

Effect of thermal processing of hemp seeds (*Cannabis Sativa* L.) on its compositional and functional parameters

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SUMMARY: Hemp seeds (*Cannabis Sativa* L.) are increasingly recognized as a functional food due to their rich nutritional composition and bioactive compounds. Roasting, a widely used processing method, enhances flavor, modifies texture, and alters nutritional attributes. This study examines the effects of roasting on the proximate composition, antioxidant activity, and functional properties of hemp seeds. Roasting significantly reduced moisture content (from 0.99 to 0.36%), leading to an increase in fat (from 26.96 to 28.64%) and protein (from 31.98 to 35.04%). The total phenolic content (TPC) increased by approximately 155% (from 47.00 to 120.10 mg GAE/100g), contributing to enhanced antioxidant activity. While Ferric Reducing Antioxidant Power (FRAP), Reducing Power Activity (RPA), and ABTS-RSA radical scavenging activity increased significantly, DPPH-RSA activity showed a slight decline. Functional properties, including oil-holding and water absorption capacity, improved after roasting, whereas bulk density remained relatively unchanged. Overall, the findings support the use of roasted hemp seeds as a functional ingredient with applications in innovative food formulations.

KEY-WORDS: Antioxidant enhancement; Bioactive compounds; Functional foods; Hemp seeds; Nutritional composition; Roasting process.

RESUMEN: Efecto del procesamiento térmico de semillas de cáñamo (*Cannabis Sativa* L.) sobre sus parámetros composicionales y funcionales. Las semillas de cáñamo (*Cannabis Sativa* L.) son cada vez más reconocidas como un alimento funcional debido a su rica composición nutricional y compuestos bioactivos. El tostado, un método de procesamiento ampliamente utilizado, mejora el sabor, modifica la textura y altera los atributos nutricionales. Este estudio examina los efectos del tostado en la composición proximal, la actividad antioxidante y las propiedades funcionales de las semillas de cáñamo. El tostado redujo significativamente el contenido de humedad (del 0,99 % al 0,36 %), lo que resultó en un aumento de la grasa (del 26,96 % al 28,64 %) y la proteína (del 31,98 % al 35,04 %). El contenido fenólico total (TPC) aumentó aproximadamente un 155 % (de 47,00 a 120,10 mg GAE/100 g), lo que contribuyó a una mayor actividad antioxidante. Si bien el poder antioxidante reductor férrico (FRAP), la actividad de poder reductor (RPA) y la actividad de eliminación de radicales ABTS-RSA aumentaron significativamente, la actividad de DPPH-RSA mostró una ligera disminución. Las propiedades funcionales, como la retención de aceite y la capacidad de absorción de agua, mejoraron tras el tostado, mientras que la densidad aparente se mantuvo prácticamente sin cambios. En general, los hallazgos respaldan el uso de semillas de cáñamo tostadas como ingrediente funcional con aplicaciones en formulaciones alimentarias innovadoras.

PALABRAS CLAVE: Alimentos funcionales; Composición nutricional; Compuestos bioactivos; Proceso de tostado; Potenciador de antioxidantes; Semillas de cáñamo.

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1. INTRODUCTION

Cannabis, a wind-pollinated plant from the *Cannabaceae* family, has its origins in central Asia and has been utilized since ancient times for food, fiber, medicine, and psychotropic substances. The two main species are *Cannabis Sativa* L. and *Cannabis indica* L., with ongoing debates among botanists regarding their distinctions (Rashida *et al.* 2020). In India, *Cannabis sativa*, known as Indian hemp, has historical significance, and was mentioned in the Atharva Veda as one of the five sacred plants. It has been used for recreational, religious, and spiritual purposes, as well as for its fiber, food, oil, and medicinal properties. However, the British government of India imposed restrictions on cannabis resin consumption in the 1930s, leading to a decline in the cultivation and use of *C. sativa* (Bapat, 2015).

