

Chemical composition and secondary metabolites of Egyptian faba bean (*Vicia faba* L.) effected by germination and soaking

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ABSTRACT:

Two Egyptian faba bean (*Vicia faba* L.) varieties (Sakha-1 and Giza-843) were soaking in distilled water for 24, 48 and 78 hours to dry seeds, and germination. For both varieties, the seed composition for determining moisture, lipid, ash, nitrogen, protein and carbohydrates contents was similar. Secondary metabolites concentrations were estimated, total phenolic compounds, total flavonoids, total alkaloids, phytic acid and tannins. Analytical data for proximate composition of faba bean seed varieties (Sakha-1 and Giza-843) showed that, Giza-843 seeds contain higher level of moisture, ash content, crude fiber and total carbohydrates (7.53, 3.75, 6.81 and 50.61 %, respectively). The Chemical compositions of Sakha-1 seeds were 1.65, 5.03 and 31.44% for crude lipid, total nitrogen and crude protein respectively. Results showed that the variety Sakha-1 was higher than Giza-843 variety in total phenolic compounds (TPCs), total alkaloids (TAs) and phytic acid. Total flavonoids (TFs) in the Giza-843 seed (81.34 mg/100g) were higher than those found in the Sakha-1 seeds (80.23mg/100g). Also, tannins were higher in Giza-843 seed (653.25 mg/100g) than in Sakha-1 seeds (635.59mg/100g). Soaking process of variety (Sakha-1 and Giza-843) in distilled water for (24,48 and 72 hours) significantly lowered total ash content and crude fiber compared with control but, it was increased moisture, crude lipid, crude protein and total carbohydrates compared with control. Data of soaked seeds in water showed a slight reduction in TPCs, TFs and tannins contents, while the results of total alkaloids and phytic acid were increased compared with control. Germination processes caused a decrease of moisture, crude lipid and total carbohydrates. But, it caused an increasing in total ash content, crude fiber and crude protein compared with control. Germination processes caused a drop in Total phenolic compounds, total flavonoids, total alkaloids and phytic acid.

Keywords: Faba bean (*Vicia faba* L.); Chemical composition; Secondary metabolites; Soaking process; Germination process.

INTRODUCTION

The faba bean (*Vicia faba* L.), known as Horse, Broad, or Field bean, is a protein-rich legume use for human consumption and animal feed (Sudheesh *et al.*, 2019).

Like other food legumes, the faba bean are distinguished by their unusually high amounts of protein and carbohydrates content. Crude lipids, crude fibre, vitamins and minerals are also present in large amounts (El-Tinay, 1993 and Alonso *et al.*, 2000). High levels of antioxidant activity conferred by polyphenolic compounds are associated with multiple biological activities including anti-inflammatory, anti-aging, apoptotic, anti-cancer, antioxidant, cardiovascular and other chronic diseases (Perez-Hernandez *et al.*, 2021).

The seeds of the faba bean are regarded as a superior functional foods source. Like other legumes, faba bean have to go through various manufacturing processes like soaking and cooking before they can be eaten. These procedures enhance the flavor and aroma of seeds, boost the protein digestibility and remove the raffinose sugars that cause uncomfortable sensations and bloating (Wainaina, *et al.*, 2021). Potassium, phosphorus, iron, and zinc are among the dietary minerals and trace components found in Faba bean seeds (Meng *et al.*, 2021).

People frequently utilize soaking, a domestic technological procedure, to create supplementary foods at home. Studies conducted in the past (Mubarak, 2005; Vijayakumari and Vadivel, 2007) have demonstrated that soaking greatly decreased the

concentrations of phytates and tannins. Legumes seed coats (hulls) are frequently inedible (Wang *et al.*, 2009). Simple techniques like heating, soaking and germination or autoclaving render the majority of the anti-nutritional agents ineffective (Nowak and Haslberger, 2000).

