Sustainability, Agri, Food and Environmental Research, (ISSN: 0719-3726), vol 12(2), 2024
http://dx.doi.org/10.7770/safer-V13N1-art729

Effect of soaking, germination and roasting on Nutritional, functional and Anti-nutritional characteristics of date (*Phoenix dactylifera*) seeds.

Efecto del remojo, germinación y tostado sobre las características nutricionales, funcionales y antinutricionales de las semillas de dátil (*Phoenix dactylifera*)

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ABSTRACT

Date seeds are by-products of date palm fruit and are considered as an agro-waste and hence go underutilized. They are an excellent, natural and inexpensive source of dietary fiber, proteins, minerals, antioxidants and phenolic compounds. The present study was undertaken to assess the changes in the nutritional, antinutritional and functional characteristics of Mazafati date seeds after the process of soaking, sprouting and roasting. The date seeds were soaked for 48 hours and sprouted for 15 days at room temperature and then roasted at 220^{o}C for 15 minutes in a hot convection oven and ground into a powder. The various quality characteristics of non-sprouted date seeds powder (NSDSP) and Sprouted date seeds powder (SDSP) was further analyzed for its. It was found that SDSP had a significant ($p<0.05$) increase in moisture, protein, dietary fiber, Total ash, phenolic and Flavonoid content and a significant improvement in the Anti-oxidant capacity compared to NSDSP. A significant decrease in the carbohydrates, fat, tannins and phytates was also observed in SDSP when compared to NSDSP. Hence, bio-processing technique like sprouting can enhance the nutritional value of date seeds and can be utilized as a functional ingredient in various foods products and can contribute in agro-waste reduction.

Keywords: Date seeds, Germination, Functional properties, Roasting, Antinutrients, sprouting.

RESUMEN

Las semillas de dátil son subproductos del fruto de la palmera datilera y se consideran un desperdicio agrícola y, por lo tanto, se subutilizan. Son una fuente excelente, natural y económica de fibra dietética, proteínas, minerales, antioxidantes y compuestos fenólicos. El presente estudio se llevó a cabo para evaluar los cambios en las características nutricionales, antinutricionales y funcionales de las semillas de dátil Mazafati después del proceso de remojo, brotación y tostado. Las semillas de dátil se remojaron durante 48 horas y germinaron durante 15 días a temperatura ambiente y luego se tostaron a 220oC durante 15 minutos en un horno de convección caliente y se molieron hasta convertirlas en polvo. Se analizaron más a fondo las diversas características de calidad

del polvo de semillas de dátiles no germinadas (NSDSP) y del polvo de semillas de dátiles germinadas (SDSP). Se encontró que el SDSP tuvo un aumento significativo ($p<0,05$) en la humedad, proteínas, fibra dietética, contenido de cenizas totales, fenólicos y flavonoides y una mejora significativa en la capacidad antioxidante en comparación con el NSDSP. También se observó una disminución significativa en los carbohidratos, grasas, taninos y fitatos en SDSP en comparación con NSDSP. Por lo tanto, la técnica de bioprocesamiento, como la germinación, puede mejorar el valor nutricional de las semillas de dátiles y puede utilizarse como ingrediente funcional en diversos productos alimenticios y puede contribuir a la reducción de los desechos agrícolas.
Palabras clave: Semillas de dátiles, Germinación, Propiedades funcionales, Tostado, Antinutrientes, germinación.

Abbreviations:

- NSDSP - Non-Sprouted Date seeds Powder
- SDSP- Sprouted Date seeds powder
- AOAC Association of Official Analytical Chemists

INTRODUCTION

Dates (*Phoenix dactylifera*) are considered one of the oldest fruits cultivated and consumed by man for many years. They are monocotyledonous plants belonging to the Arecaceae (previously known as Palmaceae). They are fruits with a fleshy pericarp, mesocarp, and epicarp and a ventrally grooved elongated seed, also known as pits. There are more than 2000 species and varieties of dates vary in their nutritional composition, origin, or harvesting processes. The highest producer countries for date palm fruits are Egypt, Iran, and Saudi Arabia, with 1.3, 1.0, and 0.9 million tons per annum, respectively (Abdul Qadir, et.al., 2011). Since the production of date fruit is high, many products are being developed, such as jams, syrups, date bars, and other products, therefore during its processing which includes operations like chopping, pitting, or extraction of syrups, tons of date seeds that are the byproducts that constitute 10-15% of the total weight of the fruit are considered as waste material and disposed of due to their low market value, or it used for animal feeds.