In July 2018, the Indian government authorized the commercial growth of hemp, marking a significant step towards industrial hemp production, particularly in Uttarakhand. This state became the first to reinterpret industrial hemp laws, allowing for the licensing of premium hemp fiber production. Villages in the Pauri Garwal region have begun cultivating non-narcotic hemp to establish a seed bank, potentially providing locals with sustainable livelihoods. The hemp sector in Uttarakhand is projected to generate approximately 240 crores rupees annually, with farmers earning around 1 million rupees from hemp sales (Dasila *et al.* 2020).

Hemp seeds (*Cannabis sativa* L.) are increasingly recognized as a functional food due to their rich nutritional profile, often labeled as “superfood.” They are an excellent source of high-quality protein, essential fatty acids, fiber, and various bioactive compounds that promote health and well-being (Callaway, 2004). Unlike marijuana, hemp seeds contain only trace amounts of tetrahydrocannabinol (THC), making them a safe and legal dietary option with no psychoactive effects (Rodriguez-Leyva and Pierce, 2010).

Hemp seeds are nutritionally beneficial, and are rich in polyunsaturated fatty acids (PUFAs), especially omega-3 and omega-6 in a favorable 3:1 ratio, which supports cardiovascular health. They also serve as a complete protein source, containing all nine essential amino acids, making them an excellent alternative to animal proteins for vegetarians and vegans (Tang *et al.*, 2006).

Roasting is an effective food processing technique which reduces anti-nutrients in seeds while enhancing their flavor and aroma. Studies indicate that roasting hemp seeds not only increases their oil and protein content but also serves as beneficial pre-treatment before oil extraction, potentially boosting oil production (Babiker *et al.*, 2021). The roasting process affects the antioxidant capacity of oil seeds by breaking down naturally occurring antioxidant compounds and creating new Maillard Reaction Products (MRPs) with antioxidant potential. Generally, roasted seeds have higher antioxidant levels compared to unroasted ones, as roasting facilitates the release of phytochemicals from the seed matrix. Previous research has shown that roasting seeds optimizes the extraction of antioxidant compounds (Akele *et al.*, 2024).

Given their rich nutrient composition and wide-ranging health benefits, hemp seeds have become a subject of scientific interest for their potential applications in nutrition and medicine. This paper aims to explore the nutritional composition of hemp seeds and examine their health benefits, focusing on their impact on cardiovascular health, metabolic function, and inflammation. This study will highlight the potential of hemp seeds as a valuable dietary supplement for promoting human health. The objective of this study is to evaluate the effects of roasting on the proximate composition, antioxidant capacity, and functional properties of hemp seeds.

2. MATERIALS AND METHODS

2.1. Raw material

Hemp seeds were purchased from the local market of Haldwani, Uttarakhand, India.

Raw hemp seed were cleaned to remove unwanted extraneous matter. They were then stored at room temperature until use. Roasted hemp seeds: Raw hemp seeds were roasted in a pan at 160 °C for 10 minutes. The roasting time and temperature were selected based on preliminary trials to ensure a balance between enhanced antioxidant properties and preserving the structural integrity of the seeds.

2.2. Sample preparation

A five-g sample of raw and roasted hemp seeds was weighed and ground into a fine powder. The powdered seeds were extracted with 80% acidified methanol thrice using an orbital shaker for 30 minutes at room temperature. After extraction, the mixture was centrifuged at 4000 rpm for 10 minutes, and the supernatant was collected for analysis.

2.3. Proximate composition analysis

Moisture content was determined using the AOAC method (2010). The estimation of fat was done using an automated Soxhlet apparatus by Pelican SOCS-PLUS. Protein content was analyzed using a Kjeldahl apparatus (Kjelplus SUPRA LX VA). The ash of *Cannabis sativa* L. seeds was prepared according to the method given in AOAC, (1997). Carbohydrate content was calculated using the following formula, as described by Gbenga-Fabusiwa *et al.* (2018).

$$\text{Carbohydrate} = 100 - (\text{Moisture} + \text{Fat} + \text{Ash} + \text{Protein})$$

2.4. Analysis of antioxidant activity

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method by Singleton and Rossi (1965), and the results were expressed as milligrams of Gallic acid equivalent per 100 g of hemp seeds (mg GAE/100 g). Flavonoid content was measured using the aluminum chloride colorimetric method and expressed as milligrams of Rutin equivalent per 100 g of hemp seeds (mg RE/100 g) by Singleton *et al.* (1999).

