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Effect of solvent concentrations on antioxidant activity and biochemical parameters of adzuki bean (*Vigna angularis*) sprouts at different germination times

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Abstract

In this study were determined DPPH radical scavenging power, metal-ion chelating activity, gamma aminobutyric acid and phytic acid levels, total phenolic substance content, extraction yield in water and various organic solvents (acetone, n-hexane and ethanol), concentrations (50, 70 and 90%) and germination times (0, 24, 48, 72, 96 and 120 h) of adzuki bean (Vigna angularis) sprouts. The extraction yield ranged from 11.47% (H3) to 28.55% (E1). The highest DPPH radical scavenging capacity was determined at E2 concentration (2.978 µmol/g DW) for 120 h (P<0.05). E3 (1.744 mg EDTA equivalent/100 g) and A3 (1.145 mg EDTA equivalent/100 g) showed the highest metal chelating activity after 48h of germination. This activity decreased in the germination period from 48 h to 120 h (P<0.05). When different solvent concentrations were compared no significant change (P>0.05) in gamma aminobutyric acid and phytic acid contents at 0, 24 and 48 h analyses. The highest gamma aminobutyric acid content was detected to A1 (67.29 mg/100 g DW) and H1 (69.17 mg/100 g DW) concentrations at 120 h (P<0.05). No significant changes were found in total phenol content in all solvent concentrations in 48 h (P>0.05). At the end of 120 h, total phenolic components were determined in the lowest levels in W and the highest concentrations in E2 (P<0.05). These results showed that adzuki bean seeds may be more effective in these parameters, depending on the increase in the activities of bioactive components and the decrease in anti-nutritional factors, and the concentration in water and aqueous organic solvents with the increase of germination time.

Keywords: *Germination time, adzuki bean, solvent concentrations, sprout, antioxidant activity, phytic acid, gamma aminobutyric acid*

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Farklı çimlenme sürelerinde adzuki fasulyesi (*Vigna angularis*) filizlerinin antioksidan aktivite ve biyokimyasal parametreleri üzerine çözücü konsantrasyonlarının etkisi

Öz

Bu çalışmada, farklı çimlenme sürelerinde (0, 24, 48, 72, 96 ve 120 saat) elde edilen adzuki fasulyesi (Vigna angularis) filizlerinin, su ve çeşitli organik çözücülerde (aseton, n-heksan ve etanol) ve farklı konsantrasyonlarda (% 50, 70 ve 90) ekstrakte edilerek DPPH radikal süpürme gücü, metal-iyon şelatlama aktivitesi, gama aminobütirik asit ve fitik asit düzeyleri, toplam fenolik madde içeriği belirlenmiştir. Ekstraksiyon verimi %11.47 (H3) ila %28.55 (E1) arasında değişmiştir. En yüksek DPPH radikal süpürme kapasitesi, 120 saat boyunca E2 konsantrasyonunda (2.978 µmol/g DW) belirlendi (P<0.05). E3 (1.744 mg EDTA eşdeğeri/100 g) ve A3 (1.145 mg EDTA eşdeğeri/100 g), 48 saatlik çimlenmeden sonra en yüksek metal şelatlama aktivitesini gösterdi. Bu aktivite, çimlenme periyodunda 48 saatten 120 saate düştü (P<0.05). Farklı çözücü konsantrasyonları karşılaştırıldığında, 0, 24 ve 48 saatlik analizlerde gama aminobütirik asit ve fitik asit içeriklerinde önemli bir değişiklik (P>0.05) olmadı. En yüksek gama aminobütirik asit içeriği, 120. saatte A1 (67.29 mg/100 g DW) ve H1 (69.17 mg/100 g DW) konsantrasyonlarında tespit edildi (P<0.05). 48 saatte tüm solvent konsantrasyonlarında toplam fenol içeriğinde önemli bir değişiklik bulunmadı (P>0.05). 120 saat sonunda, toplam fenolik bileşenler su ekstraksiyonunda en düşük seviyelerde ve E2'de en yüksek konsantrasyonlarda belirlendi (P<0.05). Bu sonuçlar, çimlenme süresinin artmasıyla birlikte biyoaktif bileşenlerin aktivitelerindeki artışa, anti-besinsel faktörlerin azalmasına, su ve sulu organik çözücülerdeki konsantrasyona bağlı olarak adzuki fasulyesi tohumlarının belirlenen parametrelerde daha etkili olabileceğini göstermiştir.

Anahtar kelimeler: *Çimlenme süresi, adzuki fasulyesi, çözücü konsantrasyonları, filizlenme, antioksidan aktivite, fitik asit, gama aminobütirik asit*

1. Introduction

Recently, people's awareness about healthy nutrition has increased their orientation to natural food sources. This orientation has enabled the rapid growth of the functional food sector, which has gained popularity thanks to the beneficial components it contains, which attracts the attention of the consumer who is trying to achieve a healthy life through diet [1]. Studies have shown that functional food industry in the world has reached a size of 30-60 billion dollars and has an average increase of 10% every year [2]. Studies have shown that as a result of the sprouting of seeds with high nutrient and low water content, the bioavailability of vitamins, proteins and various nutritional elements increases, while the effectiveness of toxins and enzyme inhibitors decreases [3]. Seed sprouts are a simple, inexpensive and environmentally friendly method for producing plant-derived foods with functional properties. Meanwhile, the nutritional and medicinal values of seeds may change during sprouting. For example; the levels of anti-nutritional and anti-digestibility factors such as protease inhibitors and lectins can be reduced, and also the accumulation of secondary metabolites characterized as antioxidants can be induced [4]. The most

important anti-nutritional factor seen in seeds with high nutrition is amount of phytic acid. Phytic acid and some polyphenolic compounds, which prevent nutrients from being metabolized by binding minerals, cause difficulty in digestion for the organism with their anti-nutritional properties [5]. These anti-nutritional factors can be partially or completely stopped by applying various processes. Sprouting helps regulate physiological activities as well as biochemical events such as the conversion of carbohydrates to simple sugars, the breakdown of proteins, oxidation of lipids, nucleic acid and protein synthesis, cell differentiation [6]. At the end of these physiological processes, it has been determined that seeds have higher nutritional value by obtaining high biological value protein content, higher polyunsaturated fatty acid content and vitamin content. Therefore, germination can be thought of as a type of pre-digestion that helps break down high molecular complex materials into their building blocks.

