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# The effect of different germination times on some nutritional and anti-nutritional properties of green lentil sprouts

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## Abstract

In the developing, changing and constantly renewing food and beverage sector, different nutrition trends are becoming more important day by day. One of these is the consumption of germinated legumes, which has attracted attention in the world recently and has many practitioners. The germination process is an effective process on the nutritional values of legumes and can make them even more valuable. The aim of this study was to determine the effects of germination time (3, 6, 9 days) on some nutritional and anti-nutritional contents contained in green lentils sprouts. Both sprouting period and cultivars significantly influenced the nutritional quality of lentil sprouts. The values of protein, starch, phytic acid, condensed tannins, total phenolic, total flavonoid, free radical scavenging activity contents of sprouting periods ranged between 26.4 (Raw) to 27.8% (9 day), 48.8 (raw) to 45.5% (9 day), 0.59 (6 days) to 1.09% (raw), 0.60 (3 days) to 0.77% (9 days), 3.97 (raw) to 10.24 mg GA/g (9 days), 0.55 (raw) 4.58 mg QE/g (9 days), 9.32 (raw) to 13.50% (9 day), respectively. The highest ash, Fe, Cu, Mn and Zn contents were obtained from raw grains with 2.76%, 5.96 mg/100g, 0.95 mg/100g, 1.34 mg/100g and 4.09 mg/100g values. Germination process improved quality of lentils by enhancing the nutritive value and digestibility of nutrients and reducing the anti-nutrients.

## Introduction

Lentil (*Lens culinaris* Medic.), which is grown in temperate and sub-tropical climates between 58º North and 40º South latitudes in the world (Alghamdi et al., 2014), is grown in almost every region of Turkey. Lentil is produced 5.7 million tons in the world. It is produced 353 thousand tons in Turkey and with this value, it ranks 3rd in the world's lentil production (FAO, 2019). Lentils, as a pulse crop, are a very important component of agriculture especially in developing countries and nutritional rich-food. Lentil is widely used in human nutrition because of its high protein content, rich vitamin and mineral content, low level of nutrition-hindering factors and shorter cooking (Yadav et al., 2007).

Today, healthy life and healthy food consumption have particularly become the lifestyle of people with high-income levels. While searching for new sources of functional and healthy foods, special attention has been paid to sprouts from the pulses which are more and more frequently used in human diets worldwide (Ghumman et al., 2016). Ready-to-eat seedlings, commonly known as sprouted seeds that are harvested at the earliest and earliest plant growth stages, play a special role in healthy living (Benincasa et al., 2019).

Sprouted seeds (germinated seeds), which have been known for centuries in many cultures, are widely used because it is technologically can be done without advanced equipment, cheap, has a quick production cycle, simple processing technique, and provides fairly high yields (Delian et al., 2015). This practice is reported to be associated with improvements in the nutritive value of seeds. Sprouted seeds have owned by higher contents of nutrients (amino acids, protein, vitamins, and minerals), and lower levels of nonnutrients (trypsin inhibitors, tannins, phytates, and lectins) (Troszyńska et al., 2011). Therefore, the sprouts may thus become a potential source of nutritious food or food ingredients. Lentil sprouts contain many functional and health-promoting components. Therefore, its consumption reduces the risk of many diseases such as cardiovascular diseases, diabetes, obesity, inflammation and cancer (Świeca et al., 2013).

The aim of this study was to determine the effects of germination time on some nutritional and anti-nutritional contents contained in green lentils sprouts.

## Materials and Methods

### Plant materials and germination

Raw green lentil seeds of the Ceren and Ankara Yesili cultivars were provided from Field Crops Central Research Institute in Ankara, Turkey. While the Ankara yesili is a coarse-grained cultivar with a thousand-grain weight of 55.8 g, the Ceren is a small-grained cultivar with a thousand-seed weight of 31.3 g.