Ferric Reducing Antioxidant Power (FRAP) Assay was performed according to Benzie and Strain (1996), where the absorbance was measured at 593 nm. The results were expressed as milligrams of Trolox equivalent per 100 g of hemp seeds (mg TE/100 g). DPPH Radical Scavenging Activity (DPPH-RSA) was determined according to the method of Brand-Williams *et al.* (1995), with absorbance measured at 517 nm. Results were expressed as milligrams of Trolox equivalent per 100 g. ABTS Radical Scavenging Activity (ABTS-RSA) was assessed according to Rice-Evans (1997), and the results were expressed as milligrams of Trolox equivalent per 100 g. Reducing Power Assay (RPA) was determined using the method given by Oyaizu, (1986).

2.5. Functional Properties

Oil-holding capacity (OHC), water absorption capacity (WAC) and bulk density (BD) were estimated using the techniques described by Mall and Patel (2023).

2.6. Statistical analysis

All experiments were performed in triplicate ($n = 3$). Statistical analysis was carried out using M.S. Excel 2016 with a paired t-test. Results are presented as Mean $\pm$ Standard Deviation (SD). Charts and scatter graphs were also prepared using M.S.Excel 2016.

RESULTS

3.1. Proximate composition of raw and roasted hemp seeds

The moisture contents in raw and roasted hemp seeds was 0.99 and 0.36%, respectively. Fat content was 26.96% in raw seeds and 28.64% in roasted seeds. Protein content increased from 31.98% (raw) to 35.04% (roasted). Carbohydrate content was 31.93% in raw seeds and 27.29% in roasted seeds. The ash contents in raw and roasted seeds were 7.49 ± 0.03 and 8.69 ± 0.04 , respectively (Table 1).

TABLE 1. Proximate composition of raw and roasted hemp seeds

Sample	Moisture %	Fat %	Protein %	Ash %	Carbohydrates %
Raw Hemp Seeds	0.99 ± 0.025	26.96 ± 0.61	31.98 ± 0.83	7.49 ± 0.03	31.93 ± 1.26
Roasted Hemp Seeds	0.36 ± 0.029	28.64 ± 0.47	35.04 ± 1.22	8.69 ± 0.04	27.29 ± 1.36
t value	251**	-3.17**	-3.56**	-43.15**	3.62**

Values are Mean ± SD of three observations

**indicates significant difference at $p \leq 0.05$

3.2. Total phenolic content (TPC) and flavonoid content (FLV)

The TPC values for raw and roasted hemp seeds were 47.00 mg GAE/100 g and 120.10 mg GAE/100 g, respectively. Flavonoid content was 102.44 mg RE/100 g in raw seeds and 101.83 mg RE/100 g in roasted seeds (Figure 1).