Gamma aminobutyric acid (GABA) is a non-protein amino acid found in plants [7]. The amount of GABA in plants can be affected by endogenous factors such as adverse conditions, hormonal changes, factors affecting growth and development, cytoplasm pH, nitrogen and carbon levels [8], as well as exogenous factors such as seed germination mechanical effect, acidity, salt, extreme heat and cold, drought, viral and microbial infections increase in the content of polyphenols and various stress sources. In the literature research, there are no reports on how germinated seeds change the GABA amounts and phenolic component ratios at different solvents and concentrations [9].

Leguminous grains, which are among the edible seeds, constitute an important part of the human diet. As the FAO notes, only a few hundred of the world's more than 50,000 edible plant species are important and nutritious food sources. Legumes are of global importance due to their high protein, vitamin, mineral and dietary fiber. This food group generally contains amino acid composition complementary to cereals. Therefore, consumption of legumes improves the protein quality in the daily diet of people in some developing countries [10]. Adzuki bean (*Vigna angularis*) from legume sprouts is widely preferred because it is easy to produce sprouts. It is consumed as the second most popular legume after soybean in Asian countries, especially in Japan, and is among the 12 most important legumes grown worldwide [11]. Edible seeds have many biological functions thanks to their rich phytochemical content. Various analysis methods are used to determine these components in plants such as soxhlet extraction, maceration, supercritical fluid extraction, and ultrasound-assisted extraction. Besides, the extraction yield and antioxidant activity depend not only on the extraction method but also on the solvent used for extraction. The presence of various antioxidant compounds with different chemical properties and polarities may or may not be soluble in a particular solvent [12]. The aim of this study was to determine effects of extraction of adzuki bean at different germination times such as 0 h (pre-sprouting) and 24, 48, 72, 96 and 120 h, water and various organic solvents (acetone, *n*-hexane and ethanol) and concentrations (50, 70 and 90%) on antioxidant activity and some biochemical parameters. Furthermore, with this study, it is aimed to determine the use value of sprouted foods as healthy functional foodstuffs and to obtain basic research data for the development of high added value materials that can be used industrially.

2. Material and methods

2.1 Seed sterilization and sprouting

Adzuki bean seeds to be used in the study were obtained from a local legume seller in Aksaray, Turkey. To prevent the formation of microbial activities in the seeds, seed

sterilization was performed in 0.07% sodium hypochlorite (NaClO) solution and 70% ethanol for 3 minutes. For hydration before the sprouting stage, seeds were soaked at room temperature (25°C) at a 1:10 ratio of seed weight (g)/water volume (mL) for up to 12 h. The seeds soaked during germination were provided with the necessary daylight, temperature (25ºC), humidity (80%) in an artificial climate incubator. To promote growth, the seeds were soaked daily with deionized water to retain relatively high humidity. Samples taken randomly at 0 (pre-sprouting), 24, 48, 72, 96 and 120 h of germination were frozen at -20ºC. The freeze-dried samples were ground in a blender.

2.2 Extraction and solvent fraction procedure

Lyophilized adzuki bean sprouts (5 g) were homogenized with acidified methanol (10 mL, 0.1% HCl). After standing for approximately 1 h (4°C), it was subjected to centrifugation (10.000 rpm, 30 minutes). Up to 50 mL of distilled water was added to the supernatants obtained. Solvent fractions were prepared as follows; 0.5 g of the prepared extract was taken from the sample, and it was extracted at 50, 70 and 90% concentrations by adding 10 mL of each solvent (acetone, *n*-hexane and ethanol) at 200 rpm with the help of a mixer for 1 day at 24 ± 1 °C. Then, the obtained extracts were centrifuged at 2500 rpm for 25 min. and the supernatant portions were stored in a deep freezer (-20°C) until analysis. The abbreviations of solvent and concentration used in the study are expressed as follows: water (W), acetone 50% (A1), 70% (A2), 90% (A3); *n*-hexane 50% (H1), 70% (H2), 90% (H3); ethanol 50% (E1), 70% (E2), 90% (E3).

2.3 Extract yield percentage

Extraction efficiency is the calculation of the yield of the solvent to separate the specific components from the original product. This yield is calculated by comparing the amount of obtained dry extract as the mass with the total amount of the original product and is determined as a percentage (%).

2.4 DPPH (2,2 Diphenyl-1-picryl-hydrazyl) radical scavenging activity

Free radical scavenging activity was performed according to the method described by Brand-Williams [13]. DPPH solution ($6x10^{-5}$ M) was prepared with ethanol. Then, 1 mL of DPPH solution and 500 µL of seed sprout extract were mixed. The solution was incubated for 45 minutes at room temperature and then measured by spectrophotometer at 517 nm. Free radical scavenging activity was expressed as µmol/g.

2.4 Metal-ion chelating activity

Metal chelating activity was determined according to the method of Dinis et al. [14]. The stock solution was prepared as 800 µL extract +100 µL solution (2 mM $FeCl_2$). To initiate reaction, 400 µL (5 mM) of ferrozine was added and incubated at room temperature for 10 minutes. Absorbance values were measured spectrophotometrically at 562 nm. Results were expressed as mg EDTA equivalent/100 g extract.

2.5 GABA content

Adzuki bean sprouts was analyzed with the plant GABA ELISA Assay Kit (MBS2700393, MyBiosource company, Southern California, San Diego (USA). The procedures were applied in full compliance with kit usage instructions determined by the company. The sprout homogenates prepared for analysis were carried out at a concentration of 0.1 M (pH=7.2) in cold conditions. Results are expressed as mg/100 g DW.

2.6 Phytic acid levels

Phytic acid levels in seed sprouts were analyzed by the method specified by Haug and Lantzsch (1983) [15]. A mixture was prepared by adding 1 mL of ferric solution (0.2 g ferric ammonium sulfate dissolved in concentrated HCl (98%, 100 mL) and the solution made up to 1 L) onto the supernatant (0.5 mL) obtained from the extraction. The mixture was heated for 30 min., then cooled in ice water for 10 minutes. 0.5 mL of sample from the stock solution was added to the 2,2'-bipyridine solution (1 mL). The final mixture prepared was measured spectrophotometrically at 519 nm and the results were expressed as mg/100 g.

2.7 Total phenolic substance

The total phenol amount was calculated by the Folin-Ciocalteu method using the gallic acid standard [16]. 2.5 mL of Folin-Ciocalteu and 2 mL of 40 g/L (V/V) sodium carbonate were added to extracts (500 mL). After the samples were allowed to stand in the dark for 2 h, the absorbance was measured by spectrophotometer at 764 nm. The results were expressed as mg GAE/100 g FW.

