ±0.07	9.67 ^c ±0.81	18.10 ^{bc} ±0.85	2.93±0.18	7.93 ^b ±0.23	59.09 ^b ±0.0	0.80 ^b ±0.02
3	58.25 ^a ±0.01	8.25 ^b ±0.16	7.62 ^c ±0.13	15.49 ^c ±0.32	3.03±0.24	9.51 ^a ±0.26	63.63 ^a ±0.0	0.87 ^a ±0.06

All values for protein, fat, crude fiber and ash are presented on moisture free basis. Results are mean of three determinations±SD. Values with same letters (a, b, c and d within columns) are not significantly different at $P < 0.05$. ^b db: Dry basis.

while seed moisture increased ($P < 0.05$). The moisture content significantly varied from 4.4 to 58.2% in germinated seeds. As germination proceeded, the seeds took up water from the surrounding in order to commence the metabolic process (Megat-Rusydi *et al.*, 2011).

A significant decrease in protein content was observed as germination progressed. The decline in protein content during germination was in agreement with observations reported on seed germination of safflower seed (Kim *et al.*, 2008). The reduction in protein content may attribute to its utilization in germination process.

The oil content of safflower seeds dropped steadily throughout the germination. As shown in Table 2, following germination for three days, a significant decline in lipid content from 29.67 to 7.62% (about 74.31% decrease) was observed ($P < 0.05$). The lipids stored in seed oleosomes are the major source of energy during germination and the early periods of seedling growth. In fact, lipids are being degraded into fatty acids which are utilized to produce energy through β -oxidation. The crude fat content of sesame seed significantly decreased accompanied by germination time (Liu *et al.*, 2011).

In this study no difference between ash content in different stages of germination was observed. This result was similar to Badifu (2001) who reported that no noticeable changes were found in ash content of *Cucurbitaceae* seeds during germination.

Crude fiber decreased significantly from 31.8 to 15.4% during germination. As germination progressed, partial utilization of cell wall carbohydrate can occur and consequently, the content of structural carbohydrates such as lignin and cellulose can be affected negatively with the duration of germination. Also, Hooda and Jood (2003) reported a decrease in soluble dietary fiber of fenugreek seeds on germination because of enzymatic activity of α -galactosidase.

Total Phenolic Content

Table 2 shows that phenolics content gradually increased with germination time (from 4.72 to 9.51 mg g⁻¹). This may be due to the fact that in early stages of germination, the seed requires high oxygen concentration and therefore phenolics can protect the cells against potential oxidation-induced stress (Andarwulan *et al.*, 1999). Liu *et al.* (2011) found that the TPC of sesame seed changed from 0.51 to 13.42 mg GAE g⁻¹ dry matter after 5-day germination.

Antioxidant Properties

The extract of 3-day germinated seed showed the highest radical scavenging activity (63.6%) compared to un-germinated (50%) seed (Table 2). This may be due to the presence of highest TPC in three-day germinated seeds, since the % DPPH inhibition is directly correlated with TPC (Allothman *et al.*, 2009).

The iron reducing power of seed extracts was also determined and shown in Table 2. As observed in DPPH inhibition, germinated seed extract exhibited an increase in reducing power over the period. Phenolic compounds are essential to the plant growth and stress response and there is a close correlation between the content of total phenolic and its antioxidant activity due to the singlet oxygen quenching and free radical scavenging properties (Gujral *et al.*, 2011).

Malondialdehyde

During seed germination and development, the cellular levels of reactive oxygen species (ROS) would increase and malondialdehyde (MDA) is considered as a sensitive marker commonly used for assessing membrane lipid peroxidation (Bailey *et al.*, 1996). MDA content increased gradually (from 1060 to 2735 mol g⁻¹ FW) during the 3-day germination which implied

that lipid peroxidation increased as germination proceeded (Figure 1).

Vitamin C

Figure 2 shows that ascorbic acid concentration increased on the first day from 4.22 to 7.04 mg 100 g⁻¹ while after that it was constant until the end of germination. Ascorbic acid has been directly implicated in the modulation of plant growth, including the early stage of embryos germination (Tommasi *et al.*, 2001). Some researchers showed that the increase in vitamin C content during germination was pertained to the increase in activity of key enzymes in ascorbic acid biosynthesis pathway such as *L*-Galactono- γ -lactone dehydrogenase (GLDH, EC 1.3.2.3) (Pérez-Balibrea *et al.*, 2011).

