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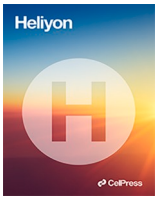

Research article

Exploring Cold plasma technology: Enhancements in Carob seed germination, phytochemical Composition, and antioxidant activity

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ABSTRACT

The cultivation of carob tree does not need many climatic and ecological requirements. The main limit to its large-scale cultivation is the defects for propagation with seeds. Addressing this, our study evaluated the effect of cold plasma pretreatment on carob seed germination.

Impressively, cold plasma showcased beneficial effects by significantly increasing water uptake in seeds (CS: 1.71 ± 0.59; PS/3.99 ± 1.56) and decreasing the contact angle from 80.7° to 57.9°, enhancing the seed surface's hydrophilicity. While the germination rate enhancement was subtle, the treatment presented an innovative route to modifying the seed's physiochemical properties. Specifically, storage proteins like albumin, globulin, and prolamin were notably reduced (Albumin (from 7.67 to 4.95 mg/g DW), Globulin (from 8.52 to 5.80 mg/g DW) and Prolamin (from 3.53 to 1.66 mg/g DW)). Additionally, there was a decline in the overall content of polyphenols (from 846.88 to 760.94 mg GAE/100g DW) and flavonoids (from 790.93 to 502.95 mg GAE/100g DW) and a decrease in the ferric reducing power (from 34.48 to 26.39 mg AAE/g DW). However, radical scavenging activity remained consistent. Intriguingly, FTIR-ATR spectral analysis post plasma treatment indicated oxidative alterations in the seed coat, marked by a distinctive intensity at 1732 cm^{-1}.

This investigation suggests that the application of eco-friendly technology could provide improvements in seed surface's hydrophilicity, but appropriate conditions could be chosen to increase germination efficiency.

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1. Introduction

The international community has chosen to improve the quality of life and eradicate famine by 2030. To meet this goal, the World Food Program is investigating approaches to enhance food and encourage sustainable agriculture [1]. We seek to increase agricultural output to satisfy the world's consumers, who are steadily growing in number. The agricultural sector produced a significant amount of the world's food production, and seeds were critical components of sustainable agriculture and the food industry [2]. New technologies for farming are playing a more and more significant part in seed quality enhancement, leading to entirely new technological innovations [3].

The conventional way to improve seed germination is by using magnetic fields, ultraviolet light, and other mechanical processes or by saturating seeds with chemical substances, fungicides, and hormones before planting [4]. Still, every single one of these techniques has particular drawbacks, including being slow, complicated, and risky for the environment [5]. Cold plasma is currently used to treat plant seeds effectively [6]. This procedure may influence the wetting characteristics of seeds, resulting in much quicker germination and more efficient production [7]. The cold plasma method enhances seedling growth and germination by improving seed wettability, antioxidant enzyme activity, soluble carbohydrate, and protein content, and reducing membrane degradation from lipid peroxidation [8]. As a result, the cold plasma technique may be used to protect seeds from the effects of drought stress. The cold plasma treatment can help to reduce seedling mortality and increase seed germination rates [2].

Plenty of research projects have been conducted to examine the impact of cold plasma on germination [4,6,8–14]. These experiments have demonstrated that using cold plasma may significantly improve seeds germination rate and their bioactive phytochemical contents; furthermore, seeds can be preserved and kept for extended periods after cold plasma treatment.

Ceratonia siliqua, the scientific designation for Carob, is a member of the Fabaceae family. The carob tree (*C. siliqua*) is a perennial evergreen tree. It is a drought-tolerant tree that can grow in poor soil conditions [15]. It is native to the Mediterranean region and exists worldwide, including in North and South America, Africa, and Australia [16].

Carob fruit is a perfect food component with the possibility to be used in producing a wide range of health-beneficial properties [17] due to its nutritional and economic value, such as its extensive bioactive profile as well as its elevated dietary fibers [18], and their associated impact on diabetes, overweight, oxidative stress, high cholesterol levels, and inflammation [15]. However, carob trees are grown in limited land areas, due to some defect for seeds propagation [19], which was associated with *Ceratonia* seeds natural dormancy [18]. Several plant families exhibit this type of dormancy, especially *Leguminosae, Malvaceae, Geraniaceae, Chenopodiaceae* [20]. However, carob seeds were considered one of the most challenging species to germinate. The seed coat is rigid and incapable of absorbing water, implying that carob has challenges with natural regeneration. Consequently, carob seed germination is difficult without seed coat scarification [21].