In the study, three different germination times (3, 6, 9 days) were applied on two green lentil cultivars. Germination experiments were carried out the factorial arrangement of completely randomized design with 4 replications. For the purpose of surface sterilization of lentil seeds, they were first kept in 1% sodium hypochlorite solution for 15 minutes and then washed several times with distilled water. 200 g of surface-sterilized seeds were placed in separate containers and 1000 ml of distilled water was added to them and kept at room temperature for 6 h. Then the seeds were filtered and kept on blotting paper at room temperature for 2 h. The seeds that are kept to drain the water were placed in germination containers with two layers of filter paper in a way that they would not overlap. They were watered with fresh distilled water when necessary.

The study was carried out at 20˚C in the temperature, light and humidity controlled Climate Room of Bilecik Seyh Edebali University Faculty of Agriculture and Natural Sciences. The seeds were germinated in dark conditions for 3, 6, and 9 days. The sprouted seed samples were harvested. During this time, the radicle of the seed came out, and the seed coat was torn. Sprouted seeds were frozen for 12 h to stop the germination process. After thawing at room temperature, then raw and sprouted seeds were dried in a draught oven at 40 ˚C. The dried samples were ground to pass through a 0.5 mm sieve. Samples were preserved in at +4 °C until analysis. All analyses were performed in 3 replicates.

### Chemical analysis

The raw and sprouted samples were analyzed for crude protein (N × 6.25) and ash as described in AOAC, 2000. Total starch and phytic acid was determined enzymatically using a Megazyme Total Starch Assay kit and phytic acid Assay Kit (Megazyme International, Ireland), respectively. The total flavonoid content was determined by using Arvouet-Grand et al. (1994) with some modifications. The effect of each sample on 2,2-diphenyl-1-picryl-hydrazylhydrate (DPPH) radical was identified according to Gezer et al. (2006) and Yıldırım et al. (2021). Total condensed tannin: A 6 ml of tannin solution was added to 0.01 g of ground seed then placed in a tube and mixed on a vortex. The tubes were tightly capped and kept at 100 ° C for 1 hour, and the samples were allowed to cool. Then, they were read at a spectrophotometer at the absorbance value of 550 nm (Bate-Smith, 1975). Condensed tannins were calculated by the following formula: Absorbance (550 nm x 156.5 x dilution factor) / Dry weight (%) (Yıldız et al., 2021). Mineral element concentrations of grain were measured using inductively coupled plasma - mass spectrometry (ICP - MS). All samples were analyzed in duplicate and the mean was used for the statistical analysis (Başaran et al., 2021). The following minerals were quantified: iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn).

### Statistical analysis

All experimental results were subjected to two-way analysis of variance (ANOVA) using Minitab Statistical Software. Duncan test was employed to draw the comparison between means and the significance was accepted at $p<0.05$.

## Results and Discussion

The mean of squares and significance for cultivars, sprouting periods, and their interactions of all experiments are given in Table 1. Both sprouting period and cultivars significantly influenced the nutritional quality of lentil sprouts.

**Table 1.** Mean squares of investigated experiments of two green lentils on raw and different sprouted period

| Parameter | Source (DF) | | | |
|---|---|---|---|---|
| | Cultivar (C) (1) | Sprouting period (SP) (3) | Rep (3) | C × SP (3) |
| Protein | 17.13** | 2.88** | 0.07ns | 0.03ns |
| Starch | 18.98** | 16.18** | 0.06ns | 1.35* |
| Phytic acid | 0.009ns | 0.369** | 0.003ns | 0.026** |
| Condensed tannin | 0.005ns | 0.062** | 0.0026ns | 0.027** |
| Total polyphenols | 3.09** | 61.31** | 0.10ns | 2.92** |
| DPPH radical scavenging | 25.45** | 30.74** | 1.92ns | 5.37** |
| Total Flavonoids | 0.45* | 23.88** | 0.09ns | 0.33* |
| Ash | 1.18** | 1.72** | 0.03ns | 0.02ns |
| Iron (Fe) | 0.004ns | 1.53** | 0.02ns | 0.13* |
| Copper (Cu) | 0.0007ns | 0.005ns | 0.002ns | 0.0004ns |
| Manganese (Mn) | 0.35** | 0.08** | 0.002ns | 0.011* |
| Zinc (Zn) | 1.24** | 3.93** | 0.004ns | 0.32** |