Date seeds are considered an excellent, natural, and inexpensive source of dietary fiber compared to date flesh. Dietary fibers are non-digestible cell wall components that play a significant role in human health and have considerable health benefits. It possesses positive effects on health as well as appropriate functional properties which make it suitable to be used in different food products (Gavzan & Taghizadeh, 2016). The consumption of fiber-rich foods is also associated with prevention of many lifestyle disorders like obesity, cardiovascular diseases, and cancers. It helps in lowering the LDL or bad cholesterol levels, provides satiety, and hence has anti-obesity and anti-diabetic properties. It also enhances gut health by adding faecal bulk to reduce the risk of constipation, hemorrhoids, and colon cancers. In contrast, whole grains containing bran are deemed the wealthiest source of

insoluble fiber, but due to milling, the grains get depleted from the bran, which contains all the good properties. Therefore, many other sources have the potential to be a rich source of dietary fiber but are not commonly used and go underutilized. One among them is date seeds, that are considered an agro-waste rather than a resource. Due to a large quantity of dietary fiber, they are deemed to have potential health benefits for humans as prebiotics. High consumption of dietary fiber delivered through functional foods containing date pits could be claimed to fulfil recommended daily intakes (RDI) given by the FDA and other health organizations (Mahmood et al., 2015).

In addition to this, bioprocessing techniques like soaking and germination are the simplest, effective and the most common methods to enhance the nutritional quality and to reduce the anti-nutritional factors of this legume (Handa et al., 2017). Germination activates seeds from their dormancy, restoring the seed's metabolic activities and leading to biochemical, nutritional, and sensorial changes in the seeds (Dogra et al. 2013). Whereas, roasting is a traditional processing method, which can enhance nutritional value and sensory quality of various foods. Furthermore, roasting affects the antioxidant capacity of products (Peng et al., 2021). Various studies have also shown that germination brings about the accumulation of different bioactive compounds, including GABA, vitamins, and polyphenols. Phenolic compounds are metabolites naturally found in plants that tend to protect the cell components in the body from oxidative damage by acting as free racial scavengers. Phenolic compounds of fruit seeds, such as phenolic acids and flavonoids, have been shown to possess many beneficial effects, including antioxidant, anticarcinogenic, antimicrobial, antimutagenic, and anti-inflammatory activities, and can help in the reduction of cardiovascular diseases (Shahidi, et al., 2015). Regardless of dietary fibre, date seeds also contain bioactive components, which add potential value to food products; hence, it becomes crucial to develop foods that can be a potential source of antioxidants and phytochemicals. Hence date seeds can serve as a cheap source and high nutritional value dietary fiber supplement and promising functional food.

Chronic diseases (diabetes, cardiovascular disease, and diabetes) contribute to 60% of all deaths globally (WHO report, 2014). Since the diet is a modifiable factor that can prevent such diseases, it becomes essential to identify and develop and utilize novel prebiotic and functional foods as an alternative approach to reducing the risk of nutrition-related diseases. Therefore, date seeds and their bio-processing by germination may help contribute towards agro-waste reduction and enhancement of nutritional value by increasing the bioactive compounds and decreasing anti-nutrients present in the date seeds. There are many studies that reported the presence of nutritional, anti-nutritional and functional characteristics and the effect of roasting at different temperatures on date seed of various varieties, but the effect of combination of bioprocessing techniques such as soaking, germination and roasting on the nutritional, antinutritional and functional properties of date seeds and in for particular the Mazafati date seeds variety is still undocumented, which needs to be further explored. Hence, the goal of the present study was to determine effect of soaking, germination and roasting on Nutritional, functional and Anti-nutritional characteristics of Mazafati date (*Phoenix Dactylifera*) seeds.