2.8 Statistical analysis

The study was carried out in 3 replications and the statistical analysis of the research data, SPSS (20.0) statistical program was used. Differences found statistically significant in the analysis of variance data were compared with Duncan multiple comparison test ($P < 0.05$).

3. Results and discussion

3.1 Extraction yield

Extraction efficiency can be affected by the nature of the phytochemicals in the composition of the herbal products, the extraction method applied, particle size of material, the solvent used, as well as other interacting substances. It may also vary depending on polarity, pH, temperature, extraction time. In this study, extracts of adzuki bean sprouts were obtained by using water and different concentrations of aqueous acetone, aqueous *n*-hexane, aqueous ethanol (50, 70 and 90%). Extraction yields were evaluated from 0 h to 120 h at all solvent concentrations. The extraction yield increased at 120 h for all concentrations compared to 0h. The yield range in the concentrations ranged from 11.47% (H3) to 28.55% (E1) (Figure 1). The sprouting times of 120 h and the extraction yields of various solvents in % from the highest to the lowest, respectively; E1> A1 > E2 > H2 >A2 > H1 > W> E3 >A3 > H3. According to obtained results, it can be said that as polarity of solvent selected for extraction increases, extraction efficiency increases in parallel. These data also indicate that the increase in the amount of water in the solvents also increases the extraction efficiency. This may contribute to an increase in yield as a result of the extraction of compounds other than phenolic groups.

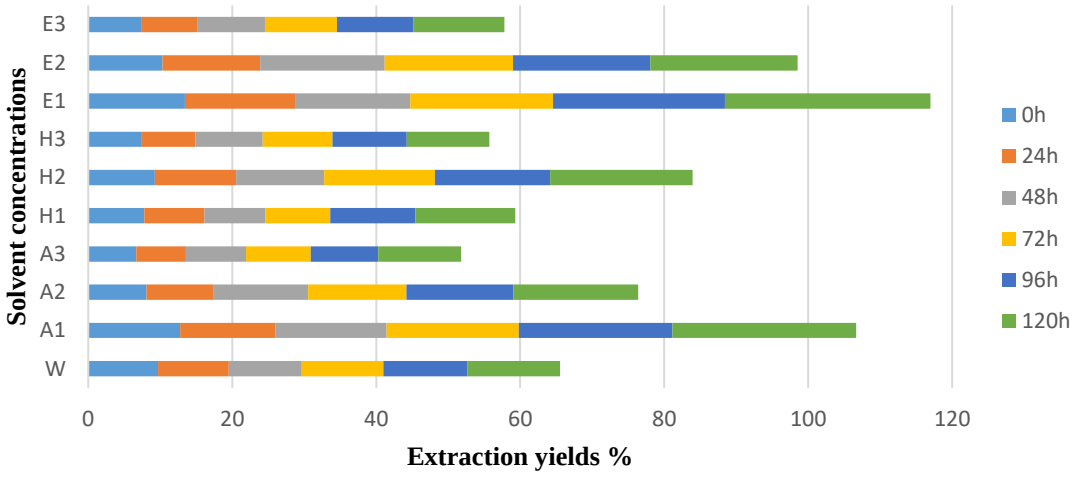

Figure 1. Extraction yield (E.Y) of lyophilized adzuki bean sprout extract at germination times at different solvents and concentrations. Extraction yield as 100 x (g dry extract/g adzuki bean sprout)

3.2 DPPH radical scavenging activity

The antioxidant activities of seed and sprouted adzuki bean seeds at different germination times at various solvent concentrations were determined by measuring the DPPH radical scavenging ability (Figure 2). Seed extracts (0 h) in solvent concentrations differed in terms of antioxidant activities. The highest radical scavenging activity at 0 h was detected in E2 (0.754 µmol/g DW). DPPH scavenging activity increased at all solvent concentrations after 120 hours of germination compared to raw seed. E2 and A3 concentrations had the highest DPPH radical scavenging capacity with of 2.978 and 2.541µmol/g DW, respectively (Figure 2). The researchers reported that bean species had low pre-germination antioxidant levels and increased capacity with increasing time [3,4,17,18]. Han et al. (2013) explained highest DPPH radical scavenging activity at various solvent concentrations in the germination periods (4, 8 and 12 days) of radish buds (*Raphanus sativus*) sprouts in the water extract (86.67%) and lowest scavenging in the acetone extract (77.23%) on the 4th day [19]. Wu et al. (2012) found ORAC activity of 800-2000 mmol TE/100 g FW before sprouting and 500-2000 mmol TE/100 g FW after germination of adzuki bean seeds for 1-4 days [18]. The DPPH activity of mung bean and soybean, a different bean species, was measured at 24-120 h, 0.03 before germination and 0.01 after germination, 0.12-0.20 and 0.03-0.13mmol TE/g DW, respectively [3]. In another study, it was reported that the DPPH activity of jack bean (*Canavalia ensiformis* L.) seeds germinated for 4 days was measured 1.50 TE/g DW before and 1.70 mmol TE/g DW after germination [4]. In the study of Pajak et al. (2014), the DPPH activity of Mung bean sprout (*Vigna radiata* L. Wilczek) was found to be 0.11 at seed stage and 1.41 mg TE/g DW at the end of 5 days [20]. The increase of DPPH with germination time, may be related to the fact that the phenolic components of bean varieties differ in quality and quantity. However, we can say that in the current study, besides the germination times, the solvent and concentration differences used in the extraction also cause positive effects on the radical scavenging activity.

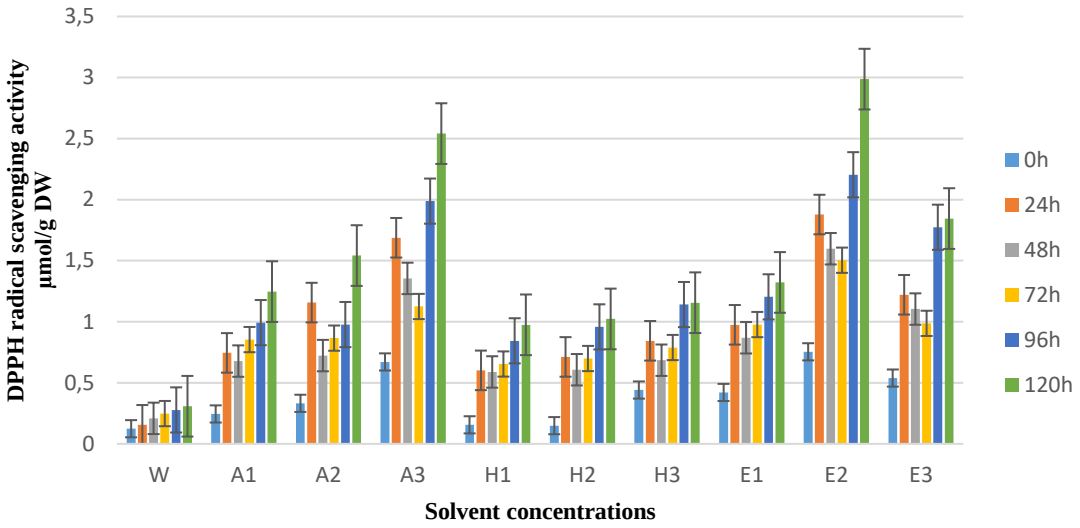

Figure 2. DPPH activity of adzuki bean sprouts at different solvent concentration extract and germination times. Results are expressed as µmol/g DW.