*: Significant at the p<0.05 probability level, **: Significant at the p<0.01 probability level, DF: Degree of Freedom

The mean value for cultivars, sprouting periods, and their interactions of all experiments are given in Table 2, Table 3 and Figure 1. Protein content varied between 26.4 (Raw) to 27.8% (9 day) with an average value of 26.9%. Sprouting time caused significant increases in protein content. Ankara Yesili cultivar (27.67%) had higher protein content than Ceren cultivar (26.20%) (Table 2). The increase in protein content is thought to be due to losses in dry weight, especially carbohydrates, along with respiration during germination. The increase of crude protein content during lentil germination has been reported by other researchers (Fouad & Rehab, 2015; Xu et al., 2019).

**Table 2.** Some quality experiments of lentil sprouts grown under different germination times

| Experiments | PC | SC | PA | CT | TP | TF |
|---|---|---|---|---|---|---|
| Sprouting period | | | | | | |
| Raw | 26.4b | 48.8a | 1.09a | 0.75a | 3.97d | 0.55d |
| 3 day | 26.5b | 47.3b | 0.80b | 0.60b | 8.66c | 3.29c |
| 6 day | 27.0b | 46.5c | 0.59d | 0.62b | 9.13b | 3.63b |
| 9 day | 27.8a | 45.5d | 0.70c | 0.77a | 10.24a | 4.58a |
| Cultivars | | | | | | |
| Ceren | 26.20 b | 46.3b | 0.78 | 0.69 | 8.31a | 3.13a |
| Ankarayeşili | 27.67 a | 47.8a | 0.81 | 0.68 | 7.69b | 2.89b |
| Mean | 26.9 | 47.0 | 0.80 | 0.69 | 8.00 | 3.01 |

PC: protein content (%), SC: starch content (%), PA: phytic acid (%), CT: Condensed tannin (%), TP: total phenolic (mg GA/g), TF: total flavonoid (mg QE/g)

**Table 3.** Some quality experiments of lentil sprouts grown under different germination times

| Experiments | DPPH | AC | Fe | Cu | Mn | Zn |
|---|---|---|---|---|---|---|
| Sprouting period | | | | | | |
| Raw | 9.32c | 2.76a | 5.96a | 0.95 | 1.34a | 4.09a |
| 3 day | 12.32b | 2.43b | 5.81b | 0.93 | 1.24b | 3.22b |
| 6 day | 13.34a | 2.17c | 5.37c | 0.90 | 1.17c | 2.89c |
| 9 day | 13.50a | 1.66d | 5.16d | 0.89 | 1.11d | 2.43d |
| Cultivars | | | | | | |
| Ceren | 12.95a | 2.06 b | 5.59 | 0.92 | 1.11 b | 3.36 a |
| Ankarayeşili | 11.29b | 2.45 a | 5.56 | 0.91 | 1.32 a | 2.96 b |
| Mean | 12.12 | 2.30 | 5.58 | 0.92 | 1.21 | 3.16 |

DPPH: Free radical scavenging activity (%), AC: ash content (%), Fe: iron content (mg/100g), Cu: copper content (mg/100g), Mn: manganese content (mg/100g), Zn: zinc content (mg/100g)

Starch content in raw grain and in samples obtained at germination times of raw, 3, 6 and 9 days was 48.8%, 47.3%, 46.5%, and 45.5%, respectively. Ankara Yesili cultivar (47.8%) had higher starch content than Ceren cultivar (46.3%) (Table 2). According to C X SP interaction, the highest starch content was obtained in raw seed of Ankara Yesili cultivar with 49.1% (Figure 1). Although the reduction in starch varied due to different species and germination conditions, these decreasing trends are close with the previous studies in lentils (Ghavidel & Prakash, 2007; Fouad & Rehab, 2015). Some enzymes activated during the germination of legumes seeds are responsible for the conversion of starch into oligosaccharides or monosaccharides resulting in reduction (Olaerts et al., 2015). It is thought that this situation causes significant decreases in starch content during sprouting time, as in our study.

