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Original Article

# Study and Comparison of Chemical and Antioxidant Properties of Mazafati Date Seed Germ and Seed Powder

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## Abstract

Background and Objective: Ever since the concept of functional foods was introduced and consumer awareness of the relationship between health and food consumption increased, there has been a rise in demand for super healthy foods. There is a considerable amount of dietary fiber in date seeds, which plays a key role in disease prevention. Iran is one of the top date producers in the world, and considering the nutritional value of this product, the application of date seed, as a byproduct of the date industry, in human and animal nutrition has attracted great interest. Materials and Methods: In this study, date seed germ was prepared and compared with date seed in terms of antioxidant properties (Scavenging activity to DPPH radical, Total phenol determination), fiber, proteins, fats, and mineral salts. Results: The results showed an increase in antioxidant properties (Scavenging activity to DPPH radical, Total phenol determination), amount of fiber, protein, fat and minerals in date seed germ compared to date seed powder. Conclusion: Therefore, both date seed powder and date seed germ can be used in food formulation as functional foods.

**Keywords:** Germ, Date seed, Antioxidant, Fiber

## Introduction

With the introduction of functional foods in recent years and the increase in consumer awareness of the relationship between health and food consumption, there has been a growing demand for super healthy foods. Researchers are trying to fortify food products using micronutrients such as omega-3 fatty acids, phytosterols, and liquid fibers in order to improve their health effects and prevent illnesses such as cancer [1].

Iran is one of the main producers of dates in the world and, according to the latest reports, it produces 1,326,130 tons of dates annually with an economical value of 415,702 USD. Seedless dates are prepared every year in date producing regions for both domestic consumption and export [2]. The date seed constitutes about 10-14% of the date fruit weight and contains a significant amount of carbohydrates and fiber. Therefore, the application of date seed, as a byproduct of the date industry, in human and animal nutrition has attracted great interest. The addition of date seed to bread can significantly increase its nutritional value and is specifically important for increasing fiber in the end product [3]. Recent studies have addressed chemical composition and application of date seed in different industries. According to the findings, there is a high content of dietary fiber in date seed, which plays a significant role in preventing diabetes and hyperlipidemia. It also plays a protective role against hypertension, coronary heart disease, cholesterol, colon cancer, prostate cancer and bowel disorders [4]. Free radicals are the most important factors contributing to food oxidation, and studies have shown the activity of different reactive oxygen species (ROS) in reactions leading to growth inhibition and in pathogenic processes such as aging, cancer, coronary heart disease, and Alzheimer's disease. Antioxidants are responsible for neutralization of the excess of free radicals such as ROS in the body [5]. Antioxidant activities have a close relation with the phenolic compound content in plants. Phenolic compounds comprise a large group of secondary plant metabolites that contains nearly 8,000 different substances. The main function of these compounds in relation to oxidation is neutralizing free radicals and creating complexes with metal ions. Phenolic compounds donate electrons to free radicals and control oxidation reactions of fats [6]. Phenols are useful compounds in

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date seed and usually show high antioxidant activities [7]. Karimi *et al*. (2013) used a completely randomized design in their study to evaluate the effects that adding date seed powder to wheat flour at various ratios (5%, 10%, and 15%) in loaf bread production had on the rheological properties of dough and on physicochemical properties of the loaf bread. The results showed that increases in date seed powder added to wheat flour improved water absorption, dough stability time, and Farinograph quality number decreased dough development time and degree of looseness of dough. They showed that date seed powder could be used in bread production. In doing so, only 5% of wheat flour could be replaced with date seed powder because higher percentages resulted in stiff dough and changed bread appearance, flavor, taste and color [8].

Fahloul *et al*. (2010) replaced sucrose with date fruit powder in the production of biscuits at 0%, 20%, 40%, and 60%. Then, they evaluated the resulting biscuits in terms of moisture content, final water activity, color, and hardness. The results showed that this replacement had no effects on moisture content but reduced hardness and increased color [9]. Cereal germ contains proteins, fats, vitamins, and minerals. It is relatively cheap to remove the germs from cereal grains [10]. The germs are a rich source of dietary fiber and can help with prevention and treatment of certain digestive disorders [11,10].