As reported in literature, carob seeds showed variable germination characteristics according to pretreatments, seed origin, seed genotype and seed gender [20,22,23]. Thus, carob germination studies are necessary to obtain general information about seed germination and to describe the relation between pretreatment and seed proprieties (origin, genotype, gender). Several treatment procedures were elaborated to accelerate germination process and to maximize germination rate by raising moisture uptake and gas exchange.

Treating carob seeds with hot water, sulfuric acid (H_2SO_4), gibberellic acid (GA3), or tap water can all enhance germination [22].

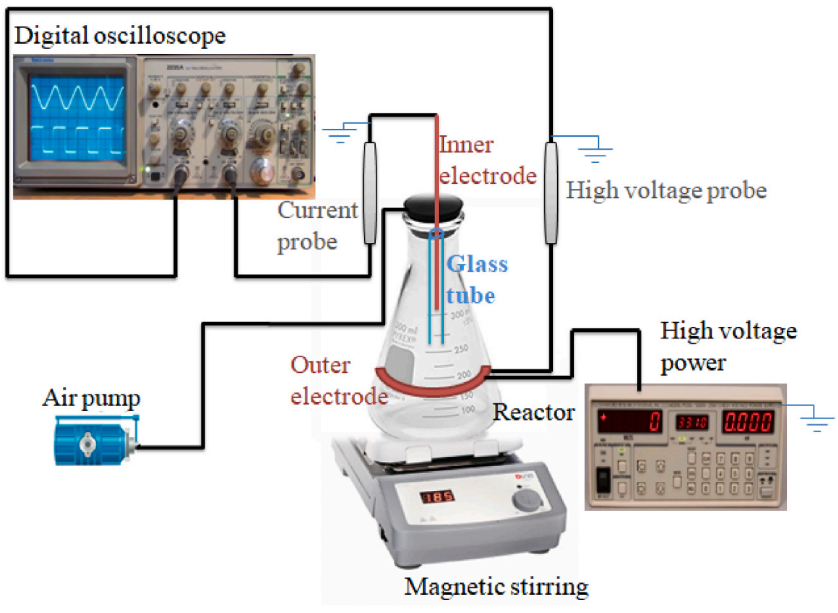


Fig. 1. Experimental setup diagram for Cold plasma treatment.

Mechanical or acid scarification is commonly used to break hard seed coat and improve seeds germination [24]. Gibberellin and other plant hormones were also used for this purpose [25]. All these practices can damage seeds and produce alterations in the embryo and endosperm [26].

In pursuing innovative and innocuous strategies to improve seed germination, this study pioneers the exploration of cold plasma technology tailored for the *Ceratonia siliqua* seeds. Cold plasma, a relatively uncharted terrain in seed treatment, promises a confluence of biophysical alterations that might be the key to overcoming the innate germination challenges these seeds present. The primary goal is to unravel the transformative impacts of cold plasma on seed germination and subsequent seedling growth. The present investigation delves into the intricate changes on the seed surface post-plasma treatment, gauging metrics like water uptake, moisture content, and water contact angle. Simultaneously, the study focused on storage proteins and the intricate tapestry of phytochemicals and antioxidants, drawing attention to possible biochemical changes within the seeds. Through this comprehensive approach, the aim of this study is to explore the application of Cold Plasma Technology for enhancing carob seed germination, a species known for its difficulty in germination. Additionally, we aim to investigate the phytochemical changes occurring after treatments.

2. Materials and methods

2.1. Plant material

Mature carob pods of a cultivated genotype were collected in July 2023 from a local farm in Gabes, Tunisia (latitude 10°05′26.80″ E and longitude 33°52′49.29″ N). These pods, specifically chosen based on their uniformity in size and appearance, were stored under standardized conditions to minimize external variables. Seeds were extracted manually, ensuring minimal damage, and stored in a dark environment at room temperature until further use.

2.2. Cold plasma treatment

Fig. 1 details the different parts of the experimental configuration. The plasma assembly included a DBD reactor and a high-voltage AC power supply. Two high-voltage and ground electrodes form the DBD plasma reactor used for this study. To generate the DBD plasma, an aluminum rod constituted the internal electrode. A second ground electrode was placed in a glass tube and immersed in the reactor at a distance of 10 mm from the solution's surface to be treated. The thickness of the dielectric medium, the reactor wall, was 4 mm. The high electrical voltage applied was a sine wave of approximately 30,000 V/40 mA. Indeed, a high voltage amplifier (TREK-30 kV model 30/20A, 8 USA) coupled to a voltage generator (BFi OPTILAS, France). The DBD plasma is obtained by subjecting the electrodes to a high sinusoidal voltage of 18 kV (peak to peak) at a frequency of 500 Hz. This voltage is due to an amplification of a signal generated by an applied voltage delivered by the generator reaching 10 V. It was then amplified by the amplifier to reach 18 kV. To control the input and output voltage, a digital oscilloscope (Lecroy Wave Surfer 24 Xs, 200 MHz) was used.