By causing the production of enzymes the remove or lessen the anti-nutritional and indigestible components in legumes, germination also increases the nutritional value of legumes (Bau *et al.*, 1997). Germination is a widespread technological application as it can decrease the level of anti-nutritional factors in legume seeds and improve the concentration (Alonso and Marzo, 1998 and Vidal-Valverde *et al.*, 2002). The chemical physical and gastronomic characteristics of the seed meal change during germination reducing his nutritional rate (Rozan and Lambein, 2000 and Cen *et al.*, 2023). Therefore, this inquiry was conducted to ascertain the influence soaking and germination on chemical structure and secondary metabolites of two Egyptian faba bean seed varieties.

MATERIALS AND METHODS

Samples

Faba bean (*Vicia faba* L.) seed varieties Sakha-1 and Giza-843 was provided who Malloway Agricultural Research Station, Minia, Egypt.

Soaking

Faba bean seeds (500 g) of each variety were soaked in water at a ratio of 1:10 for 24, 48 and 72 hours. These are the typical soaking times used in various houses when cooking beans for a meal. The seeds were dried in an oven at 55°C for around 6 hours. The dried seeds were sieved through a 60-mesh sieve and ground using a hammer mill. Samples of sieved flour were used to assess the chemical composition and some secondary metabolites concentrations.

Germination

The samples were cleansed and rinsed with tap water, then left to soak at room temperature (28°C) for six hours. Samples were placed beneath moist muslin fabric after six hours and they were allowed to germinate for 48 hours at room temperature (28°C) without being exposed to the sun (Yasmin *et al.*, 2008).

Approximate analysis

The association of official analytical chemists official procedures were followed in the analysis of the Chemical composition of broad bean seeds. All tests were carried out in triplicate and mean values are reported. The official procedure (AOAC, 2000) was followed for the determination of moisture content and ash content. Crude lipids were determined according to (AOAC, 1984); the Kjeldahl method was employed to calculate the total nitrogen. After that, protein was estimated by multiplication the nitrogen concentration by 6.25 as a faba bean specific component. AOAC (1990) was used to determine the amount of Crude fibre.

Determination of total Carbohydrates

The following equation was used to determine the differences in carbohydrate composition

Carbohydrates = 100 - (moisture +total ash + lipids + fibre + Protein)

Qualitative determination of Secondary metabolites concentrations in Faba bean

Determination of tannins

The method outlined by Burns (1971) was used to determine the amount of tannins.

Determination of total phenolic compounds and total flavonoids

The procedure outlined by Singleton and Rossi, (1965) was modified to determine the amounts of TPCs in the methanol extracts as follows:-

1ml of the sample was added for 1 ml phenol reagent from Folin & Ciocateau's. The total flavonoids (TFs) content was defined in catechin equivalents (CE) (6.25–200µg/mL) and was expressed in gram of CE/100 gram according to the mode of Zhishen *et al.*, (1999) method described.

Determination of the total alkaloids

The alkaline precipitation gravimetric method published by Harborne, (1973) was used to determine the alkaloid concentration. The amount of total alkaloid in the sample was determined and reported as a percentage of its weight. In comparison to a blank, the absorbance was at 565 nm.

Extraction and determination of phytic acid content

The following Ellis *et al.*, (1977) instructions, two faba bean samples were used to extract Phytic acid. The method outlined by Wheeler and Ferrel, (1971) was used to determine the amount of phytic acid in the raw and processed samples.

Statistical Analysis

The data was calculated using Microsoft Excel to determine the mean \pm SD for each correlation. The experimental device was arranged completely randomized. The statements acquired were subject to an analysis of variance and mean treatments compared to the LSD test by Gomez and Gomez (1984).