MATERIAL AND METHODS

Raw Material: Mazafati date seeds were collected in batches from a Ronak Enterprises, Rajkot, Gujrat which manufactures dates products. These date seeds were considered as a waste for the manufacturer which was not being utilized, hence date pulp was removed and the date seeds were segregated, and cleaned to remove the adhering mucilage from the date pulp and skin. The date seeds were divided into two batches of 200 g each, for the further processing of non-sprouted date seeds powder (NSDSP) and Sprouted date seeds powder (SDSP).

Processing of the samples: For the processing of NSDSP 200 g of date seeds were dried for 24 h after cleaning and the dried samples were roasted at 220^0 C for 15 minutes in a hot convection oven. The roasted samples were then crushed using a mortar and pestle and finely ground using a mixer and then sieved using a 60 mesh to obtain a fine powder. The SDSP was processed by soaking 200 g of date seeds in 500 ml of water for 48 h at room temperature. The water was then drained and the soaked seeds were then placed on a wet muslin cloth covered and kept in a cool dark place for sprouting for 15 days with frequent monitoring and sprinkling of water. The sprouted seeds were weighed and dried at room temperature for 24 hours and roasted at 220^0C for 15 minutes. The roasted samples were then crushed using a mortar and pestle and finely ground using a mixer and then sieved using a 60 mesh. Both the NSDSP and SDSP were then stored in an air tight container in a cool place, away from any moisture, or light, for further analysis.

Physiochemical Analysis: The weight of the seeds was determined using an analytical balance. The pH was estimated following the AOAC (method No. 981.21, AOAC 2005) and the moisture was determined as per (AOAC 2005).

Nutritional Components Determination: The protein content was calculated as nitrogen x 6.25, which was estimated by kjeldhal method (AOAC 2005). Total Carbohydrate content was determined calorimetrically by Anthrone method (AOAC 1980). Total Fat was estimated by Soxhlet Method (AOAC 2005) and Total Ash by (AOAC 2005). Soluble, Insoluble and Total Dietary fiber was determined by gravimetric method by enzyme digestion of starch and protein (IS: 11062 – 1984)

Estimation of Functional Properties and Anti-oxidant capacity: The extracts for the determination of Total phenolics, Total Flavonoids and Antioxidant activities were prepared by stirring each of the powdered samples in a conical flask with 20 ml of methanol at 35^0C, 150 rpm /min for 12 h which was then filtered through a whatman filter paper and stored at 4^0c and this was served as a working solution. The Total phenolics in the extracts of both the samples was determined by Folin-Ciocalteau reagent and was expressed as Gallic acid equivalents in mg/g dry weight (Maurya & Singh, 2010). Total flavonoids were determined by Aluminum chloride method (Sahu & Saxena, 2013). The free radical scavenging action of antioxidants was determined by DPPH and was expressed as percent inhibition. (Tepe et al., 2004).

Antinutrients analysis: Tannin was determined calorimetrically by following the AOAC method (AOAC 2005) and was expressed as tannic acid equivalents in mg/100 g dry weight and phytate content was determined by using AOAC method (AOAC 2003).

Statistical Analysis: The analysis was carried out in triplicates for all the above procedures. The statistical analysis was conducted using SPSS. The different tests used included mean, standard deviation and for the analysis of variance one way ANOVA was used followed by Duncan's test to verify the significance of each group ($p<0.05$).