The indirect discharge consists of a plasma generated in the gas phase and in contact with water vapor, using both the energies of the excited species and the plasma's electrons. An electric arc forms between the two electrodes, bringing a voltage difference. This arc is repelled from the ignition point by the feed gas flow, sweeps the maximum length of the electrode gap, and forms a non-thermal plasma zone. Then, a new arc appears and expands using the same procedure. An air pump was used to generate the airflow. It supplied air to the reactor via a polyurethane pipe, which passed through the rubber plug of the reactor. The solution to be treated by plasma contains the grains. After treatment time, the grains were recovered for experimental study and characterization.

2.3. Germination test

Before the germination test, fresh seeds were subjected to cold plasma treatment, and the seeds that were not treated with plasma were used as control. Fifteen seeds were placed in three glass Petri dishes containing two layers of moistened filter paper [14]. To maintain the moisture level constant during the germination test, water treated with plasma and distilled water were added to each experiment, and the dishes were covered with plastic wrap. Germination samples were placed in a sterilized incubator to ensure environmental conditions (temperature and photoperiod) (sup Fig. 1). The germination rate (eq. (01)), germination index (eq. (02)) and seed vigor index (eq. (03)) were calculated using the following equations:

$$G = (n^{*}100)/N \quad \text{(eq. 01)}$$

$$GI = \Sigma\, \mathrm{n/d} \quad \text{(eq. 02)}$$

$$SVI = GI^{*}S \quad \text{(eq. 03)}$$

where, n is the number of seeds germinating on day d, N is the total number of seeds, d is the number of days since the test's start, and S is the shoot length.

2.4. Determination of humidity, water uptake and contact angle

The humidity level (%) was determined based on the method prescribed by AOAC (1995) [27]. Each sample (2.0 g) was dried in an

oven at103 ± 2 °C for 24 h. The estimation of seeds humidity was determined based on equation 4 (eq. (04)):

$$H(\%) = (WI - WF)*100/WI \quad \text{(eq. 04)}$$

where WI is the sample's initial mass (before drying), and WF is the sample's final mass (after drying).

The water uptake of studied seeds was evaluated based on the method reported by Turk and Tawaha [28]. Seeds of each group were weighed (2.0 g), moistened with 20 mL of deionized water, and incubated at 25 °C. The seeds were blotted dry after 6 h of the imbibition procedure, then weighed, and the water uptake was calculated according to equation 5 (eq. (05)):

$$WU = (FW - FD)/N \quad \text{(eq. 05)}$$

where FW is fresh weight, DW is the dry weight of the seed, expressed in mg, and N is the number of seeds.

The contact angle of the seeds was examined before and after plasma treatment using the DSA25E Drop Shape Analyzer from KRÜSS. A tiny droplet of deionized water, approximately 4 μL in volume, was carefully dispensed onto the seed surface to evaluate the wetting characteristics. It is worth mentioning that the surface tension of the water used in the experiments was measured at 72.0 mN/m. The reported wetting values were the average results obtained from three independent measurements.

2.5. Quantification of seed storage proteins

The Bradford method remains a commonly used technique for the spectrometric quantification of seed protein [29]. Both groups of seeds were directly used for protein extraction, as previously described by Elfalleh et al. [30]. Ground sample (250 mg) was mixed with 5 mL distilled water (pH 6.5), stirred for 20 min, and centrifuged for 15 min at 10,000 rpm. The supernatant is recovered and stored under the name of albumin fraction. The remaining insoluble residue was used again to produce the globulin fraction by using 5 mL of an aqueous NaCl solution with a 5% (w/v) concentration. The prolamin fraction and the glutamin fraction were extracted with a 70% (v/v) aqueous ethanol solution and a 0.2% NaOH solution, respectively.

The Coomassie Brilliant Blue G-250 solution was prepared by using ethanol (95%) and phosphoric acid (85%). After filtration, the obtained solution was used as color reagents for protein quantification. A calibration standard was performed using BSA concentrations (0–500 mg/mL). The absorbance was performed after incubation (5 min) at a wavelength of 530 nm.