RESULTS AND DISCUSSIONS

Chemical composition

Chemical composition of data of Giza-843 seeds is shown in (Table 1). Results indicated that a higher level of moisture, total ash content, crude fiber and total carbohydrates were observed (7.53, 3.75, 6.81 and 50.61 % respectively) for Sakha-1 seed variety. Also, the results (Table 1) shows that Sakha-1 seeds contain a higher content of crude lipid, total nitrogen and crude protein level (1.65, 5.03 and 31.44% respectively) and lower levels in Giza-843 seeds. These findings coincide with those obtained by Iqbal *et al.*, (2006), and Mehanni *et al.*, (2021). Similar observations were mentioned by El-Sayed (1994), EL-Skeikh *et al.*, (1999) and Mittal *et al.*, (2012) which they reported that, in Egypt legume such as faba beans because their nutritional value, are widely consumed and the seeds also contain cheap and rich complex carbohydrates, crude protein, crude fiber, minerals, and vitamins.

Secondary metabolites concentrations in some variety Faba bean seeds

Results presented in (Table 2) showed that seed of Sakha-1 variety was higher than in Secondary metabolites concentrations. Total phenolic compounds (355.73), total alkaloids (90.00) phytic acid (872.52) and total flavonoids (80.23) in Sakha-1 seeds. But, the same metabolites concentrations in Giza-843 seed were TPCs (350.95 mg/100g), TAs (89.53), phytic acid (831.52mg/100g), TFs (81.43mg/100g) in Giza-843 seeds.

Tannins were higher (653.25 mg/100g) in Giza-843 seeds than the Sakha-1 seeds

(635.59mg/100g) in Table (2). Tannins and phytic acid are considered to be the most important anti-nutritional in faba beans. Legumes, grains and 27 types of oil seeds are rich in phytic acid, according to studies by El-Sheikh *et al.*, (1999) and Alonso *et al.*, (2000). The levels of phytic acid in faba beans were lower than those published by Alonso *et al.*, (2000) who recorded 2170 mg/100g, but these were comparable to those obtained by Carnovale *et al.*, (1987), who found a range of 710-1150 mg/100g.

Effect of soaking on chemical composition of faba bean seeds:

Results given in Table (3) showed that the impact of soaking in moisture, total ash, crude lipid, crude fiber, crude protein and total carbohydrates in faba bean seeds. Soaking of faba bean variety seeds in distilled water for (24, 48 and 72 hours) significantly lowered ash content and crude fiber compared with control. These decrements might be attributed to their prolixity in the water during the soaking process.

Given data in Table (3) indicate that soaking of faba bean varieties seeds in distilled water for (24, 48 and 72 hours) increase moisture, lipid, protein and total carbohydrates compared with control. These results agree with that obtained by Bau *et al.*, (1997) for soybean. It could be explained the decrease and the loss of compounds by diffusion way in water. Augustin, (1989) reported that during soaking seeds of legumes many constituents were decreased.

Effect of soaking on some secondary metabolites concentrations of Faba bean seeds

Results of the impact soaking of faba bean seeds on some secondary metabolites (TPCs, TFs, total alkaloids, phytic acid and tannins mg/100g) are shown in Table (4). Soaking in distilled water for (24, 48 and 72 hours) reduced the values of (TPCs, TFs, total alkaloids, phytic acid and tannins mg/100g), and the reduction proportionally were increased with increasing soaking timetable (4). According to Boateng *et al.*, (2007) soaking seeds in water only significantly reduced the amount of phenolic compounds content present in broad bean overall. Similar studies reported that the soaking seeds in water reduced tannins contents Oliveira *et al.*, (2001) and Mubarak, (2005).

The amount of polyphenols and flavonoids, in faba bean seeds was significantly reduced after soaking (Meital, *et al.* 2023). Ibrahim *et al.*, (2002) indicated that the soaking and germination processes reduced the amount of phytic acid in legume seeds. Rehman and Shah, (1996) on the other hand, reported that different procedures led to a noticeable decrease in the overall phenolic compounds levels of several legumes. These reductions are mainly due to increased level of phytase activity which causes phytates to be soluble (Camacho *et al.*, 1992).