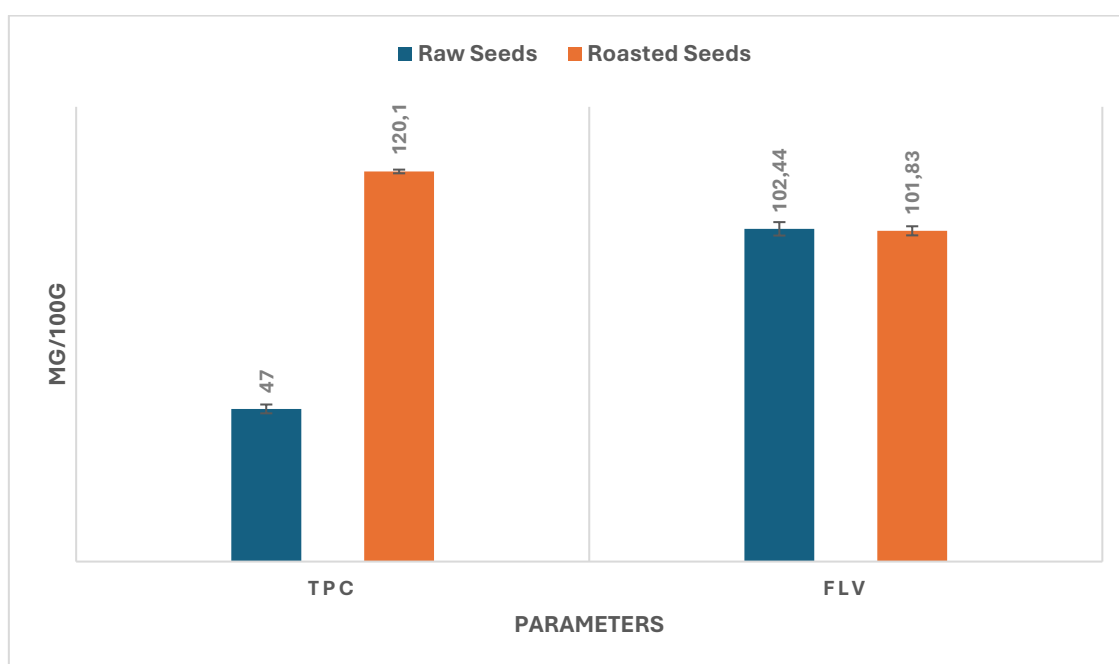


FIGURE 1. Total Phenolic Content (TPC) and Flavonoid Content (FLV) in raw and roasted seeds. The bar chart compares TPC and FLV values for raw (blue) and roasted (orange) seeds, showing a significant increase in TPC after roasting, while FLV content remains nearly unchanged. Error bars represent standard deviations. Values are Mean ± SD of four observations

3.3. Antioxidant activity of raw and roasted hemp seeds

The FRAP values for raw and roasted hemp seeds were 61.12 mg TE/100 g and 82.18 mg TE/100 g, respectively. The DPPH-RSA values were 50.05 mg TE/100 g (raw) and 44.18 mg TE/100 g (roasted). The ABTS-RSA values were 26.01 mg TE/100 g in raw seeds and 31.65 mg TE/100 g in roasted seeds. The RPA values for raw seeds and roasted seeds were 78.92 mg TE/100 g and 189.31 mg TE/100 g, respectively (Table 2).

TABLE 2. Antioxidant activity of raw and roasted hemp seeds

SAMPLE	FRAP (mg TE/100 g)	DPPH-RSA (mg TE/100 g)	ABTS-RSA (mg TE/100 g)	RPA (mg TE/100 g)
Raw Hemp Seeds	61.12 ± 1.62	50.05 ± 0.31	26.01 ± 0.19	78.92 ± 2.43
Roasted Hemp Seeds	82.18 ± 0.42	44.18 ± 0.35	31.65 ± 0.37	189.31 ± 2.51
t value	-25.09**	24.59**	-26.47**	-68.81**

Values are Mean ± SD of three observations

**indicates significant difference at $p \leq 0.05$

3.4. Functional properties of raw and roasted hemp seeds

The oil-holding capacity was 1.24 g/g in raw seeds and 1.64 g/g in roasted seeds. The water absorption capacity of raw seeds was 1.94 g/g and 2.46 g/g of roasted seeds. Bulk density was measured as 0.50 g/mL in raw seeds and 0.51 g/mL in roasted seeds (Figure 2).