Considering the high nutritional value of date palm seed and the fact that it is discarded as waste, and also taking into account the nutritional value of cereal germs, this study examined the chemical and antioxidant properties of date seed and germ to introduce them as functional compounds to be used in food formulation and also create economic value.

## Material and Methods

### Materials and Equipments

2,2-diphenyl1- picryl hydrazyl (DPPH), the Folin–Ciocâlteu reagent (FCR), standard gallic acid (from Sigma Aldrich, Germany), methanol, sulfuric acid, hydrochloric acid, and hexane (from Merck, Darmstadt, Germany) were obtained. All the solutions were prepared using deionized water. Equipment included a Labnics spectrophotometer, an AG-A20 hammer mill, a Van Soest fiber analyzer model of 1991.

### Methods

#### Seed Grinding

Sample date seeds were first cleaned and washed. After removing the impurities, they were soaked in water for 24 hours, air-dried, and ground using a hammer mill (AG-A20, made in China) to pass through a 1mm sieve. The resulting powder was refrigerated until testing.

#### Seed Germination

Sample date seeds were first cleaned and washed. After removing the impurities, they were soaked in water for 24 hours and then air-dried. They were poured in a container that was covered with a wet cloth to prevent seed surface from drying up. Water was added occasionally during storage to keep the seeds moist. Germs started to show after two weeks. They were removed by hand once they were 2-3 cm long and placed in brine to remove the bad odor. After rinsing, they were dried at 50 °C and then ground and stored in a refrigerator [12].

### Comparative Tests

#### Fiber Measurement

One gram of each cake was taken and its protein and fat were removed using appropriate solvents. Then, the amount of total raw fiber in the sample was measured by weighing the remainder, using a fiber analyzer (Van Soest 1991, made in Sweden), and calculating the weight difference after drying and ashing it at 550 °C for 12 hours in an electric oven [13].

#### Protein Measurement

Protein measurement was performed according to national standard No. 104 by oxidizing the organic compounds of the sample by concentrated sulfuric acid and converting total nitrogen to ammonium sulfate and finally calculating the amount of the ammonia released in titration [13,14].

#### Measurement of Mineral Salts

Zinc, iron, potassium, and sodium, magnesium and calcium minerals were measured by atomic absorption spectroscopy [16,15].

#### Fat Measurements

Fat measurements were performed based on the ISIRI 2862 Standard by sample hydrolysis using hydrochloric acid in the presence of ethanol and formic acid, and the lipids were extracted using hexane as the solvent [17, 13].

#### Scavenging Activity to DPPH Radical

The antioxidant activity of the date seed germ and seed powder were measured by the 2,2 diphenyl picrylhydrazyl method based on the percentage of free radical scavenging. First, 0.5 g of each powder was dissolved in 100 ml of methanol. Then, the extract was centrifuged for 15 minutes. Concentrations of 0.5, 0.2, 0.1, 0.01 and 0.02 were prepared from this extract. Then, 200 µL of 0.06 mM diphenyl picrylhydrazyl solution was added to 4 ml of the extract and the absorbance of the sample was read by spectrophotometer at 517 nm using methanol as the control. The percentage of free radical scavenging was calculated through the following formula [18,19].

%RSC= (A blank- A sample)/(A blank)×100 (Equation 1)

IC50 is a parameter commonly used to compare the free radical scavenging activity of different extracts. IC50 refers to the half-maximal inhibitory concentration of the extract against the free radical in the reaction medium [20]. The amount of IC50 Obtained using Graph Pad Prism8 software.

Total Phenol Determination

Total phenolic compounds were measured according to the method of Petropoulos *et al*. (2017). A quantity of 1 ml of the stock prepared from each cake during the previous test was added to 5 ml of the Folin–Ciocâlteu reagent and after 5 minutes, 4 ml of 7.5% sodium carbonate was added to it. After 60 minutes in the dark, the light absorption of the tubes was measured by a spectrophotometer at 765 nm, and the total phenol content was calculated using the equation obtained from the standard curve of gallic acid. The results were expressed as milligrams of gallic acid per gram of the dry weight of the sample [21,18].