2.6. Analysis of total polyphenols and flavonoids

One gram of each ground sample was incubated with 10 mL of 70% ethanol for 48 h to extract phytochemical compounds from treated and untreated carob seeds. The Folin-Ciocalteu method was used to quantify the amount of the total polyphenol [31]. Flavonoid contents were analyzed according to the colorimetric assay by Ben Othman et al. [32]. The total polyphenols and flavonoids contents were measured according to the standard curve method and expressed as milligram gallic acid equivalent (GAE) per 100 g fresh weight (DW) of sample and milligram of rutin equivalents (RE) per 100 g of fresh weight of sample, respectively.

2.7. Determination of antioxidant activities

The antioxidant activities of both studied extracts were determined using the DPPH and ABTS radical scavenging method and Ferric reducing power assay as reported by Benchikh et al. [17]. For DPPH and ABTS solutions, the decrease in the absorbance was monitored at 517 nm and 734 nm, respectively. The DPPH and ABTS radical scavenging activity were expressed as milligram of Ascorbic acid equivalents (AAE) per g fresh weight.

The reducing power of both extracts was detected by the change in the yellow color of the $FeCl_3/K_3Fe(CN)_6$ present in the solution to green and blue, depending on the reducing power of samples. The absorbance was measured at 700 nm using a UV spectrometer, and the activity was expressed as milligram Ascorbic acid equivalents (AAE) per g fresh sample weight.

The antioxidant activities were calculated according to equation 6 (eq. (06)):

$$\text{AA (mg AAE / g DW)} = \frac{CCs*Vt}{Vs*Mt}*100 \quad \text{(eq. 06)}$$

where CCs is the concentration of the samples obtained by used calibration curve, Vt is the volume of solvent used for the preparation of the extract, Vs is the volume of the sample used for the reaction and Mt is the mass of seed used for the preparation of the extract.

2.8. FTIR analysis

FTIR-ATR analysis of the samples was performed using a PerkinElmer two-spectrometer system, covering the spectral range of 450–4000 cm^1. Data acquisition involved averaging 24 scans with a 2 cm^{-1} resolution. Spectrum 10 Spectroscopy Software (version 10.4.4) was used to conduct baseline corrections and normalize the spectra to enhance the quality of the spectral data.

2.9. Statistical analyses

All the analyses were carried out in triplicate, and the data were reported as the means and standard deviations (±SD). All the

studied parameters were subjected to analysis of variance (ANOVA), which was determined using XLSTAT 2014 software. Duncan's multiple range test conducted significant differences between the group means ($p < 0.05$).

3. Results and discussion

3.1. Effect of cold plasma treatment on carob seed germination

The influence of cold plasma treatment and plasma-activated water on the germination of *Ceratonia siliqua* seeds is presented in Fig. 2. Our data showed no marked difference in germination between the treated and untreated seeds. By day 3, both germination and vigor indices revealed no notable differences. However, by day 7, the vigor index for the control samples treated with plasma-activated water showed a significant rise (Fig. 3). These results align with the findings of Volin et al. [10], who observed no considerable effect of plasma on the germination rates of soybean (*Glycine* max (L.) Merr.), corn (*Zea mays* L.), and bean (*Phaseolus vulgaris* L.) seeds. Furthermore, extended plasma exposure was linked to potential damage to seed morphology, subsequently leading to a drop in germination rates [11].

Contrastingly, several studies have highlighted the beneficial impact of cold plasma treatment on seed germination. Ling et al. [12], for instance, reported a notable boost in the germination rate of oilseed rape seeds, 6.25% for Zhongshuang 7 and 4.44% for Zhongshuang 11, following plasma treatment. Similarly, both Los et al. [13]and Ling et al. [14] also found enhanced germination rates in wheat (*Triticum aestivum*) seeds and soybean seeds, respectively, post-plasma treatment. Moreover, Jiafeng et al. [33] even pinpointed an optimal plasma treatment power of 80 W to amplify the wheat seed germination rate by 6.7%. Such variability in the results across species might be attributed to the plasma source, specific treatment conditions, and the seed type itself [11].

CP generates reactive oxygen and nitrogen species, such as ozone and nitric oxide, which can modify the seed surface chemistry. This activation enhances water imbibition and nutrient uptake, facilitating the initiation of germination processes. In general, the seed germination improvement through cold plasma treatment could be attributed to several factors: (1) increase of surface roughness and microstructuring of the outer layers of the seed coat [4], (2) presence of nitrogen dioxide, nitrate, and nitrite in cold plasma, which are able to break seed dormancy [6] by regulating gibberellin biosynthesis and abscisic acid catabolism [8], (3) improve of the accessibility of active oxygen/nitrogen species to the embryo in the seeds [2], (4) stimulate some enzymes involved in the metabolism of proteins and soluble sugars [8].