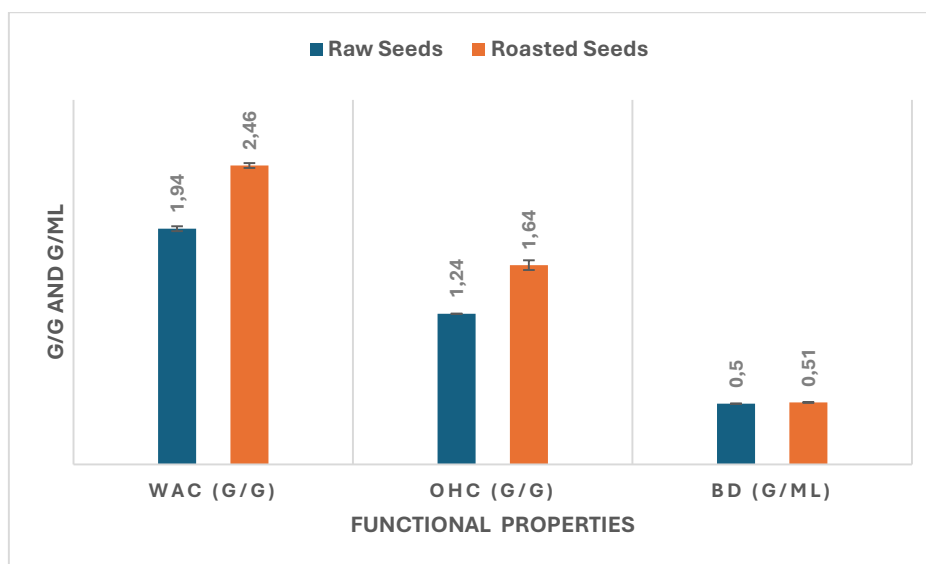


FIGURE 2. Oil Holding Capacity (OHC), Water Absorption Capacity (WAC), and Bulk Density (BD) of raw and roasted seeds. The bar chart illustrates that roasting increases both OHC and WAC, with a significant rise in WAC, while BD remains nearly unchanged. Error bars represent standard deviations. Values are Mean ± SD of four observations

3.5. Regression analysis between total phenols and antioxidant activity

A regression analysis revealed a strong positive correlation between total phenolic content and flavonoid content in both raw and roasted hemp seeds. (Figure 3). A regression analysis revealed a strong positive correlation between total phenols and antioxidant activities (FRAP, RPA, and ABTS-RSA). In raw seeds, total phenols accounted for 85% of variation in FRAP and 80% in RPA. However, the ABTS-RSA assay exhibited a weaker correlation. After roasting, the correlation with FRAP strengthened to 89%, while the relationship with RPA remained moderate. ABTS-RSA activity demonstrated a minimal correlation with total phenols after roasting (Figure 4a and 4b).