Abdel-Aleem *et al.*, (2019) reported a similar set of findings. Soaking in distilled water reduced phytic acid, polyphenol and tannins of lentils reported that by Vidal-Valverde *et al.*, (1994). According to Alonso *et al.*, (2000) soaking faba bean in distilled water dramatically reduced the amount of phytic acid, polyphenol and tannins. They also reported that soaking processes hardly decreases the in total alkaloids by Onwuka (2006) and Mahmoud *et al.*, (2016).

Effect of germination on chemical composition of faba bean seeds

The results of moisture, total ash, crude fiber, crude lipid, crude protein, carbohydrates and control as affected by germination of faba bean varieties Sakha-1 and Giza-843 are presented in Table (5), Figures (1) and (2).

From these data showed that germination processes lead to a reduction in moisture, crude lipid and total carbohydrates. These decreases might be explained by their utilization as an energy source to launch the germination process. However, germination caused increased in ash content, crude fiber and crude protein compared with control. This rise was mostly brought on by the germination process's use of seed components. A similar result were reported by El-Beltagy, (1996), Bau *et al.*, (1997) and El-Adawy (2002) in germinated mung bean seeds, soybean seeds, and chickpea seeds, respectively.

Effect of germination on some secondary metabolites concentrations of Faba bean seeds:

The Impact of germination on some secondary metabolites concentrations (TPCs, TFs, TAs, phytic acid and tannins contents) of germinated faba bean seeds is showed in Table (6). These data confirmed that the germination process resulted in a reduction of all the secondary metabolites concentrations mentioned above compared with control Figures (3) and (4).

According to Ibrahim *et al.*, (2002), the phytic acid content of legume seeds decreased during the germination process. Vidal-Valverde *et al.*, (1994) indicated that the germination of two lentil varieties is reduced phytic acid and tannin by 44% and 66%, respectively. According to Alonso *et al.*, (2000) germination lasting 24 and 72 hours causes the tannin and phytic acid content of faba bean seeds to decrease. Similar results were described by El-Adawy (2002) for germinated chickpea. Mahmoud *et al.*, (2016) found that the germination processes decreases the total phenolic and total alkaloids in lupine seeds.

CONCLUSION

Legumes, particularly faba bean seeds, are considered a good source of protein, carbohydrate and crude fiber. Consuming of faba bean seeds has numerous health benefits as the seeds are rich in natural total phenolic compounds and alkaloids with antioxidant activities and anti-mutagenic properties.

Also, it could be concluded that soaking process for (24, 48 and 72 hours) and germination process were necessary to produce a significant reduction in total phenolic compounds, total flavonoids, total alkaloids, phytic acid and tannins levels in both varieties of faba bean under study. The soaking and germination processes are simple and inexpensive in energy, terms of time, and fuel and can be used to treat a variety of pulses at home.

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Table 1: Analytic statements for proximate composition of some variety seeds Faba bean.

Constituents	Sakha-1(%)	Giza-843(%)
Moisture	7.31±0.5505	7.53±0.2527
Total ash content, TAC	3.29±0.1623	3.75±0.4375
Crude fiber, CF	5.72±0.9078	6.81±0.5383
Crude lipid, CL	1.65±0.0925	1.30±0.07
Total nitrogen, N	5.03±0.9127	4.80±0.12
Crude protein CP	31.44±0.2568	30.25±0.4375
Total Carbohydrates	50.59±0.2745	50.61±0.2863

Data are expressed as mean ± SD values given represented means of three determinations.

Table 2: Quantitative analysis of secondary metabolites in faba bean seeds.