RESULTS AND DISCUSSION

Effect of soaking, sprouting and roasting on the physicochemical properties of Sprouted and non-sprouted date seeds powder. The results in table 1 showed the variance in the mean physiochemical properties of the non-sprouted date seeds powder (NSDSP) and sprouted date seeds powder (SDSP). It shows that the mean score for moisture of NSDSP and SDSP was (0.62±0.54 %) and (2.82±2.44 %) respectively. The SDSP had a higher moisture when compared to the NSDSP. The increase in moisture could be due to the soaking process before the germination, as the entry of water(imbibition's) in the seed coat causes the seed to swell and initiate sprouting (Devi, et.al.,2015). The highest weight gain was observed in sprouted date seeds (312.66±0.577 g) when compared to non-sprouted date seeds (199.66±0.57 g) and they were significantly different ($p<0.05$), the increase in weight could be due to the water absorption during soaking and the weight of the sprouts during germination. The Moisture and weight gain are positively correlated (r-+1.0) which indicates due to the increase in the moisture content there was an increase in the weight of the seeds after soaking and sprouting. The date seeds after sprouting had a significant ($p<0.05$) decrease in their pH from (4.36±0.57) to (3.10±0.0). According to a study there is a positive correlation between pH and maximum germination, with no germination observed below pH 4 (Turner, et.al., 1988)

Effect of soaking, sprouting and roasting on the nutritive values of Sprouted and non-sprouted date seeds powder. Table 2 illustrates the proximate composition of NSDSP and SDSP. The results indicated that mean energy (kcal) values of NSDSP (197.23±0.05) kcal was Significantly($p<0.05$) higher than SDSP (181.74±0.55) Kcal. The reduction in the energy value might be due to breakdown of starches and fat during the germination process. (Murata *et al*,1968). A statistically significant difference($p<0.001$) was observed after sprouting. The carbohydrate content of SDSP (15.72±0.35) g was less as compared to NSDSP (17.03±0.03) g by a small numerical difference of 1.31g. The results obtained were similar to another study where the carbohydrate content of the lentils decreased from 48.70% in raw samples to 41.69 % in sprouted samples of 6 days (Fouad & Ali, 2015). The alpha-amylase breaks down complex carbohydrates to simpler and more absorbable sugars which are utilized by the growing seedlings during the early stages of germination for energy. Germination brings about a change in the insoluble nutrients in the cotyledons to soluble nutrients by hydrolysis of macromolecules. (Enuji-ugha, *et.al*., 2003). The

protein content was slightly high in SDSP (8.18±0.42) g when compared to NSDSP (7.52±0.60) g. The increase in the protein could be due to the synthesis of new proteins by proteases during germination (Bau *et al.*1997). Since the date seeds were sprouted for a higher period of 15 days there would have been a greater loss of dry weight which might have reawakened the protein synthesis upon imbibition's, which lead to increase in the protein content of sprouted seeds (Nonogaki, *et. al*., 2010). The fat content of NSDSP (11.00±0.57) g decreased to (9.46±0.94) g in SDSP. The loss in the fat could be due to total solid loss (Wang *et al*. 1997) or fat being used as energy for the germination process (El-Adawy, 2002). The data also showed that there was significant ($p < 0.001$) impact of sprouting on the dietary content of date seeds with the mean values of (88.85±0.01) g and (87.59±0.01) g NSDSP and SDSP respectively. There was an increase in the dietary fiber content in SDSP which was similar with the finding of (Chung *et al,* 1989) where the fiber content increased from 3.75% in unsprouted barley seed to 6% in 5-day sprouts. The change might be due the loss of starch. (Ranhotra, *et.al*.,1977). Mineral Content in the seed was analyzed by estimation of the total Ash content which gives the inorganic residue remaining after the removal of organic matter and water by heating. It was observed that the ash content of NSDP (0.96±0.57) % had a significant ($p>0.001$) increase after sprouting to (1.76±0.57) % in SDSP. The increase could be due to increase in the phytase enzyme activity during germination that would hydrolyze the bond between protein-enzyme and minerals become free and their availability increases. (Narsih et al., 2012)