3.3 Metal-ion chelating activity

The metal chelating activities of solutions of adzuki bean sprouts prepared at different solvent concentrations were shown in Figure 3. In this method, which is expressed as the decolorization rate of red color in reaction mixture depending on reducing capacity of various iron ions in extracts, results were expressed as mg EDTA equivalent/100 g. It was determined that E3 (1,744 mg EDTA equivalent/100 g) and A3 (1,145 mg EDTA equivalent/100 g) showed the highest metal chelating activity after 48 h of germination from acetone, *n*-hexane and ethanol extracts. Decreases in activity were observed from the 48 h germination period to 120 h. When the biochemical data of living organisms are examined, it has been found that increased iron levels increase the risk of various vascular diseases, cancer derivatives, and some neurological system disorders, thus increasing the risk of various diseases [21]. The high reactivity of iron, which is one of the transition metals, gives it a pro-oxidant feature. Although Fe^{3+} (ferric) ion is ten times lower than Fe^{2+} in terms of reactivity, it produces peroxide. Reducing the iron-ion concentration in the organism can prevent the occurrence of fenton-type reactions, reducing ROS production and thus preventing cellular molecular damage [22].

We need to consider the fact that iron-mediated free radical production, which causes DNA damage and lipid peroxidation, and the fact that increased iron in the blood goes beyond its oxygen-carrying function and creates toxicity. Lentil cultivars are known to have the highest Fe^{2+} content among seeds. It was determined that these concentrations decreased significantly after germination. It has been reported in studies that the germination factor and Fe levels decreased in various legume seeds such as soybean and kidney bean [23]. The results of the study showed that the post-germination decline in iron levels in seeds may vary depending on germination times and other environmental factors. Adzuki bean seed sprouts showed a strong chelating effect on iron (Fe^{2+}) ions at various solvent concentrations. In this study, we can say that adzuki bean seed sprouts are effective in terms of their ability to reduce iron ions in the solution, especially after 48 h, with the effect of different solvents and concentrations at various germination times. The sprout extracts obtained from adzuki bean seeds under different stress conditions showed the highest Fe^{2+} chelating ability.

In a research, it has been stated that there is a synchronization between the phenolic component concentration in seed sprouts and iron ions. Contrary to the results of this study, it was stated that the total phenolic component concentration of broccoli and radish sprouts decreased in response to iron-ion chelating activity. In the same study, it was reported that the increase in the iron ion concentration of the shoots had an effect on growth and germination [6]. It has been shown that the iron concentrations used are not at concentrations that may adversely affect growth and germination. In addition, the increase in iron production in plants may cause photosynthesis to not be carried out regularly, resulting in oxidative and cellular damage [24].

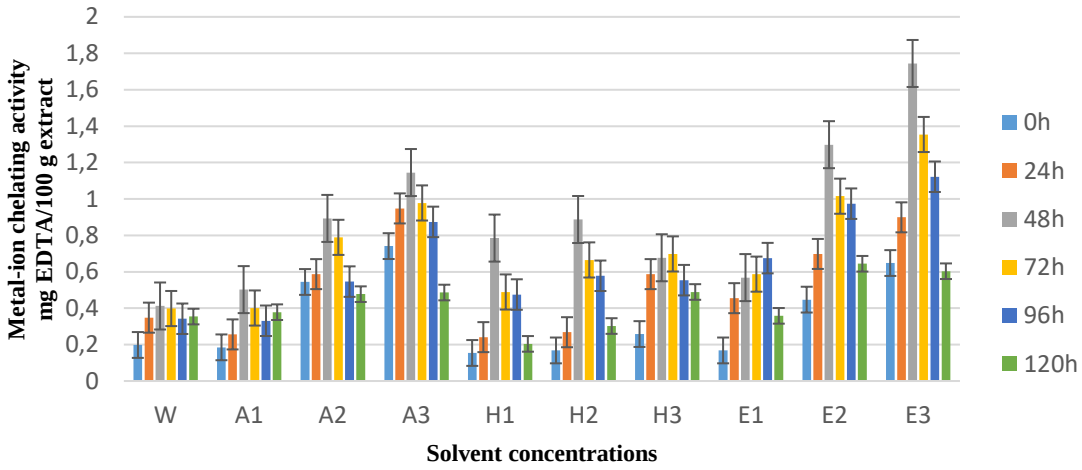

Figure 3. Metal-ion chelating activity of adzuki bean sprouts at different solvent concentration extract and germination times. Results are expressed as mg EDTA/100 g extract

3.4 GABA

Recent studies have emphasized importance of mechanisms that reveal the potential effects of bioactive components in seed sprouts. It has been shown that by sprouting, the accumulation of bioactive compounds such as phenolic compounds, glucosinolates, vitamins and GABA can increase. During the sprouting process, some enzymes produced or induced such as amylase, protease, phytase, and β-glucanase can help break down large molecules such as starch, protein, and GABA, dietary fibers and vitamins can be formed [25]. It has been reported that GABA provides beneficial effects in reducing blood pressure in terms of human health [26]. It has been reported that an increase in GABA production is observed in plants with the effect of stress factors such as hypoxia, acidity increase, cold stress, mechanical effect, insufficient or excessive water, and darkness [27]. Kihara et al. (2007) reported the accumulation of GABA in sprouted barley seeds. Although there have been studies on the determination of GABA levels in some plant seed sprouts under various stress and germination conditions, no research has been found examining the effects of germination times and solvent concentrations of seed sprouts on GABA accumulation [28]. The amount of GABA before and after germination of the sprouted extracts prepared at various solvents and concentrations from the samples taken at the end of different germination times is shown in Figure 4. When different solvents and their concentrations were compared, no significant change was observed in GABA content in 0, 24 and 48 h applications, while these values increased in 72, 96 and 120 h periods. Notably, A1 (67.29 mg/100 g DW) and H1 (69.17 mg/100 g DW) concentrations

were highest at approximately 120 h, compared to pre-sprouting (0 h) GABA levels. In another study, Ding et al. (2016) found that the GABA content of hulled rice increased significantly under hypoxic conditions (6 h) after germination [29]. Yang et al. (2015) reported that the GABA content increased (43.4%) when ultrasonic treatment of soybean sprouts was applied during the soaking treatment (30 minutes) [30]. Studies have reported that germination process increases GABA levels in adzuki beans, kidney beans, soybeans, lentils, lupines, sesame seeds, peas, brown rice, buckwheat and oats [10,31]. Also, Lin et al. (2015) examined the effect of different germination conditions on rice and reported that oxygen stress increased the GABA content by 15 times [32]. In another study, Saikusa et al. (1994) found that soaking on cultivar japonica rice and hybrid indica increased the GABA content 8-fold [33].