Statistical Analysis

Data were analyzed by the Duncan test at the 95% significance level ($p < 0.05$), using SPSS 22 software. Excel 2007 software was used to draw graphs. IC50 values were calculated using Graph Pad Prism 8 software. All tests were performed in three replications.

## Results and Discussion

Fiber Measurements

Figure 1 shows the results of measuring raw fiber content in date seed and date germ. A significant difference can be seen in the fiber content between these two treatments.

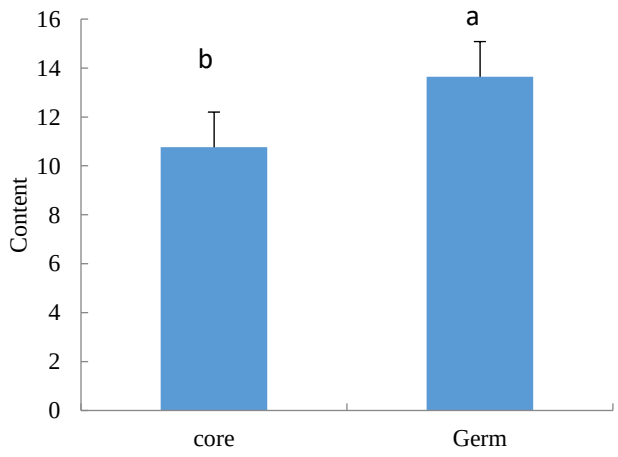


**Fig. 1** Fiber content in date seed and date germ
*Different small letters represent a significant difference in the level of 5%

These results are consistent with those of other studies indicating a high dietary fiber content in date seeds, which is very beneficial in terms of nutrition. This compound has a desirable effect on regulating visible peristaltic waves and may also possess antioxidant properties [22]. Moreover, Almana and Mahmoud showed that if the date seed was ground properly, it could be used as a substitute dietary fiber source without negatively affecting the sensory quality of bread [3]. According to Al-Farsi and Lee (2011), the fiber content in date seed was 5.64-15.8 g/ 100 g fresh weight. Some of the health benefits of dietary fibers are that they reduce blood cholesterol, regulate blood glucose to control diabetes, and reduce digestive diseases [23]. Asgharipour *et al.* (2018), stated that increasing date seed powder in Cheetos formula increased raw fiber content in the product [24].

Protein Measurements

Figure 2 shows the results of measuring protein content in date seed and date germ. A significant difference can be seen in the protein content between the two treatments.

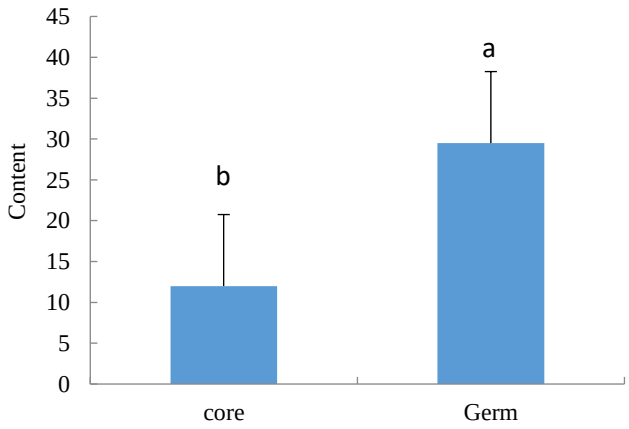


**Fig. 2** Protein content in date seed and date germ
*Different small letters represent a significant difference in the level of 5%

Attalla and Harraz (1996) studied the chemical composition of the pits of 11 date palm cultivars in Qassim region of Saudi Arabia and found that the date pits under study had 57.7-68.9% total carbohydrate, 3.8-5.8% simple sugar, and 5.1-7.5% raw protein. They also pointed to insignificant contents of phosphorus (0.19-0.26%) and concluded that the phytic acid concentration in date seed was significantly lower compared to cereals and oilseeds [25]. Hashemi *et al*. (2014) reported that protein content in wheat germ was 29.5%. Shurpalekar and Rao (1976) found wheat germ protein content to be 7.3-21-35% [26].