Effect on the Functional Properties of sprouted and non-sprouted date seed powders. Table 3 illustrates the mean total phenolic content and Total flavonoid content of NSDSP and SDSP. The phenolic content of NSDSP was found to be (20.15±0.53) mg/100g which was in contrast to the results obtained by (Ghezi1 *et al*., 2020) where the phenolic content of Al Hillawi, Al Khadhrawi and Al zahdy date varieties ranged from 56.6, 65.6 and 65.32 mg/ml for methanolic extract respectively and 27.88 to 40.06 mg/ml for aqueous extract. The decrease in the phenolic content in the NSDSP in our study might be due to the roasting process (Lemos *et al*., 2012). There was a slight statistically significant($p<0.001$) increase in the phenolic content of the SDSP (22.02±0.20) mg/100 g when compared to NSDSP. The increase could be due to biosynthesis or bioaccumulation of phenolic compounds as a defense mechanism to survive under stress (Randhir *et al*., 2004). The maximum level of Phenolic content of lentil seeds increased till 5 days of germination and decreased on the 6th day (Fouad & Ali, 2015). The decrease might be due to mobilization of stored phenolics by the activation of enzymes like polyphenol oxidase during sprouting process (Vadivel & Biesalski, 2012). The flavonoid content of NSDSP of the Mazafati date seeds was 3.85±0.15 mg/100g which was in accordance with the results obtained by (Rehman, 2017), where the highest total flavonoid content (3.1 mg/g dry weight at 100%) was seen in Qush Basrah date seeds from Bahla. Sprouting of seeds is known to increase the nutritive value such as phenolics and flavonoids and the health qualities of foods in a natural way. (Kim *et al*., 2012) Our results are in accordance with others as a significant increase was seen in SDSP (5.61±0.05) when compared to NSDSP. The increase might be due to enzymatic activity induced by heat during roasting. (Kim *et al.,* 2017).

Effect of soaking, germination and roasting on the DPPH free radical scavenging activity in sprouted and non-sprouted date seeds powder

The Fig.1 depicts the percentage inhibition of the DPPH scavenging activity of NSDSP and SDSP and it was observed that the percentage inhibition increased with the increase in the concentration. The concentration range was between 200 µ/ml to 1000µ/ml. The percentage of inhibition at a lower concentration of 200µ/ml for NSDSP and SDSP was found to be 25.19 % and 27.48% respectively. And at the highest concentration of 1000µ/ml the percentage of inhibition for NSDSP and SDSP was found to be 81.68 % and 83.21% respectively. SDSP had a significant higher DPPH radical scavenging activity compared to NSDSP. The increase in the antioxidant activity with sprouting is one of the metabolic changes which takes place upon sprouting of seeds due to increase in the activity of hydrolytic enzymes. (Cuadrado, 1997). The increase might also be due to the synthesis of compounds such as vitamin C or tocopherols which are responsible for antioxidant activity. (Sharma & Gujral, 2010).

Effect of soaking, germination and roasting on the Anti-nutrient composition in sprouted and non-sprouted date seed powder. Table 4 depicts the Means and standard deviation values of Anti-nutrients present in the NSDSP and SDSP. Antinutrients are commonly found in plant foods and have an adverse effects or health benefits. A change in the tannin content of NSDSP was observed after the process of sprouting. It was observed that Tannin content in SDSP (0.95±0.01) mg/100g had a significant ($p<0.001$) decrease when compared to NSDSP. The decrease in the tannins after soaking and sprouting might be due formation of tannin-enzyme and tannin-protein complex or due to binding and leaching of tannins with organic compounds like carbohydrates. (Rusydi.M, *et al,*2012). A change in phytates content of NSDSP (0.56±0.02) mg/100g was observed which decreased significantly ($p<0.001$) in SDSP (0.36±0.00) mg/100g. The reduction in phytic content during soaking may be due to the leaching of phytate ions in water due to diffusion. (Duhan A*, et.al;* 2002). Overall reduction of both tannins and phytates maybe attributed to the combined effects of soaking and germinating treatments. (E. Sangronis, *et.al,* 2007).

Correlation between antioxidant activity and Antinutrients in NSDSP and SDSP. Table 5 depicts a correlation between antioxidant activity and the antinutrient levels. A negative correlation (r= (-0.992) was observed between the antioxidant activity an Antinutrients. It was observed that as the Antioxidant activity increased in NSDSP and SDSP, there was a decrease seen in the antinutrients like tannins and phytates.