According to germination time, phytic acid and tannins content of genotypes ranged from 0.59 (6 days) to 1.09% (raw) and 0.60 (3 days) to 0.77% (9 days), respectively. The sprouting process caused significant decreases in phytic acid and tannins content up to day 6 of germination (Table 2). According to C X SP interaction, the highest phytic acid content was obtained in raw seed of Ceren and Ankara Yesili cultivars with 1.10% and 1.08%, respectively. The highest tannins content was obtained in raw seed of Ceren cultivar with 0.83% (Figure 1). Antinutrients, extensively found in plant part, have both health advantages and disadvantages. For example, phytic acid binds some minerals and forms insoluble complexes. Germination significantly reduced the phytic and tannin contents as previously observed by Fauad & Rehab (2015) in lentil seeds. Khattak et al., (2007) indicated that the decrease in phytate content in lentil seeds during germination is associated with an increase in phytase activity. Shimelis & Rakshit (2007) and Saharan et al. (2002) reported that the decrease in tannins of seeds during the germination process may be due to the leaching of tannins into water and binding of polyphenols with other organic materials.

As germination days progressed, a significant increase in the total phenolic and flavonoids content of lentil seeds were observed. According to germination time, total phenolic and flavonoid contents of genotypes ranged from 3.97 (raw) to 10.24 mg GA/g$^{-1}$ (9 days) and 0.55 (raw) 4.58 mg QE/g (9 days), respectively. For both experiments, higher values were determined in Ceren cultivar than in Ankara Yesili cultivar. According to C × SP interaction, the highest total phenolic (11.12 mg GA/g$^{-1}$) and flavonoid contents (4.71 mg QE/g$^{-1}$) were obtained in the 9-day seed of Ceren cultivar (Figure 1).

Randhir et al. (2004) reported that these increases that occur in total phenolic with the germination process could be due to the biosynthesis and bioaccumulation of phenolic compounds as a defensive mechanism to survive under environmental stresses. After germination, diverse changes in the phenolic compounds happen which are depending on many factors like the type of seeds, germination time, etc Fouad & Rehab, (2015). Flavonoids are prevalent in plant parts. In the study by Fouad & Rehab, (2015), the total phenolic and flavonoids of raw lentil seeds were significantly lower than sprouted seeds.

Sprouted seeds had significantly higher DPPH compared to raw seeds. The highest DPPH was determined in the 9-day sprout period (13.50%), while the lowest was in the raw seed (9.32%) (Table 3). According to C X SP interaction, the highest DPPH content was obtained in 9-day of Ceren cultivar with 15.5% (Figure 1). Ceren cultivar (12.95%) had higher DPPH content than Ankara Yesili cultivar (11.29%) (Table 3). DPPH is one of the most important methods to evaluate the antioxidant properties of plants and is