For our case, the control samples underwent a pre-treatment with sulfuric acid. This achievement could account for the observed inefficacy of the cold plasma treatment on germination, setting our findings apart from other studies. Gunes et al. [20], noted that 20 min immersion in concentrated sulfuric acid can effectively bolster the germination rate of carob seeds. In fact, acid scarification changed in the cell membrane's permeability and increased carob seeds germination up to 95% [22].

3.2. Effect of cold plasma treatment on humidity, water uptake and contact angle

Cold plasma treatment appeared to exert minimal influence on the humidity of carob seeds. Comparatively, both control and plasma-treated seeds showed negligible variation in seed moisture, averaging 8.4 ± 0.4% for the control and 8.5 ± 0.6% for the plasma-treated samples (Table 1).

In untreated seeds, the average contact angle (θ) between the water droplet and the seed surface was higher than 80°, as presented in Table 1. However, seeds exposed to plasma treatment showed a reduced contact angle of less than 59°. This decrease underscores a

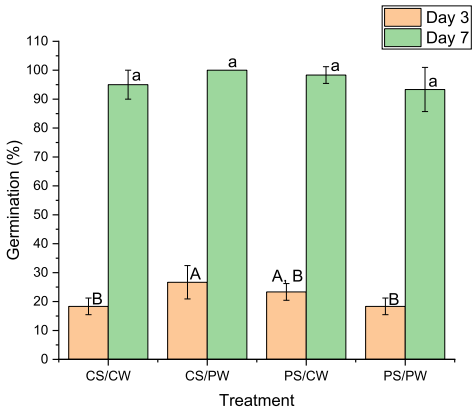


Fig. 2. Germination % of carob seed under different treatments, CS/CW: control seed irrigated with control water, CS/PW; control seed irrigated with cold plasma treated water, PS/CW: cold plasma treated seed irrigated with control water, PS/PW: cold plasma treated seed irrigated with control water. Different capital letters represent significant variation ($p < 0.05$) for the germination (%)-Day 3 between treatments. Different small letters represent significant variation ($p < 0.05$) for the germination (%)-Day 7 between treatments.

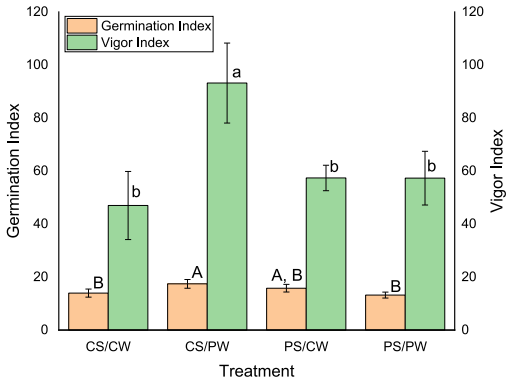


Fig. 3. Germination Index and Vigor Index of carob seed under different treatments, CS/CW: control seed irrigated with control water, CS/PW; control seed irrigated with cold plasma treated water, PS/CW: cold plasma treated seed irrigated with control water, PS/PW: cold plasma treated seed irrigated with control water. Different capital letters represent significant variation ($p < 0.05$) for the germination index between treatments. Different small letters represent significant variation ($p < 0.05$) for the vigor index between treatments.

significant uptick in the hydrophilicity of the seed surface. Srisonphan et al. [34] found similar outcomes, suggesting enhanced wettability of seed surfaces post-plasma treatment. Such changes in wettability can be traced back to modifications in the seed surface's composition and physical structure. When plasma treatments use working gases like oxygen or nitrogen, they tend to elevate the presence of polar groups on the seed surface, intensifying their hydrophilic nature. Our observed rise in hydrophilicity likely stems from the combined effects of these alterations. Additionally, Molina et al. [35] posited that the influence of plasma treatment is predominantly surface-specific, permeating only a few tens of nanometers deep. This effect means the changes brought about by the plasma were localized to the seed's outermost layers.

Water's affinity to seeds is pivotal for kickstarting germination, a widely accepted notion in seed biology. An increase in surface wettability aids in bolstering the crucial bond between water and the seed, forming a thin water layer on the seed, and fast-tracking its complete immersion. However, a protective layer in the seed coat, known as the palisade layer, can potentially obstruct this interaction. If cold plasma treatment cannot effectively penetrate the specialized 'water gap' within this palisade layer, the seed might remain impermeable, and this phenomenon was highlighted by Wong et al. [36].