Fat Measurements

Figure 3 shows the results of measuring fat content of date seed and date germ. A significant difference can be seen in the fat content between the two treatments. Date seed showed a higher fat content than date germ.

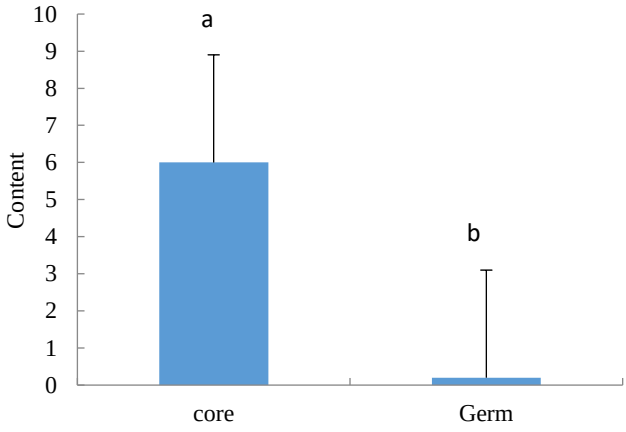


**Fig. 3** Fat content in date seed and date germ
* Different small letters represent a significant difference in the level of 5%

Attalla and Harraz (1996) studied the chemical composition of the pits of 11 date palm cultivars in Qassim region of Saudi Arabia and found that the date pits under study had 6-7-12.3% of raw fat, which is consistent with the results of the present study [25]. Hashemi *et al*. (2016) reported that fat content in wheat germ was 9.03% [26]. Shurpalekar and Rao (1976) reported wheat germ fat content to be 8.10% [27].

Minerals Measurements

The mean concentrations of mineral elements in date seed powder and date germ powder are listed in Table 1. Comparison of the means of the data using Tukey's test showed that the minerals under study were substantially higher in the seed germ treatment, with the highest increase being in potassium (21,100 mg/kg) and sodium (1,574 mg/kg).

Almost all reports of similar studies show that potassium has the highest concentration among macro minerals, which is consistent with the results of this study [28]. As for micro minerals, the results showed that iron had the highest concentration.

Results of Antioxidant (Total Phenol and DPPH) Tests

The results for total phenolic compounds expressed as mg of gallic acid equivalents/g dry seed and germ powder) are presented in Table 2. It can be seen that the date seed germ treatment had a higher phenolic compound content. There is a significant difference between the two treatments in this regard ($P<0.05$). Ardekani *et al*. (2010) reported that the pits of 14 cultivars of Iranian dates showed high antioxidant activity and could be used as a rich source of natural antioxidants for pharmaceutical purposes [29]. Platat *et al*. (2014) reported that polyphenol content and antioxidant activities in date seed was much higher than date fruit [30]. Al-Farsi (2008) reported that phenolic compound content in different cultivars of date palm was over 3,102 mg of gallic acid equivalents per 100g of date seed powder [31].

Rizzello, *et al*. (2010) reported the total phenolic compound content in wheat germ was 2.061 mmol gallic acid per gram [32]. Yu *et al*. (2010) found that wheat germ extract showed higher antioxidant activity compared to the well-known synthetic antioxidants [33].