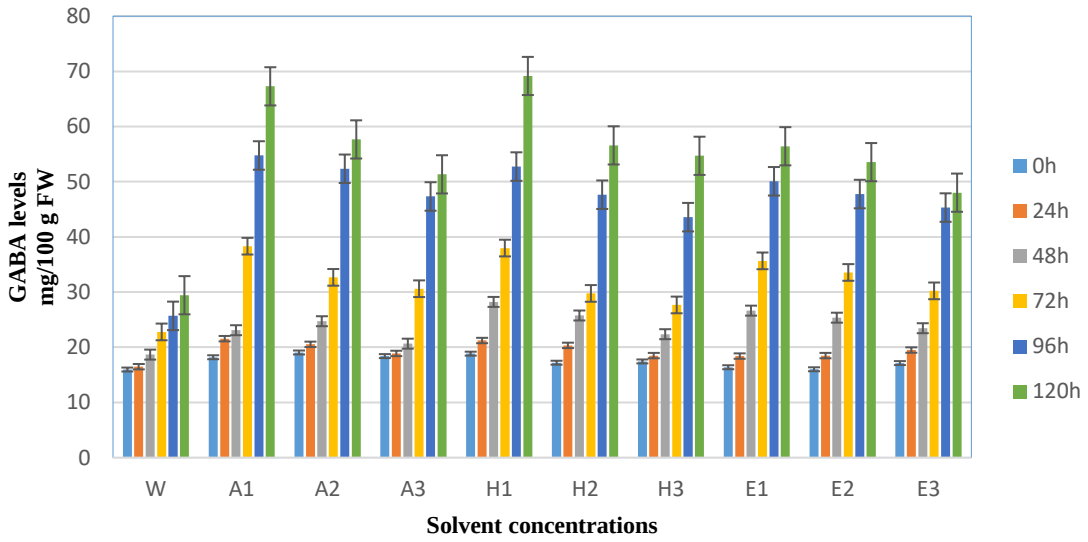

Figure 4. GABA levels of adzuki bean sprouts at different solvent concentration extract and germination times Results are expressed as mg/100 g DW.

3.5 Phytic acid

Products such as legume sprouts contain polysaccharides, proteins, dietary fibers, etc. They are very popular because they are rich in nutritional diversity. However, in addition to its nutritional properties, it also contains large amounts of phytic acid, which is known as an anti-nutritional factor. The reason why phytic acid is accepted as an anti-nutrient is that it chelates important metal ions (Ca^{2+}, Mg^{2+} and Zn^{2+}) needed by the organism and affects their bioavailability [34]. Therefore, the processing processes of foods rich in phytic acid in the food industry are critical. Phytic acid contributes to phosphorus metabolism by providing cell growth, energy demand, inositol phosphate and phosphatidylinositol phosphate signal transmission. Phosphorus accumulated in plant seeds constitutes 65-85% of the organism and is stored in the form of phytic acid [35]. Phytic acid degradation also affects bean sprout growth. It was determined that the effect of germination time and solvent concentration on phytic acid content after sprouting was significant ($P<0.05$). As seen in Figure 5, phytic acid contents did not change significantly at all solvents and concentrations up to 48 h germination ($P>0.05$). Germination was reduced by about 8-26% up to 48 hours, but reductions were found in the range of 52-73% at 120 h. However, at the end of the 120-hour germination period, the phytic acid content decreased by 73% at E1 and H1 concentrations. In a study, it was stated that chickpea sprouts germinated with chickpea grains for 4 days contain higher oil

and ascorbic acid, lower phytic acid levels, and increase protein digestibility and antioxidant capacity [36]. Doblado et al. (2007) reported that the vitamin C content of the sprouted cowpeas increased by 58-67% and the phytic acid levels decreased after sprouting [37]. In the same study, they determined that the antioxidant activity reached the highest levels in 6-day-old sprouts. They announced that after 96 h of germination, the phytic acid content was reduced by 50%. Ghavidel and Prakash (2007) reported that the phytic acid content of some leguminous (lentil, cowpea, mung beans and chickpea etc.) seeds decreased by 18-21% after germination [38]. In another study, it was determined that phytic acid levels of chickpea (*Cicer arietinum* L.) sprouts extracted with methanol decreased from 1.01% to 0.6% after 48 h of germination, and this value reached a maximum decrease of 0.9% after 120 h [39]. In the current stud, phytic acid content decreased by 26.6% after 48 h at E1 and H1 concentrations. At the end of 120 h, these values reached approximately 73%. These results showed that decrease in phytic acid content may cause a maximum decrease with the effect of germination time of the seeds as well as solvent from which they are extracted and their concentrations.