Constituents	Sakha-1	Giza-843
TPCs (mg/100g)	355.73±9.9787	350.95±8.5575
TFs (mg /100g)	80.23±3.6158	81.34±4.3668
Total alkaloids (TAs) (mg/100g)	90.00±1.030	89.53±3.4327
Phytic acid(mg /100g)	872.52±7.9312	831.52±4.1713
Tannins (mg/100g)	635.59±3.8143	653.25±5.6875

Data are expressed as mean ± SD values given represented means of three determinations

Table 3: Impact of soaking on chemical composition of faba bean seeds

Treatments	Moisture (%)		Total ash content (%)	
	Sakha-1	Giza-843	Sakha-1	Giza-843
Control	7.33	7.49	3.30	3.71
Soaking for 24 h	7.37	7.51	3.15	3.57
Soaking for 48 h	7.40	7.58	3.09	3.41
Soaking for 72 h	7.43	7.62	3.00	3.05
LSD0.05	A 0.015	B 0.279	A 0.504	B 0.644
	Crude fiber (%)		Crude lipid (%)	
Control	5.13	5.80	1.49	1.35
Soaking for 24 h	5.01	5.43	1.52	1.39
Soaking for 48 h	4.89	5.28	1.55	1.43
Soaking for 72 h	4.76	5.32	1.64	1.51
LSD0.05	A 0.856	B 0.561	A 0.219	B 0.172
	Crude protein (%)		Total Carbohydrates (%)	
Control	31.5	30.61	51.25	51.04
Soaking for 24 h	31.60	31.03	51.35	51.07
Soaking for 48 h	31.65	31.24	51.42	51.09
Soaking for 72 h	31.70	31.36	51.47	51.14
LSD 5%	A 12.400	B 21.429	A N. S	B 1.642

Data are expressed as mean ± SD values given represented means of three determinations.

Table 4: Impact of soaking on secondary metabolites of faba bean seeds

Treatments	TPCs mg/100g		TFs mg /100g	
	Sakha-1	Giza-843	Sakha-1	Giza-843
Control	350.37	349.0	80.23	81.5
Soaking for 24 h	343.5	334.2	79.07	81.0
Soaking for 48 h	340.3	335.1	75.60	80.7
Soaking for 72 h	337.7	331.5	72.6	74.87
LSD 5%	5.072	18.059	4.44	13.629
	Total alkaloids (mg/100g)		Phytic acid (mg/100g)	
Control	89.81	90.92	873.00	751.22
Soaking for 24 h	65.00	68.70	831.09	729.14
Soaking for 48 h	53.40	42.50	808.03	711.07
Soaking for 72 h	40.70	35.32	798.42	703.00
LSD 5%	9.22	68.524	10.917	1.132
	Tannins (mg/100g)			
Control	630.04	635.20		
Soaking for 24 h	620.21	622.50		
Soaking for 48 h	612.00	613.07		
Soaking for 72 h	608.03	608.05		
LSD 5%	3.589	37.299		

Data are expressed as mean \pm SD values given represented means of three determinations

Table 5: Impact of germination on chemical composition of faba bean seeds

Treatments	Control		germination	
	Sakha-1	Giza-843	Sakha-1	Giza-843
Moisture	7.33 \pm 0.5614	7.50 \pm 0.4330	7.14 \pm 0.6388	7.47 \pm 0.2491
Total ash content	3.35 \pm 0.3475	3.72 \pm 0.5465	3.40 \pm 0.360	3.85 \pm 0.8725
Crude fiber	5.75 \pm 0.7166	6.80 \pm 0.5365	5.79 \pm 0.5023	7.04 \pm 0.6948
Crude lipid	1.69 \pm 0.1035	1.30 \pm 0.070	1.51 \pm 0.2503	1.19 \pm 0.0589
Crude protein	31.75 \pm 0.8208	30.30 \pm 0.4985	32.15 \pm 2.0175	31.60 \pm 2.080
Total Carbohydrates	50.13 \pm 0.2533	50.38 \pm 0.3586	50.01 \pm 1.5175	51.23 \pm 1.8487

Data are expressed as mean \pm SD values given represented means of three determinations

Table 6: Impact of germination on secondary metabolites concentrations of faba bean seeds