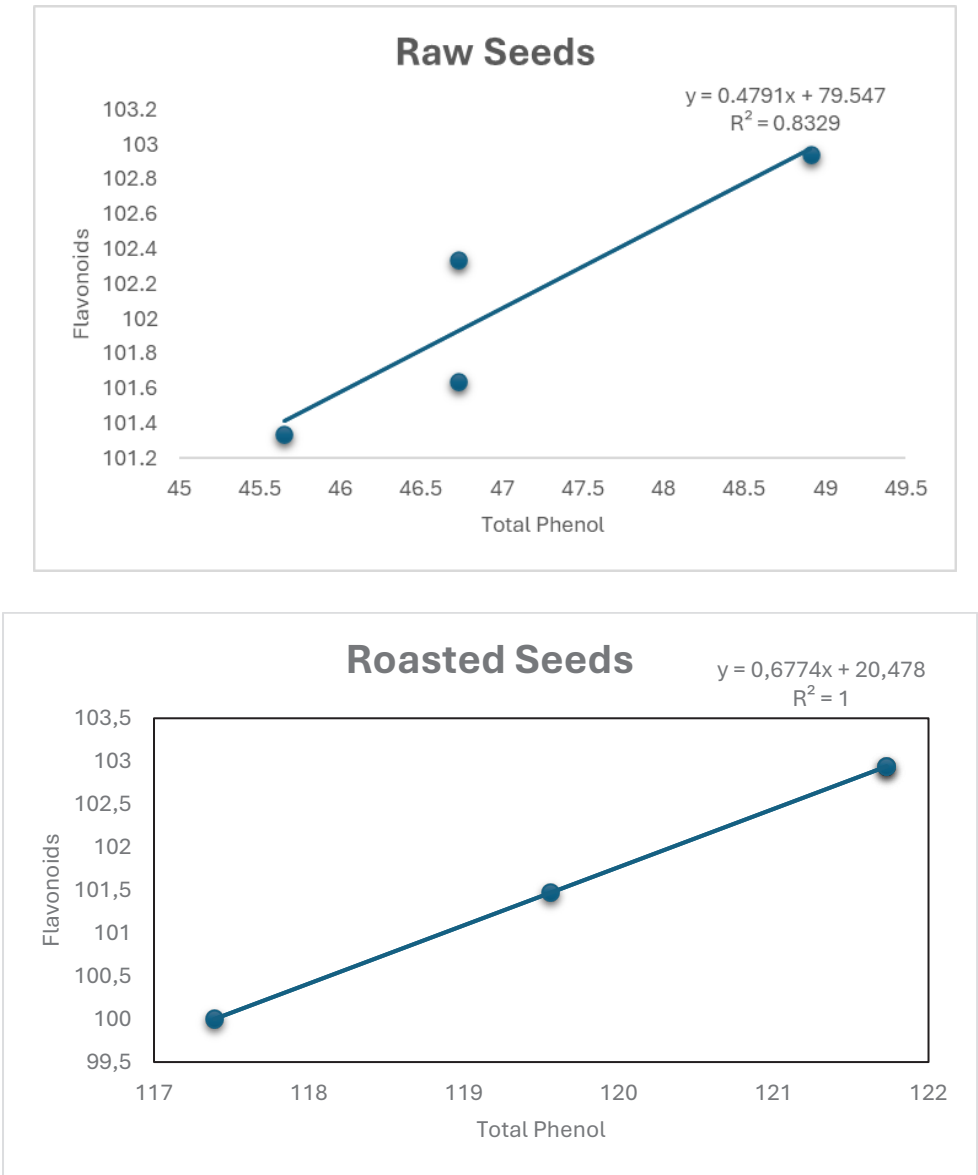


FIGURE 3. Correlation between total phenolic content and flavonoid content in raw and roasted hemp seeds. The scatter plot shows a positive linear relationship, and R^2 value indicating a strong correlation.