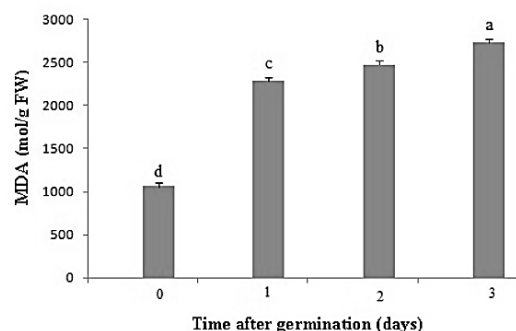


Figure 1. The changes in Malondialdehyde (MDA) content during the germination of safflower seeds (Mean \pm SD, n= 3).

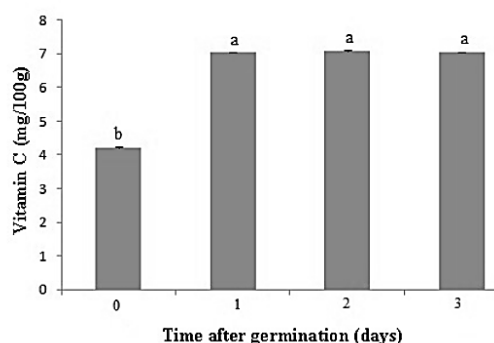


Figure 2. The changes in vitamin C content during the germination of safflower seeds (Mean \pm SD, n= 3).



Fatty Acids Profile

The percentage of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyUnsaturated fatty acids (PUFAs) in germinated and non-germinated safflower samples are shown in Table 3. Saturated fatty acids were affected by germination as they decreased from about 16% in un-germinated seeds to about 11% in germinated samples. This result was in agreement with Mariod *et al.* (2012) who reported 2% reduction in SFA and a little decrease in total monounsaturated fatty acids by germination in black cumin seeds, although in this work, oleic acid decreased from about 20 to 18% through germination. On the contrary, Hahm *et al.* (2009) reported that oleic acid was increased after germination in sesame seeds. During germination, linoleic acid content showed an overall increase of 4.07%. In general, polyunsaturated fatty acids increased from 82.3% in raw seeds to about 85% in germinated samples and so, germination improved the quality of safflower oil by increasing unsaturated and lowering saturated fatty acids content.

CONCLUSIONS

As shown in this study, germination had a significant effect on chemical composition and nutritional value of safflower seed. As sprouting progressed, ascorbic acid, total phenolic and antioxidant activity increased. Furthermore, saturated fatty acid reduced

about 5% and an increment of 2.5% in unsaturated fatty acids was observed.

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Table 3. Fatty acids composition (%) obtained from native safflower seeds and during germination.^a

Germination (Day)	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	SFAs	USFAs
0	0.61	11.94	3.41	19.94	62.14	0.20	15.96	82.28
1	0.54	9.84	2.51	17.90	66.14	0.31	12.89	84.35
2	0.61	10.82	3.18	17.97	65.87	0.81	14.61	84.65
3	0.18	7.80	3.28	18.16	66.21	0.44	11.26	84.81

^a 14:0, Myristic acid; 16:0, Palmitic acid; 18:0, Stearic acid; 18:1, Oleic acid; 18:2, Linoleic acid; 18:3, Linolenic acid; SFAs, Saturated fatty acids; USFAs, UnSaturated fatty acids.

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تغییرات ترکیبات اولیه، فعالیت آنتی‌اکسیدانی و پروفیل اسیدچرب در طی جوانه‌زنی دانه گلرنگ

ع. شیروانی، م. جعفری، س. ا. ح. گلی، ن. سلطانی تهرانی، م. رحیم ملک

چکیده

در این مطالعه، اثر جوانه زنی سه روزه بر ترکیبات اولیه، میزان ترکیبات فنولیک، پروفیل اسید چرب، میزان ویتامین C، فعالیت بازدارندگی رادیکال آزاد، محتوی مالون دی آلدهید و قدرت احیاکنندگی دانه گلرنگ مورد بررسی قرار گرفت. به غیر از خاکستر، سایر پارامترها مانند روغن، پروتئین و فیبرخام به طور معنی‌داری کاهش یافت. میزان ترکیبات فنولیک به تدریج از ۴/۷۲ به ۹/۵۱ میلی‌گرم بر گرم افزایش یافت. ویتامین C در شروع جوانه‌زنی به سرعت افزایش داشت (از ۴/۲۲ به ۷/۰۴ میلی‌گرم بر ۱۰۰ گرم پس از یک روز جوانه‌زنی) و پس از آن تغییر معنی‌داری مشاهده نشد. جوانه‌زنی کیفیت روغن گلرنگ را با افزایش اسیدهای چرب غیر اشباع و کاهش اسیدهای چرب اشباع (حدود ۵٪) بهبود بخشید. نتایج نشان داد که جوانه‌زنی می‌تواند یک فرایند زیستی مناسب جهت بهبود ترکیبات شیمیایی و ارزش تغذیه‌ای دانه گلرنگ باشد.