CONCLUSION

The study apparently showed that traditional bioprocessing techniques like soaking, germination and roasting significantly improved the nutritional, functional and antioxidant properties of date seeds and sprouting of date seeds can further enhance the already present rich nutrients and bioactive components as well as reduce the anti-nutritional factors which would inhibit absorption of the nutrients present in date seeds. Therefore,

underutilized source of wealth (sprouted date seeds powder) can be processed and incorporated into various foods and beverages to enrich them with dietary fiber or bioactive compounds which can not only serve the purpose of agro-waste reduction but also provide healthier versions of the normally consumed foods.

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Received: 14th June 2023; Accepted: 06th January 2024; First distribution: 25th January 2024.

Table 1 Effect of soaking, sprouting and roasting on the Physiochemical Analysis of Sprouted and non-sprouted date seeds powder

Parameters	NSDSP	SDSP	p-value	r- Value
Moisture (%)	0.62±0.54	2.82±2.44	<0.001*	
Weight gain (g)	199.66±0.57	312.66±0.577	<0.001*	+1.0
pH	4.36±0.57	3.10±0.0	<0.001*	

All values are means of triplicate determinants ± standard deviation (S.D). *Significantly different (<0.05) by ANOVA; R-value ranges between+1 to -1 by Pearson's correlation's; NSDSP- Non-Sprouted Date seeds Powder; SDSP- Sprouted Date seeds Powder

Table 2 Effect of soaking, sprouting and roasting on Proximate composition of Sprouted and Non-Sprouted date seeds powders

Parameters	NSDSP	SDSP	P-value
Energy (Kcal)	197.23±0.05	181.74±0.55	<0.001*
Carbohydrates(g)	17.03±0.03	15.72±0.35	<0.001*
Protein (g)	7.52±0.60	8.18±0.42	0.19^{NS}
Fat (g)	11.00±0.57	9.46±0.94	0.07^{NS}
Dietary Fiber(g)	87.59±0.01	88.85±0.01	<0.001*
Total Ash (%)	0.96±0.57	1.76±0.57	<0.001*

All values are means of triplicate determinants ± standard deviation (S.D). *P-value is <0.05 by ANOVA, NS-not significant, NSDSP- Non-Sprouted Date seeds Powder, SDSP- Sprouted Date seeds Powder

Table 3 Effect of soaking, sprouting and roasting on Functional composition of sprouted and non-sprouted date seeds powders

Parameters	NSDSP	SDSP	P-value
Total Phenols (mg/100g)	20.15±0.53	22.02±0.20	<0.001*
Total Flavonoids(mg/100g)	3.85±0.15	5.61±0.05	<0.001*

All values are means of triplicate determinants ± standard deviation (S.D). *P-value is <0.05 by ANOVA, NS-not significant, NSDSP- Non-Sprouted Date seeds Powder, SDSP- Sprouted Date seeds Powder.

Table 4 Effect of soaking, germination and roasting on the Anti-nutrient composition in sprouted and non-sprouted date seed powder

Parameters	NSDSP	SDSP	P value
Tannins(mg/100g)	0.95±0.01	0.77±0.01	<0.001*
Phytates (mg/100g)	0.56±0.02	0.36±0.00	<0.001*