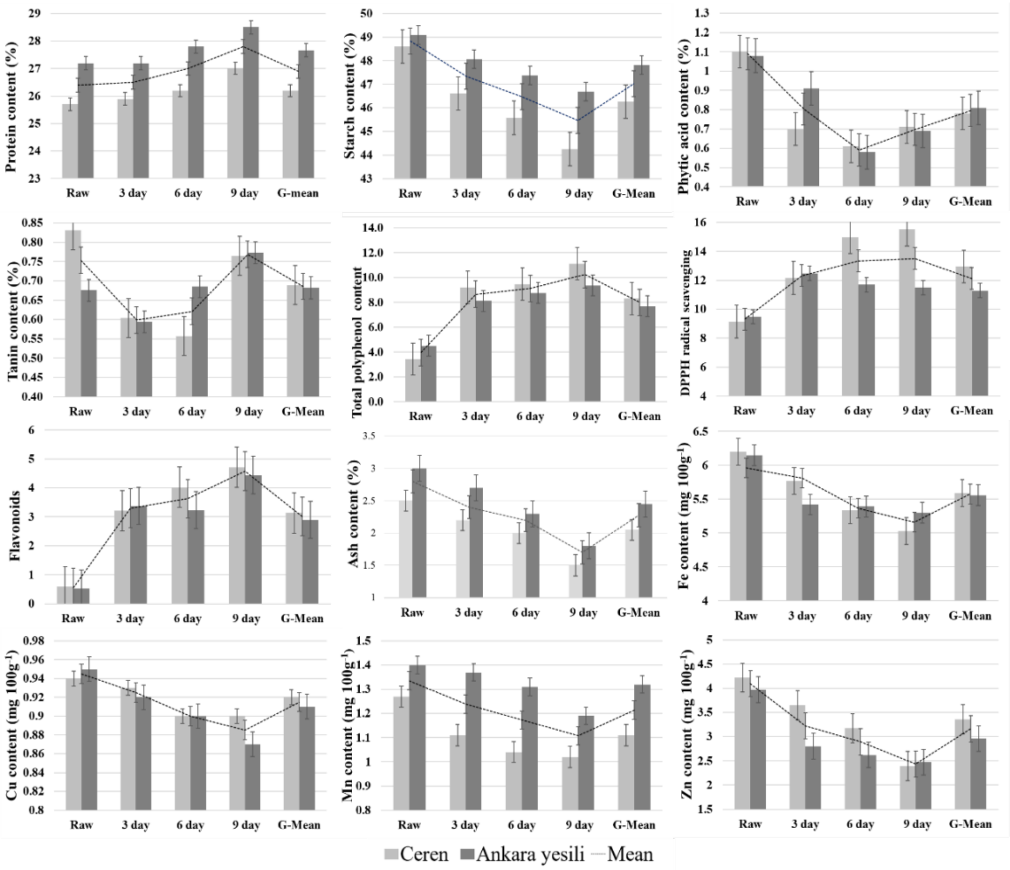


**Figure 1.** Cultivar × sprout period interaction for all experiments

desirable to be high. Phytochemicals have high DPPH, which helps to reduce the risk of some diseases (chronic diseases and cancer etc.,) (Lako et al., 2007).

As germination days progressed, a significant (except for Cu) decrease in the ash, Fe, Cu, Mn and Zn of lentil seeds were observed. The highest crude ash, Fe, Cu, Mn and Zn contents were obtained from raw grains with as 2.76%, 5.96 mg/100g$^{-1}$, 0.95 mg/100g$^{-1}$, 1.34 mg/100g and 4.09 mg/100g values. The lowest values for all of these experiments were obtained after 9 days of germination (Table 3). It was determined that the G×SP interactions of Fe, Mn and Zn contents were important (Table 1 and Figure 1). In the study conducted by Ghavidel & Prakash, (2007) on some legume seeds, they found that some minerals such as Fe, Ca and P contents decreased with the germination process, but the biological availability of these minerals increased. In addition, the same investigator also determined a decrease in ash content with germination.

## Conclusion

Lentil sprouts have become a highly desirable food product in recent years due to their nutritional content and health benefits. In this study, it was determined that the nutrient content of green lentil varieties changed with the germination process. On the 9-day of germination, the protein content increased while the tannin content decreased. The cultivars responded differently to the investigated experiments. The germination process improved the quality of lentils by enhancing the nutritive value and digestibility of nutrients and reducing the antinutrients.

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## Author Contributions

The authors contributed equally to this work.

## Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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