Treatments	Control		germination	
	Sakha-1	Giza-843	Sakha-1	Giza-843
TPCs mg/100g	350.00 \pm 20.431	349.0 \pm 17.356	290.25 \pm 19.291	295 \pm 19.00
TFs mg /100g	80.23 \pm 14.256	81.5 \pm 10.352	68.03 \pm 9.273	70.03 \pm 13.361
Total alkaloids mg/100g	89.25 \pm 8.915	90.0 \pm 6.35	79.30 \pm 9.97	81.15 \pm 1.518
Phytic acid mg/100g	873.81 \pm 11.580	751.62 \pm 9.630	839.06 \pm 12.369	747.06 \pm 7.549
Tannins mg/100g	630.32 \pm 15.32	635.83 \pm 14.96	621.37 \pm 65.311	619.82 \pm 18.017

Data are expressed as mean \pm SD values given represented means of three determinations

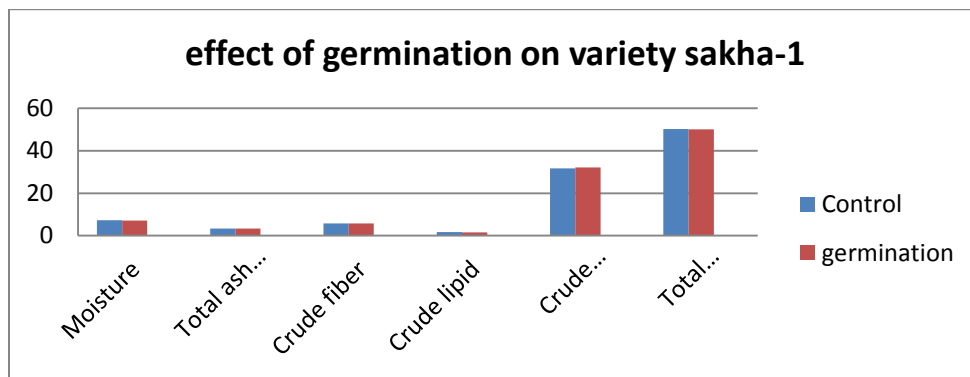


Figure 1: Impact of germination on chemical composition of Sakha-1

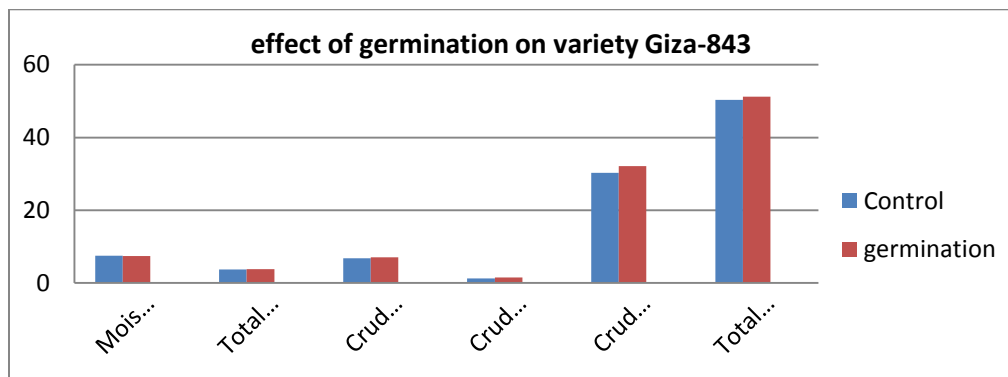


Figure 2: Impact of germination on chemical composition of Giza-843

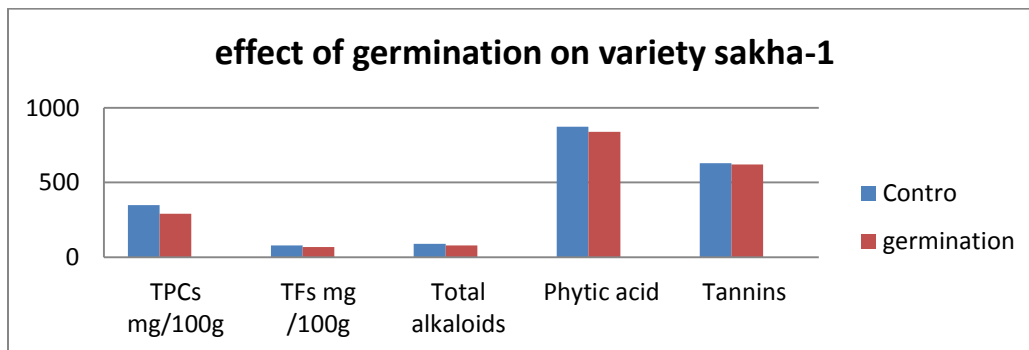


Figure 3: Impact of germination on variety Sakha-1

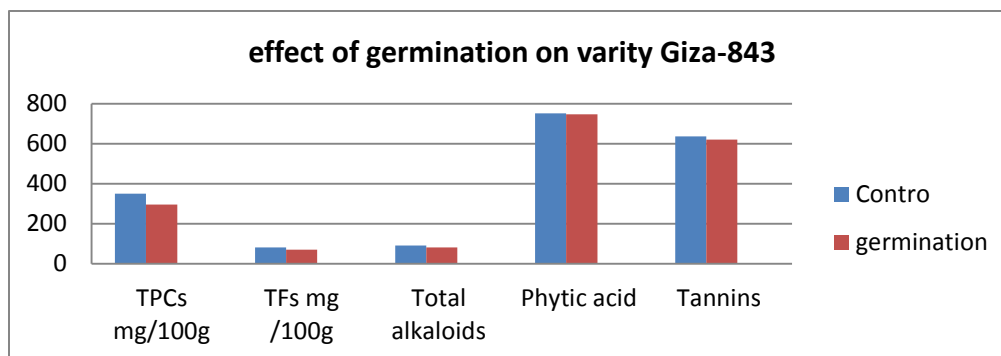


Figure 4: Impact of germination on variety Giza-843

دراسة المكونات الكيميائية والمركبات الثانوية لبذور الفول البلدى المتأثرة بالنقع والإنبات

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الملخص العربى