3.3. Effect of cold plasma treatment on storage protein contents

Carob seeds, covered with a tight-fitting brown coat, were characterized by high protein content (25.7%), with satisfactory contents of essential amino acids [17]. In carob four categories have been quantified, essentially albumin (26.4%), glutelin (23-8%), globulin (6.4) and prolamin (1.8%) [18]. Table 2 shows the effect of cold plasma treatment on four protein fractions content. The storage protein contents of treated seeds were markedly reduced. This reduction is probably due to protease activation [37] and could promote the accumulation of soluble protein [14]. Our results correlate with Pet'ková et al. [11], who noted that 10, 20, and 30 s plasma treatment positively affected the protease activity of barley grains. After plasma treatment, Sadhu et al. [38] showed increased protein content of mung beans (*Vigna radiate*). Moreover, it is suggested by Ling et al. [39] that plasma treatment can intensify the degradation of the seed's inner nutrients from a complex form (storage proteins) to a simple type (soluble proteins). The other reason for the decreased storage protein content is their involvement in some metabolic pathways [11].

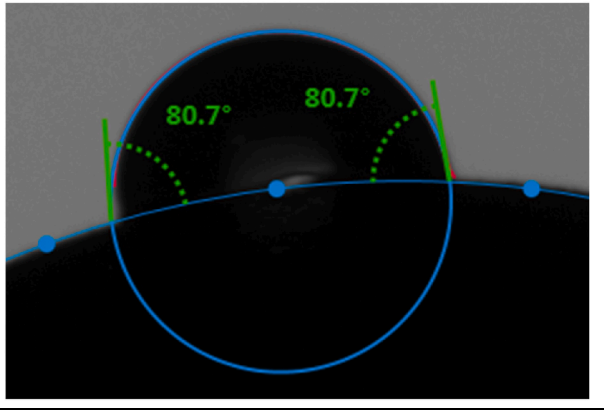
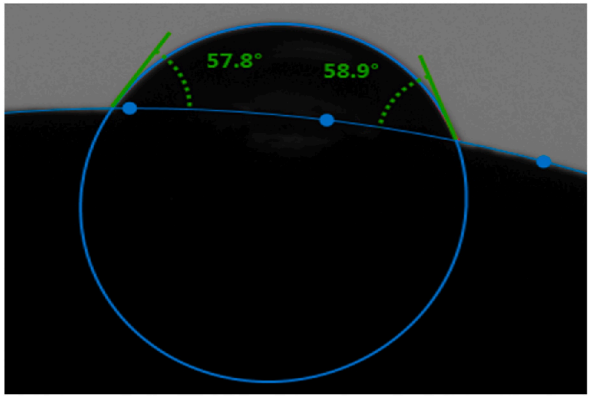
The effect of plasma treatment on the enzyme structure and function was previously reported in the literature. Sadhu et al. [38] indicated that the hydrophilicity of enzymes (amylase, protease, and phytase) has been improved after plasma treatment. Yin et al. [40] reported that the cold plasma treatment could increase α-amylase and protease enzyme activities. The sensitivity of specific enzymes to cold plasma treatment was explained by faster water imbibition in the plasma-treated samples [28,38].

3.4. Effect of cold plasma treatment on phytochemical compounds content and antioxidants activities

Phenolic compounds were considered as the first alternative for synthetic antioxidant to prevent lipid peroxidation in food systems. Carob seeds were known for their interesting contents of this phytochemical class [17]. The determination of total phenolic compound concentration was commonly related to its antioxidant potential.

Table 3 presents the scavenging phytochemicals profile and antioxidant activities of the ethanolic extracts from both treated and untreated samples. Our findings highlight a notable reduction in the total polyphenols, total flavonoid contents, and ferric-reducing power after cold plasma pre-treatment compared to the control sample. This observation mirrors plasma's previously documented impact on brown rice's phytochemical profiles and antiradical potential [9]. Specifically, it was reported that low-pressure plasma application resulted in diminished phenolic concentrations and antioxidant activities in brown rice.

Table 1
Humidity, water uptake, and contact angle of Carob seed after and before cold Plasma Treatment.

	CS	PS	*p-value*
Humidity (%)	6.56 ± 1.50^{a}	4.12 ± 2.44^{a}	NS
Water Uptake (%)	1.71 ± 0.59^{b}	3.99 ± 1.56^{a}	0.032
Contact Angle (°)	80.7 ± 1.9^{a}	58.3 ± 1.4^{b}	0.0012
Contact angle image			

CS seed of control group (untreated), PS; seed treated with cold plasma.
p-value <0.05 the difference is significant between treated and untreated seeds.