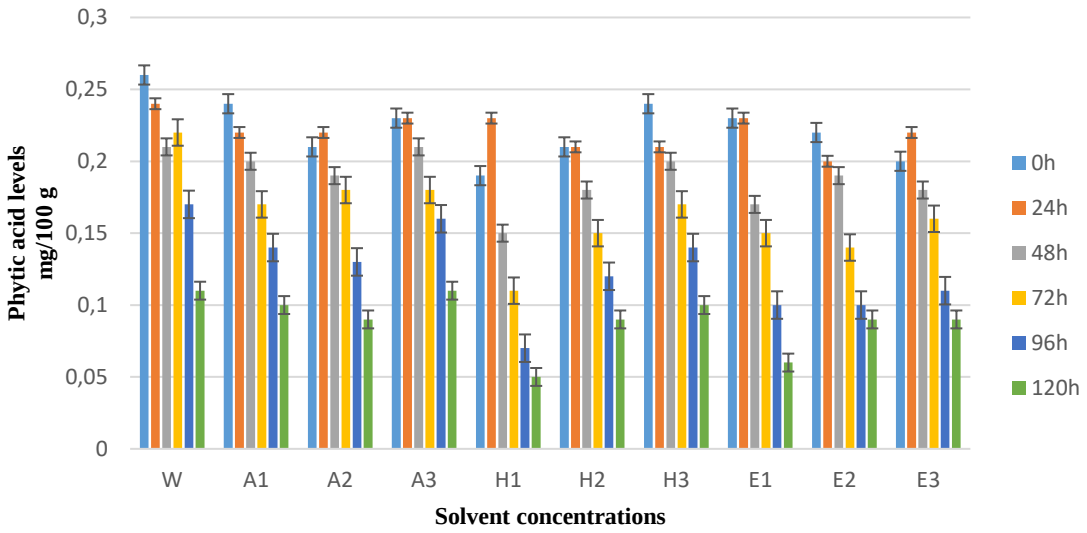

Figure 5. Phytic acid ratios of adzuki bean sprouts at different solvent concentration extract and germination times. Results are expressed as mg/100 g.

3.6 Total phenolic substance

Seed sprouts synthesize phenolic compounds under normal growth conditions, strengthen their defense mechanisms and maintain their vitality during germination. While physiological events such as sprouting occur, functional products occurring in foods may undergo structural changes. Recent studies are on mechanisms to increasing accumulation of phenolic compounds and other bioactive compounds in sprouts [10,40]. Plant polyphenols are highly electron-rich components and contribute to antioxidant mechanisms [40]. Among the most common phenolics analyzed in edible seeds and sprouts are phenolic acids, flavonoid and tannin groups [10]. Soluble forms in plant seeds can be extracted in aqueous, acidic, alkaline mediums and by enzymatic methods. The choice of analysis technique and sample preparation method are important to obtain maximum benefit from bioactive components. Extraction is one of the critical steps of the decision-making process during the determination of limit levels in the analysis of foods. In addition, the fact that foods and their target components have different physicochemical properties makes it difficult to develop methods that can analyze them with a single method. For this reason, there is a need to be extremely selective and

sensitive in the methods to be determined. The effect of solvent concentrations of adzuki bean seed sprouts before germination and at different germination times on the total amount of phenolic compounds is given in Figure 6. In general, when 0 h and other germination times were compared, no significant chenges were found in total phenol content in all solvent concentrations in 48 h. At the end of 120 h, the lowest phenolic component levels were determined in A1 and H3, the highest concentrations in E2 and H2. At the end of germination (120 h), total phenolic compounds increased approximately 6.5-7 times in E2. The present study results showed that the amount of phenolic compounds in adzuki bean sprouts increased slightly with the increase in germination times, as well as by extracting it at different solvent concentrations, and it positively affected the antioxidant capacity. Gan et al. (2016) reported that the content of total phenolic compounds in mung beans increased approximately 5.0-5.5 times after 5 days of germination [41]. We can say that the prolongation of germination times allowed the increase of soluble phenolic compounds and the synthesis and conversion of various phenolic compounds at different solvent concentrations. In fact, it has been reported that insoluble phenolics in some seeds first decreased in proportion and increased after germination [42]. The results of germinated edible seed studies have shown that total phenolic compounds can vary in the range of 30-253 mg gallic acid equivalent/100 g FW. The total phenolic content of various bean species at different times before and after germination was determined by the researchers: germinated jack bean (*Canavalia ensiformis*) 4 days 2.3-3.6 mg GAE/g DW [4], germinated sword bean (*Canavalia gladiata*) 1-4 days 40-58 mg GAE/100 g FW [18], germinated soy bean (*Glycine max*) 2-6 days 2.98-3.49 mg CE/g DW [43]. germinated kidney bean (*Phaseolus vulgaris*) 3-8 days 370- 420 mg GAE/100 g DW [44], germinated mung bean (*Vigna radiata*) 1-4 days 40-80 mg GAE/100 g FW, germinated adzuki bean (*Vigna angularis*) 1-4 days 43-80 mg GAE/100g [18]. Han et al. (2013) determined the total polyphenol content of radish buds (*Raphanus sativus* L.) sprouts at various solvent concentrations (70% ethanol, 80% methanol, 75% acetone and distilled water) at germination periods (4, 8 and 12 days) to 84.11 and 296.51 mg/g and on the 4th day ethanol extract showed the highest value with 296.51 mg/g [19]. Rafińska et al. (2019) analyzed the seeds and shoots of *Lepidium sativum* with four extraction techniques with water, supercritical CO_2 and ethanol; they determined that the extract obtained from freeze-dried sprouts with the addition of 96% ethanol as a solvent together with supercritical fluid extraction showed the best activity in terms of flavonoids, maceration with water was effective in the extraction of phenolic compounds in seeds, and 70% ethanol was more effective than 96% on phenolic compounds [45]. The increase in the synthesis of polyphenols in sprouts may be due to differences in solvent type and concentration, as well as the reduction of high molecular weight insoluble polymer formations to smaller molecules. According to the results obtained, we can say that the total phenolic content in the seeds of adzuki bean creates a high hydrophilic effect from the hydroxyl groups in the structure.

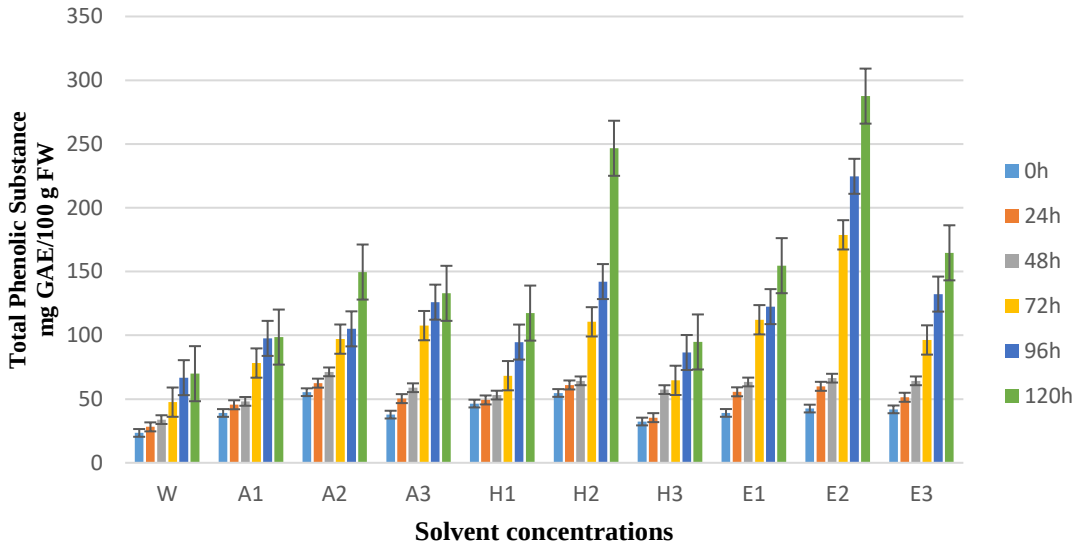

Figure 6. Total phenolic substance of adzuki bean sprouts at different solvent concentration extract and germination times. Results are expressed as mg GAE/100 g FW.