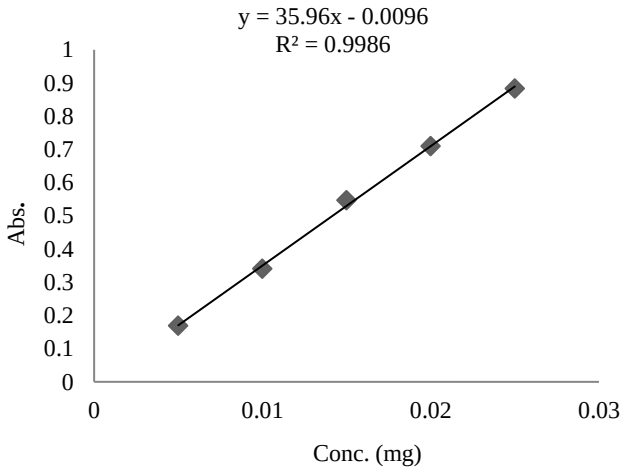


**Fig. 4** Calibration curve for absorbance of gallic acid concentrations

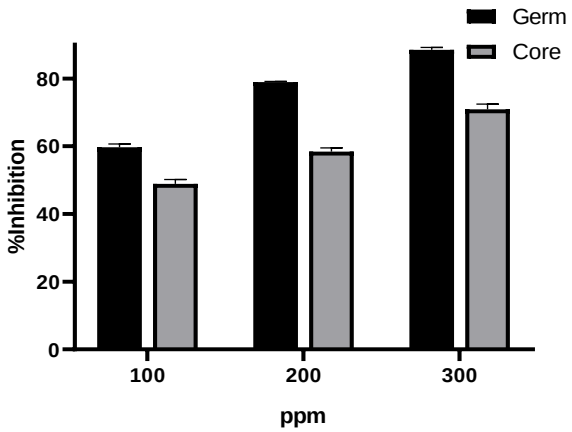


**Fig. 5** Comparison of Scavenging activity to DPPH radical in date seed and date germ

As represented in Figure 5, the extents of inhibition of free radicals at all concentrations were higher in date germ powder than date seed powder. The high extent of inhibition of DPPH free radicals in these methods is due to the higher tocopherol and phenolic compound contents. The quenching power of different extracts mostly depends on the number and position of hydroxyl groups and the molecular weights of phenolic compounds [34]. In line with other studies, the findings of this study

indicate that treatments having higher quantities of phenolic compounds show greater activity against free radicals [35,36].

**Table 1** Comparison of mineral solids content in date seed and date germ

| Sample | K (mg/kg) | Fe (mg/kg) | Ca (mg/kg) | Mg (mg/kg) | Zn (mg/kg) | Na (mg/kg) |
|---|---|---|---|---|---|---|
| Germ | 21100 a | 70.8 a | 6.6 a | 44.9 a | 17 a | 1574 a |
| Core | 3650 b | 6.72 b | 0.8 b | 18.02 b | 5.53 b | 166 b |

*Different small letters represent a significant difference in the level of 5%

**Table 2** Total phenol content in date seed powder and germ powder expressed as mg gallic acid equivalents/g dry weight of the sample

| Sample | Germ | Core |
|---|---|---|
| Total phenol | $0.09^{a}\pm 54.64$ | $0.16^{b}\pm 37.23$ |

*Different small letters represent a significant difference in the level of 5%

**Table 2** Comparison of IC50 content in date seed powder and germ powder

| Sample | Germ | Core |
|---|---|---|
| IC50 | $76.53^{a}$ | $111.4^{b}$ |

*Different small letters represent a significant difference in the level of 5%

Results on IC50 values are presented in Table 3. As expected, the lowest IC50 values were those of date seed germ treatments (76.53 µg/ml). The content of phenolic compounds is associated with antioxidant properties and increases in phenolic compounds result in increased antioxidant properties; therefore, the lower IC50 in the date seed germ treatment was not unexpected [20].

## Conclusion

Considering the high nutritional value of date palm seed and the fact that it is discarded as waste, and also taking into account the nutritional value of cereal germs, this study examined the chemical and antioxidant properties of date seed and germ to introduce them as functional compounds to be used in food formulation and also create economic value. Date seed and its germ have a high amount of mineral salts and raw fiber and antioxidant activity, and can therefore be potentially be used as cheap sources of functional compounds. Therefore, both date seed powder and date seed germ can be used in food formulations and also as functional foods.

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