Table 2
Storage protein content of Carob seed after and before cold Plasma Treatment.

Proteinic fraction	CS	CS (%)	PS	CS (%)	*p-value*
Albumin (mg/g DW)	7.67 ± 0.11[a]	37.51	4.95 ± 0.58[b]	37.02	0.004
Globulin (mg/g DW)	8.52 ± 0.56[a]	41.68	5.80 ± 0.15[b]	43.38	0.009
Prolamin (mg/g DW)	3.53 ± 0.30[a]	17.28	1.66 ± 0.27[b]	12.38	0.008
Glutelin (mg/g DW)	0.72 ± 0.21[a]	3.54	0.97 ± 0.24[a]	7.23	NS
Total (mg/g DW)	20.45 ± 0.35	100.00	13.37 ± 1.17	100.00	0.006

CS seed of control group (untreated), PS; seed treated with cold plasma.
p-value <0.05 *the* difference is significant between treated and untreated seeds.

Table 3
phenolic contents and radical scavenging activities of Carob seed after and before cold Plasma Treatment.

	CS	PS	*p-value*
Total polyphenols (mg GAE/100g DW)	846.88 ± 18.94[a]	760.94 ± 33.09[b]	0.04
Total Flavonoids (mg RE/100g DW)	790.93 ± 43.98[a]	502.95 ± 61.19[b]	0.01
ABTS+ (mg AAE/g DW)	19.13 ± 0.63[a]	19.94 ± 0.85[a]	NS
DPPH (mg AAE/g DW)	10.58 ± 0.22[a]	11.02 ± 0.24[a]	NS
Ferric reducing power (mg AAE/g DW)	34.48 ± 1.50[a]	26.39 ± 0.58[b]	0.008

CS seed of control group (untreated), PS; seed treated with cold plasma.
p-value <0.05 the difference is significant between treated and untreated seeds.

Conversely, Zielinska et al. [41] have noted the beneficial effect of plasma treatment on enhancing the accumulation of phenolic compounds and boosting antioxidant activities in okra pods. The decline in phenolic compounds post-plasma treatment could stem from either the breakdown of phenolic constituents in carob seeds or the condensation and cyclization of simple phenolic acids [42]. Such processes limit their solubility in organic solvents. Yodpitak et al. [9] identified an increase in hydrolytic enzyme activation post-cold plasma treatment. Such activation facilitates the decomposition of diverse molecules and stimulates the synthesis of novel compounds. The accumulation of active species by plasma treatment and the high activation of antioxidant enzymes in pretreated seeds could also explain the drop behavior of phenolic compound contents and antioxidant activities [8].

3.5. FTIR results

Fig. 4 displays the normalized FTIR-ATR spectra for the treated and untreated carob seeds, elucidating distinctive vibrational features. The noted vibrations include the OH stretching vibrations from 3700 to 3020 cm^{-1}, CH stretching vibrations from 3020 to 2744 cm^{-1}, and carbonyl group C═O vibrations within lipids observed prominently at 1733 cm^{-1} [43]. The spectrum also highlights an aromatic stretching band typical of lignin at 1602 cm^{-1} and a range between 1485 and 1289 cm^{-1} for CH deformations and OH bending vibrations [44]. Further characteristics include C–O stretching vibrations for carbohydrates like cellulose and hemicellulose at 1246 cm^{-1}, with C–O deformation vibrations in carbohydrates and lignin observed between 1185 and 846 cm^{-1} [45]. These vibrational features confirm the presence of crucial seed coat components: lipids and carbohydrates.

However, post-plasma treatment, the FTIR-ATR spectrum (Fig. 4) does not manifest significant shifts in spectral architecture. However, a marked decrease in the intensity of the OH group's stretching vibrations, found between 3600 and 2990 cm^{-1}, is evident. An augmented peak intensity at 1732 cm^{-1} suggests oxidative alterations within the seed coat. This increased intensity pinpoints potential oxidation, possibly involving the carbonyl groups in lipids or other entities, underscoring the plasma treatment's influence on the seed coat's chemical composition.

Upon exposure to plasma treatment, the carob seeds exhibited molecular-level alterations in their FTIR spectra. While the overall chemical composition remained broadly consistent, the pronounced reduction in the intensity of the OH stretching vibrations suggests potential reactions involving hydroxyl groups or dehydration events. More notably, the heightened carbonyl content, inferred from the increased intensity at 1732 cm^{-1}, underscores lipid oxidation, likely triggered by reactive oxygen species generated during plasma exposure. These findings show that while the primary seed coat constituents, lipids, carbohydrates, and lignin retain their presence, the plasma treatment imparts distinct oxidative modifications at the molecular level.