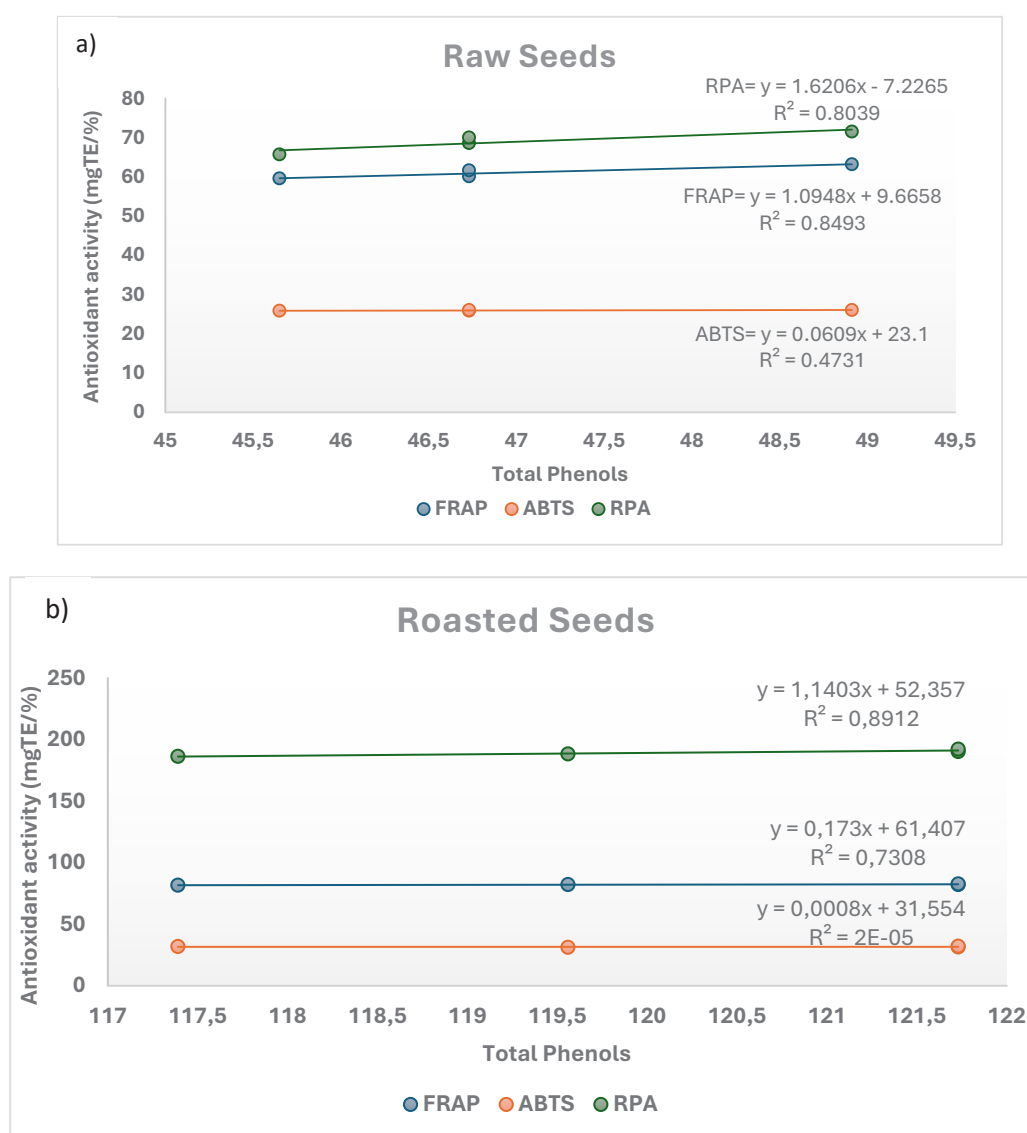


FIGURE 4. Relationship between total phenolic content and antioxidant activity in raw and roasted hemp seeds. a) Regression analysis between total phenols and total antioxidant capacity of raw hemp seeds; the scatter plot illustrates a positive correlation, as indicated by the regression equation and R^2 value. b) Regression analysis between total phenols and total antioxidant capacity of roasted hemp seeds; the scatter plot shows a clear positive trend, backed by the regression equation and R^2 value.

4. DISCUSSION

Compared to raw hemp seeds, roasted seeds had significantly increased protein and fat contents and significantly reduced moisture content ($p < 0.05$). Roasted seeds had a much lower carbohydrate content. The reduction in moisture content observed in roasted hemp seeds aligns with studies, such as Ee *et al.* (2013), which highlight the gradual evaporation of water during roasting. The increase in fat content in roasted hemp seeds is consistent with findings from Tu *et al.* (2021), who reported similar lipid concentration effects in macadamia nuts and groundnut seeds due to moisture loss and structural modifications. The observed increase in protein content corroborates research by Kumar *et al.* (2013), who found that roasting enhances protein concentration via moisture reduction and possibly due to Maillard reactions. However, Maillard reactions may also influence protein digestibility and amino acid availability. The concentration effect, rather than a real increase in total protein, is mostly responsible for the greater protein content seen after roasting. Roasting

increases the proportionate protein value by reducing moisture and heat-labile non-protein components such as tannins and phytic acid (Yadav and Bhatnagar, 2017). Additionally, heat treatment improves nitrogen extractability during analysis by denaturing seed proteins and upsetting cell structures (Waszkowiak *et al.*, 2020). Furthermore, heat treatment increases the digestibility of proteins by changing their structure and deactivating protease inhibitors, which makes proteins easier to analyze (Ohanenye *et al.*, 2022). Therefore, rather than actual protein production, the apparent increase in protein content is caused by moisture loss, decreased antinutrients, and enhanced extractability.

Roasted seeds had a significantly higher TPC than raw seeds ($p < 0.05$). There was no discernible difference in the flavonoid concentration of raw and roasted seeds. Since flavonoids are a key subclass of phenolic chemicals in plant systems, the strong correlation between total phenols and flavonoids in raw seeds suggests that changes in total phenolic content accounted for a considerable percentage of the variance in flavonoid levels (Rice-Evans *et al.*, 1997). According to reports that roasting increases the release of bound phenolics and flavonoids, this correlation continued after roasting, showing that the increase in phenolic compounds brought on by heat processing was proportionately reflected in flavonoid levels (Peng *et al.*, 2021). According to research on roasted grain and seed matrices, the regression pattern indicates that flavonoids make up a significant amount of the overall phenolic profile in hemp seeds. According to research on roasted grain and seed matrices (Hatamian *et al.*, 2020), the regression pattern indicates that flavonoids make up a significant amount of the overall phenolic profile in hemp seeds. This association is consistent both before and after roasting.