4. Conclusion

The results showed that aqueous organic solvent extracts of samples from adzuki beans at different germination times were rich in polyphenols and other antioxidants. Such processes can provide high levels of metal chelating and radical scavenging activity by adding functionality to foods. In the studies, it was stated that many seeds sprouted and caused positive changes in physiological and biochemical parameters. However, extraction at different organic solvent concentrations may allow us to obtain even more effective results in these parameters. In addition, maximum effects in terms of health can be created by determining the concentrations with high antioxidant activity results and adapting them to the industry and preparing the forms that can be used in the human diet.

References

[1] Linnemann, A,R., Benner, M., Verkerk, R., van Boekel, M.A.J.S., Consumer-driven food product development, **Trends in Food Science and Technology**, 17, 184-190, (2006).

[2] Vicentini, A., Liberatore, L., Mastrocola, D., Functional foods: Trends and development of the global market, **Italian Journal of Food Science**, 28(2), 338-351, (2016).

[3] Huang, X., Cai, W., Xu, B., Kinetic changes of nutrients and antioxidant capacities of germinated soybean (*Glycine max* L.) and mung bean (*Vigna radiata* L.) with germination time, **Food Chemistry**, 143, 268-276, (2014).

[4] Aguilera, Y., Diaz, M.F., Jimenez, T., Benitez, V., Herrera, T., Cuadrado, C., Martín-Pedrosa, M., Martin-Cabrejas, M.A., Changes in nonnutritional factors and antioxidant activity during germination of nonconventional legumes, **Journal of Agricultural and Food Chemistry**, 61, 8120-8125, (2013).

[5] Shahidi, F., Naczk, M., An overview of the phenolics of canola and rapeseed:

chemical, sensory and nutritional significance, **Journal of the American Oil Chemists' Society** 69, 917-924, (1992).

[6] Urbano, G., López-Jurado, M., Frejnagel, S.L., Gómez, Villalva, E., Porres, J.M., Frías, J., Vidal-Valverde, C., Aranda, P., Nutritional Assessment of raw and germinated pea (*Pisum sativum* L.) protein and carbohydrate by in vitro and in vivo techniques, **Nutrition**, 21, 230-239, (2005).

[7] Renault, H., El Amrani, A., Palanivelu, R., Updegraff, E.P., Yu, A., Renou, J.P., Preuss, D., Bouchereau, A., Deleu C., GABA accumulation causes cell elongation defects and a decrease in expression of genes encoding secreted and cell wall-related proteins in *Arabidopsis thaliana*, **Plant and Cell Physiology**, 52, 894-908, (2011).

[8] Nikmaram, N., Dar, B.N., Roohinejad, S., Koubaa, M., Barba, F.J., Greiner, R., Johnson, S.K., Recent advances in gamma-aminobutyric acid (GABA) properties in pulses: an overview, **Journal of the Science of Food and Agriculture**, 97, 2681-2689, (2017).

[9] Kinnersley, A.M., Turano, F.J., Gamma aminobutyric acid (GABA) and plant responses to stress, **Critical Reviews in Plant Sciences**, 19, 479-509, (2010).

[10] Gan, R.Y., Lui, W.Y., Wu, K., Chan, C.L., Dai, S.H., Sui, Z.Q., Corke, H., Bioactive compounds and bioactivities of germinated edible seeds and sprouts: an updated review, **Trends in Food Science and Technology**, 59, 1-14, (2017).

[11] McGill, J.A., Michigan-Japan and adzuki beans, Mich. **Dry Bean Digest**, 19, 4-7, (1995).

[12] Turkmen, N., Sari, F., Velioglu, Y.S., Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Foline-Ciocalteu methods, **Food Chemistry,** 99, 835-41, (2006).

[13] Brand-Williams, W., Cuvelier, M.E., Berset, C., Use of a free radical method to evaluate antioxidant activity, **LWT-Food Science and Technology**, 28, 25-30, (1995).

[14] Dinis, P., Pineda, M., Aguilar, M., Spectrophotometric quantity of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E, **Analytical Biochemistry**, 269, 337-341, (1999).

[15] Haug, G., Lantzsch, W., Methods for determination of phytate of cereal products, **Journal of The Science of Food and Agriculture**, 34, 1423-1424, (1983).

[16] Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M., Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, **Methods in Enzymology**, 299, 152-178, (1999).

[17] Lopez-Amoros, M.L., Hernandez, T., Estrella, I., Effect of germination on legume phenolic compounds and their antioxidant activity, **Journal of Food Drug Analysis**, 19, 277–283, (2006).

[18] Wu, Z.Y., Song, L.X., Feng, S.B., Liu, Y.C., He, G.Y., Yioe, Y., Liu, S.Q., Huang, D., Germination dramatically increases isoflavonoid content and diversity in chickpea (*Cicer arietinum* L.) seeds, **Journal of Agricultural and Food Chemistry**, 60, 8606-8615, (2012).

[19] Han, J.H., Moon, H.K., Chung, S.K., Kang, W.W., Comparison of antioxidant activities of radish bud (*Raphanus sativus* L.) according to extraction solvents and sprouting period, **Journal of the Korean Society of Food Science and Nutrition**, 42(11), 1767-1775, (2013).