4. Conclusion

In this research, the multifaceted effects of cold plasma treatment on *Ceratonia siliqua* seeds were investigated. While germination remained largely unaffected, there was a notable enhancement in seed surface hydrophilicity, critical for germination. Intriguingly, the plasma treatment led to a significant reduction in the seed's storage protein contents (Albumin (from 7.67 to 4.95 mg/g DW), Globulin (from 8.52 to 5.80 mg/g DW) and Prolamin (from 3.53 to 1.66 mg/g DW), possibly driven by protease activation, highlighting the significant transformative role of plasma on the seed's internal biochemistry. Concurrently, bioactive phytochemicals, renowned for their health benefits in carob, were diminished post-plasma treatment (Total polyphenols (from 846.88 to 760.94 mg GAE/100g DW) and Total flavonoids (from 790.93 to 502.95 mg GAE/100g DW)), emphasizing the need for plasma parameters optimization for each

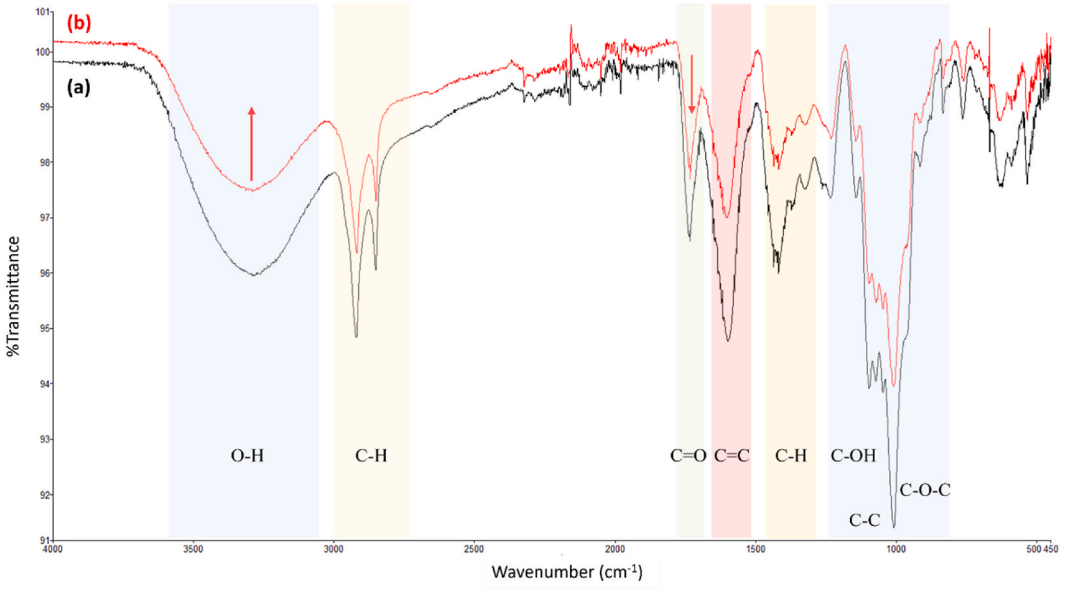


Fig. 4. FTIR Spectra of (a) untreated and (b) cold plasma treated carob seed.

species to avert undesirable outcomes. FTIR analysis further unveiled subtle chemical changes in the seed coat post-treatment. Advanced analytical techniques such as HPLC and LC-MS are imperative for a holistic understanding of the alterations post-plasma treatment and to optimize its application for carob seed germination. This study extends the frontier of seed science by shedding light on the subtle and significant modulations induced by cold plasma in seeds. It emphasizes the importance of judicious plasma application to harness its full potential.

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Data availability statement

The data presented in this study are contained within the article or Supplementary Materials.

CRediT authorship contribution statement

Khadija Ben Othman: Writing – original draft, Investigation. **Mohamed Majdi Cherif:** Writing – original draft, Investigation. **Imen Assadi:** Visualization, Methodology, Data curation. **Walid Elfalleh:** Writing – review & editing, Validation, Supervision, Conceptualization. **Lotfi Khezami:** Writing – review & editing, Funding acquisition, Formal analysis. **Achraf Ghorbal:** Writing – review & editing, Validation, Supervision, Data curation. **Aymen Amine Assadi:** Validation, Supervision, Investigation, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Walid Elfalleh is currently serving as associate editor in Heliyon Food Science and Nutrition.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28966.

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