The levels of FRAP, ABTS-RSA, and RPA were significantly higher in roasted seeds than in raw seeds ($p < 0.05$). Roasted seeds have far less DPPH-RSA activity. An antioxidant activity analysis indicates that roasting enhances FRAP, ABTS-RSA, and RPA values while reducing DPPH-RSA activity. The increase in FRAP is in agreement with Kittibunchakul *et al.* (2023), who attributed such increases to Maillard reaction products. Conversely, the decrease in DPPH-RSA activity aligns with findings by Babiker *et al.* (2021), who reported reductions in radical scavenging ability post-roasting.

The various reaction pathways involved in these tests are responsible for the rise in FRAP, ABTS, and reducing power following roasting, even if DPPH-RSA activity decreased. While roasting releases bound phenolics and produces Maillard reaction products (MRPs) and melanoidins, which have strong electron-donating and radical cation quenching abilities, it also degrades some heat-labile, small phenolics that are highly effective DPPH-RSA scavengers, resulting in a decrease in DPPH-RSA activity (Zhao *et al.*, 2022). This increases FRAP and ABTS-RSA values. According to Ajatta *et al.* (2021) these newly generated MRPs and released phenolics are more reactive in redox-based assays (like FRAP) or ABTS-RSA scavenging, but they might be structurally too big, polar, or sterically hindered to efficiently neutralize the hydrophobic DPPH radical. Thepthanee *et al.* (2024) have shown similar assay-dependent patterns in sunflower and cereal matrices upon roasting, with FRAP and ABTS-RSA increasing due to increased reducing power and DPPH-RSA declining due to loss of natural phenolics. Therefore, the products of the Maillard reaction do contribute to antioxidant activity, but their influence is more noticeable in FRAP and ABTS-RSA tests than in DPPH-RSA scavenging.

The OHC and WAC of roasted seeds were substantially greater than those of raw seeds ($p < 0.05$). Raw and roasted seeds did not significantly differ in bulk density. Enhancements in functional properties, particularly in OHC and WAC, suggest improved oil and water retention due to structural modifications. These findings are consistent with Hatamian *et al.* (2020), who observed similar trends in roasted chia seed flours. The negligible change in bulk density indicates that roasting does not significantly alter the compactness of hemp seeds, which could influence their processing applications (Iwe and Onadipe, 2001).

The regression analysis highlights the significant influence of total phenols on antioxidant activity, particularly in FRAP and RPA assays. Roasting also increased the bioavailability of phenolic compounds as evident from the relationship between total phenols and the antioxidant assay. However, the weak correlation between phenols and ABTS-RSA activity post-roasting suggests that other antioxidant mechanisms may contribute to ABTS radical scavenging.

Overall, these findings indicate that roasting enhances the nutritional profile, antioxidant potential, and functional properties of hemp seeds, rendering them suitable for various food and industrial applications. Future research should explore the impact of different roasting temperatures and durations on bioactive compound stability and bioavailability.

CONCLUSIONS

The nutritional, antioxidant, and functional qualities of hemp seeds were significantly enhanced by roasting. Fat and protein levels increased proportionately because of the roasting which caused a considerable reduction in moisture content. After roasting, antioxidant components, especially total phenolic content, Ferric Reducing Antioxidant Power, and reducing power activity significantly increased, suggesting improved redox potential. The overall antioxidant capacity increased despite the drop in DPPH-RSA activity, indicating assay-specific reactions to heat-induced chemical alterations. Functional traits, such as the ability to store oil and absorb water, also improved, indicating advantageous structural changes. These results show that roasting hemp seeds improves their qualitative characteristics and supports their prospective use as value-added components in food and nutraceutical formulations.

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DECLARATION OF COMPETING INTEREST

The authors of this article declare that they have no financial, professional or personal conflicts of interest which could have inappropriately influenced this work.

AUTHORSHIP CONTRIBUTION STATEMENT

A. H. Yadav: Conceptualization, methodology, investigation, data collection, formal analysis, writing-original draft.

V. H. Patel: Supervision, validation, project administration.

N. R. Dave: Supervision, Validation, review and editing, correspondence.

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