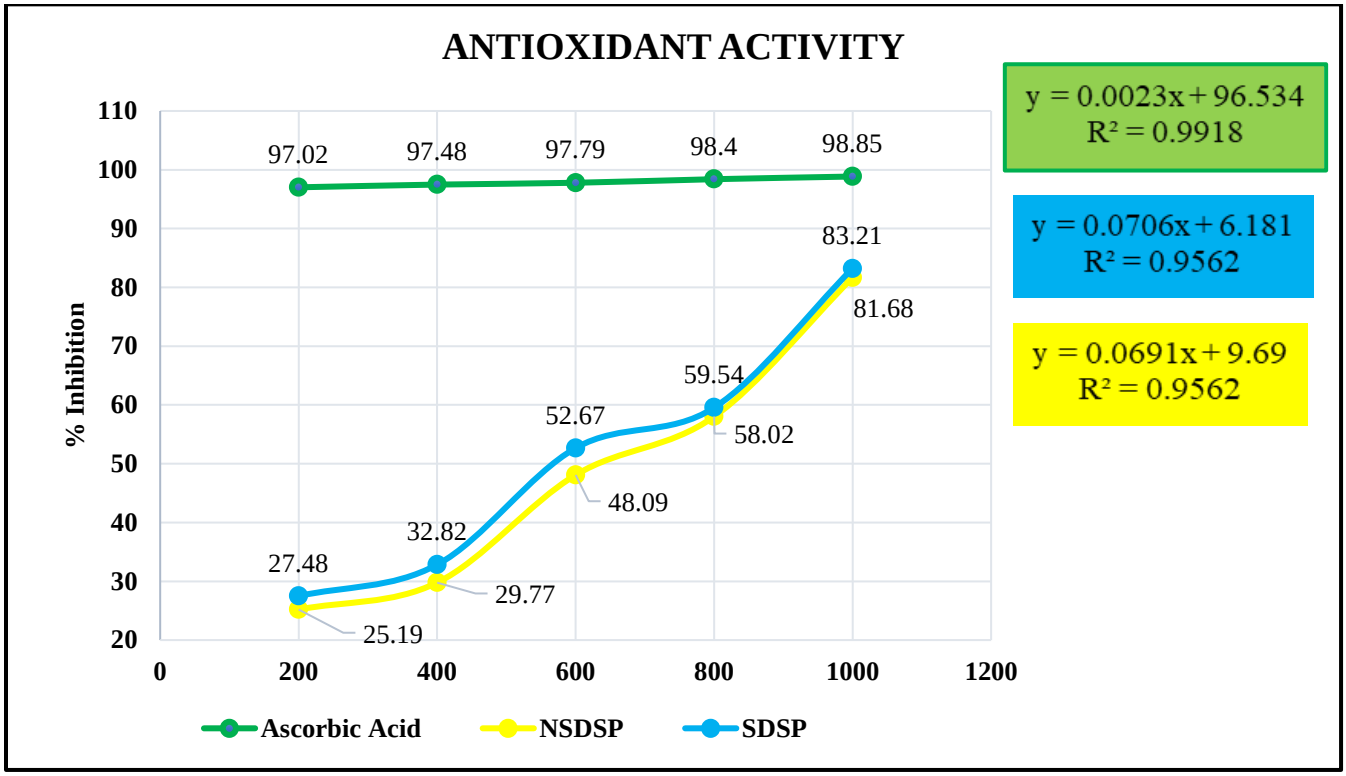
All values are means of triplicate determinants ± standard deviation (S.D); *P-value is <0.05 by ANOVA; NS-not significant; NSDSP- Non-Sprouted Date seeds Powder; SDSP- Sprouted Date seeds Powder

Table 5 Correlation between the antioxidant activity and Antinutrient levels

Parameters	NSDSP	SDSP
Antioxidant Activity (%)	48.55±0.01	53.67±0.01
Tannins (mg/100g)	0.95±0.01	0.77±0.01
Phytates (mg/100g)	0.56±0.02	0.36±0.00
R-value	-0.992	

All values are means of triplicate determinants ± standard deviation (S.D). R-value ranges between+1 to -1 by Pearson's correlation; NSDSP- Non-Sprouted Date seeds Powder, SDSP- Sprouted Date seeds Powder.

Fig.1 Effect of soaking, germination and roasting on the DPPH free radical scavenging activity in sprouted and non-sprouted date seeds powder


ANTIOXIDANT ACTIVITY
% Inhibition
110
100
90
80
70
60
50
40
30
20
0
200
400
600
800
1000
1200
97.02
97.48
97.79
98.4
98.85
83.21
81.68
59.54
58.02
52.67
48.09
32.82
29.77
27.48
25.19
y = 0.0023x + 96.534
R² = 0.9918
y = 0.0706x + 6.181
R² = 0.9562
y = 0.0691x + 9.69
R² = 0.9562
Ascorbic Acid
NSDSP
SDSP