[20] Pajak, P., Socha, R., Galkowska, D., Roznowski, J., Fortuna, T., Phenolic profile and antioxidant activity in selected seeds and sprouts, **Food Chemistry**, 143, 300-306, (2014).
[21] Siah, C.W., Trinder, D., Olynyk, J.K., Iron overload, **Clinica Chimica Acta**, 358, 24-36, (2005).
[22] Halliwell, B., Gutteridge, J.M.C., Oxygen toxicology, oxygen radicals, transition metals and disease, **Biochemical Journal**, 219, 1-4, (1984).
[23] Luo, Y., Xie, W., Jin, X., Wang, Q., He, Y., Effects of germination on iron, zinc, calcium, manganese, and copper availability from cereals and legumes, **CyTA-Journal of Food**, 12: 22-26, (2014).
[24] Park, S., Grusak, M.A., Oh, M.M., Concentrations of minerals and phenolic compounds in three edible sprout species treated with iron-chelates during ımbibition, **Horticulture, Environment,and Biotechnology**, 55(6), 471-478, (2014).
[25] Svihus, B., Newman, R.K., Newman, C.W., Effect of soaking, germination, and enzyme treatment of whole barley on nutritional value and digestive tract parameters of broiler chickens, **British Poultry Science**, 38, 390-396, (1997).
[26] Ohmori, M., Yano, T., Okamoto, J., Tsushida, T., Murai, T., Higuchi, M., Effect of anaerobically treated tea (Gabaron tea) on blood pressure of spontaneously hypertensive rats, **Nippon Nogei Kagaku Kaishi,** 61, 1449-1451, (1987).
[27] Oh, S.H., Stimulation of γ-aminobutyric acid synthesis activity in brown rice by a chitosan/glutamic acid germination solution and calcium/calmodulin, **Journal of the Science of Food and Agriculture**, 36, 319-325, (2003).
[28] Kihara, M., Okada, Y., Iimure, T., Ito, K., Accumulation and degradation of two functional constituents, GABA and β-glucan, and their varietal differences in germinated barley grains, **Breed Science**, 57, 85-89, (2007).
[29] Ding, J., Yang, T., Feng, H., Dong, M., Slavin, M., Xiong, S., Zhao, S., Enhancing contentsofγ-aminobutyric acid (GABA) and other micronutrients in dehulled rice duringgermination under normoxic and hypoxic conditions, **Journal of Agricultural and Food Chemistry**, 64, 1094-1102, (2016).
[30] Yang, H., Gao, J., Yang, A., Chen, H., The ultrasound-treated soybean seeds improveedibility and nutritional quality of soybean sprouts, **Food Research International**, 77, 704-710, (2015).
[31] Gani, A., Wani, S., Masoodi, F., Hameed, G., Whole-grain cereal bioactive compounds and their health benefits: a review, **Journal of Food Processing and Technology**, 3, 146-156, (2012).
[32] Lin, Y.T., Pao, C.C., Wu, S.T., Chang, C.Y., Effect of different germination conditions on antioxidative properties and bioactive compounds of germinated brown rice, **BioMed Research**, 608-761, (2015).
[33] Saikusa, T., Horino, T., Mori, Y., Accumulation of γ-aminobutyric acid (GABA) in the rice germ during water soaking, **Bioscience, Biotechnology and Biochemistry**, 58, 2291-2292, (1994).
[34] Thavarajah, P., Thavarajah, D., Vandenberg, A., Low phytic acid lentils (*Lens culinaris* L.): a potential solution for increased micronutrient bioavailability, **Journal of Agricultural and Food Chemistry**, 57, 9044-9049, (2009).
[35] Dorsch, J.A., Cook, A., Young, K.A., Anderson, J.M., Bauman, A.T., Volkmann C.J., Murthy, P.N., Raboy, V., Seed phosphorus and inositol phosphate phenotype of barley low phytic acid genotypes, **Phytochemistry**, 62, 691-706, (2003).
[36] Khalil, A.W., Zeb, A., Mahmood, F., Tariq, S., Khattak, A.B., Shah, H., Comparision of sprout quahty characteristics of desi and kabuli type chickpea

cultivars (*Cicer arietinum* L.), **LWT- Food Science and Teehnology**, 40, 937-945, (2007).

[37] Doblado, R., Frias, J., Vidal-Valverde, C., Changes in vitamin C content and antioxidant capacity of raw and germinated cowpea (*Vigna sinensis* var. carilla) seeds induced by high pressure treatment, **Food Chemistry**, 101, 918-923, (2007).

[38] Ghavidel, R.A., Prakash, J., The impact of germination and dehulling on nutrients, antinutrients, in vitro iron and calcium bioavailability and in vitro starch and protein digestibility of some legume seeds, **LWT- Food Science and Technology**, 40, 1292-1299, (2007).

[39] Khattak, A.B., Zeb, A., Bibi, N., Khalil, S.A., Khattak, M.S., Influence of germination techniques on phytic acid and polyphenols content of chickpea (*Cicer arietinum* L.) sprouts, **Food Chemistry**, 104, 1074-1079, (2007).

[40] Rocha-Guzman, N.E., Herzog, A., Gonzalez-Laredo, R.F., Ibarra-Perez, F.J., Zambrano-Galvan, G., Gallegos-Infante, J.A., Antioxidant and antimutagenic activity of phenolic compounds in three different colour groups of common bean cultivars (*Phaseolus vulgaris*), **Food Chemistry**, 103, 521-527, (2007).

[41] Gan, R.Y., Wang, M.F., Lui, W.Y., Wu, K., Corke, H., Dynamic changes in phytochemical composition and antioxidant capacity in green and black mung bean (*Vigna radiata*) sprouts, **International Journal of Food Science and Technology**, 51, 2090-2098, (2016).

[42] Tang, D., Dong, Y., Guo, N., Li, L., Ren, H., Metabolomic analysis of the polyphenols in germinating mung beans (*Vigna radiata*) seeds and sprouts, **Journal of the Science of Food and Agriculture**, 94, 1639-1647, (2014).

[43] Fernandez-Orozco, R., Frias, J., Zielinski, H., Piskula, M.K., Kozlowska, H., Vidal-Valverde, C., Kinetic study of the antioxidant compounds and antioxidant capacity during germination of *Vigna radiata* cv. emmerald, *Glycine max* cv. jutro and *Glycine max* cv. Merit, **Food Chemistry**, 111, 622-630, (2008).

[44] Aguilera, Y., Liebana, R., Herrera, T., Rebollo-Hernanz, M., Sanchez-Puelles, C., Benitez, V., Martín-Cabrejas, M.A., Effect of illumination on the content of melatonin, phenolic compounds, and antioxidant activity during germination of lentils (*Lens culinaris* L.) and kidney beans (*Phaseolus vulgaris* L.), **Journal of Agricultural and Food Chemistry,** 62, 10736-10743, (2014).

[45] Rafińska, K., Pomastowski, P., Rudnicka, J., Krakowska, A., Maruśka, A., Narkute, M., Buszewski., B., Effect of solvent and extraction technique on composition and biological activity of *Lepidium sativum* extracts, **Food Chemistry**, 289, 16-25, (2019).