إستخدم فى هذه الدراسة بذور صنفين من الفول البلدى هما سخا-1 و جيزه 843 وتم نقع البذور فى الماء المقطر لمدة 24 ، 48 ، 72 ساعة ثم أجريت عملية الإنبات. تم تقدير المكونات الكيميائية من الرطوبة ، والدهون ، والنيروجين الكلى ، والبروتين ، وكذلك الكربوهيدرات الكلية. كذلك تم تقدير المركبات الفينولية والفلافونيدات والقلويدات وحمض الفيتيك والتانينات. أوضحت النتائج أن بذور الصنف جيزه 843 تفوقت فى قيم المكونات الكيميائية على الصنف سخا-1 حيث إحتوت بذور الصنف جيزه 843 على 7.53، 3.75، 6.81، 50.61 % من الرطوبة والرماد والألياف الخام والكربوهيدرات الكلية. بينما إحتوت بذور الصنف سخا-1 على 1.65، 5.03، 31.44 % من الليبيدات والنيروجين الكلى والبروتين. كذلك أشارت النتائج أن بذور الصنف سخا-1 تحتوى على قيم عالية مقارنة بالقيم المتحصل عليها من بذور الصنف جيزه 843 وذلك من الفينولات الكلية والقلويدات وحمض الفيتيك والفلافونيدات، بينما إحتوت بذور الصنف جيزه 843 على كمية أكبر من التانينات (653.25 ملجم/100جم) مقارنة بمحتوى بذور الصنف سخا-1 (635.59 ملجم/100جم). أكدت النتائج أن نقع البذور لمدة 24،48،72 ساعة يحدث تغيرات معنوية ونقص فى قيم المكونات الكيميائية وكذا المركبات الثانوية. كذلك أدى إنبات البذور بعد نقعها إلى حدوث تغيرات معنوية أيضا فى المركبات الفينولية والفلافونيدات والقلويدات وكذا محتواها من حمض الفيتيك.

الكلمات الاسترشادية: