# Impact of cold plasma treatment on aflatoxin decontamination, nutritional composition, bioactive compounds, mineral content and anti-nutritional factors of groundnuts

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Groundnuts (Arachis hypogaea L.) are a globally consumed legume valued for their nutrition and affordability. Cold plasma (CP) processing, an innovative nonthermal technology, improves food safety and quality by inactivating microorganisms and reducing chemical contaminants. In the present study, groundnuts inoculated and non-inoculated with Aspergillus flavus were treated with CP at varying voltages (20-30 kV) and durations (1-15 min). CP treatment significantly reduced aflatoxin B1 levels (up to 82.1% at 30 kV, 15 min) while enhancing protein, fat, fibre, phenolics, flavonoids and mineral bioavailability. Anti-nutritional factors like phytates, oxalates and tannins decrease, improving nutrient digestibility. The present study demonstrates CP's potential as a sustainable, chemical-free method for enhancing groundnut quality and safety, with promise for large-scale application in the food industry.

**Keywords:** Aflatoxin, anti-nutritional factors, bioactive component, cold plasma treatment, groundnut.

GROUNDNUT, also known as peanut (*Arachis hypogaea* L.), is often referred to as the 'king of oilseeds' and plays a vital role as a food and cash crop, especially in India. It is also called the 'poor man's cashew nut' and 'wonder nut' due to its nutritional value, affordability and taste, making it one of the most widely consumed legumes globally<sup>1</sup>.

Groundnuts are packed with essential macro and micro minerals such as iron, zinc, phosphorus, manganese, copper, sodium, potassium, calcium, magnesium and selenium. They are also a rich source of vitamin E, B complex vitamins, monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids, dietary fibre, tryptophan and phytochemicals, all of which are known to provide significant health benefits<sup>2</sup>. Additionally, it is a good source of bioactive compounds like resveratrol, phenols, flavonoids and antioxidants<sup>3,4</sup>.

However, poor post-harvest handling, improper storage, and transportation, especially under warm and humid conditions, create a favourable environment for mould and fungal growth. This often leads to the production of mycotoxins, particularly aflatoxins, in agricultural commodities<sup>5–8</sup>. Groundnut crops are highly susceptible to fungal contamination, especially in tropical and subtropical regions where warm climates encourage the production of aflatoxins<sup>9</sup>.

Aflatoxins are toxic compounds primarily produced by two species: *Aspergillus flavus* and *Aspergillus parasiticus*, *A. flavus*, which is widespread in nature and primarily colonises the aerial parts of plants, and *A. parasiticus*, which is more soil-adapted with a limited distribution<sup>10,11</sup>. Of the 20 known derivatives of aflatoxins, the major ones include aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2), produced directly by *A. flavus* and *A. parasiticus*. Among these, aflatoxin B1 (AFB1) is the most toxic, followed by G1, B2 and G2 (B1 > G1 > B2 > G2). Aflatoxin B1 is also the most widespread and was categorised under Group 1 carcinogen by the International Agency for Research on Cancer (IARC) in 1993 (refs 12, 13).

Aflatoxins pose serious public health risks and economic challenges for farmers and consumers worldwide. These toxins can lead to acute or chronic health effects, including carcinogenic (causing cancer), mutagenic (causing genetic mutations), teratogenic (causing birth defects), immunotoxic, and hepatotoxic (liver-damaging) impacts, as well as fetal growth abnormalities<sup>14</sup>. On a molecular level, aflatoxins interfere with the immune system, DNA

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mutations, modify post-translational peptide chains, and affect protein and nucleic acid methylation. They also contribute to free radical formation, further exacerbating their harmful effects<sup>15</sup>.

Many countries have implemented regulatory measures to limit aflatoxin levels in food, such as the European Union's strict 4 ppb standard (EU, 2023). These regulations aim to protect human health but can impact global food trade<sup>16,17</sup>. Several traditional decontamination methods, including chemical treatments, thermal inactivation, and irradiation, are widely used but have notable drawbacks, such as nutrient loss, high costs, and environmental concerns<sup>18,19</sup>. As a result, researchers are exploring alternative approaches, with cold plasma emerging as a promising, non-thermal, eco-friendly technology<sup>20,21</sup>. This innovative method offers a low-cost, versatile solution that avoids the drawbacks of traditional techniques. CP processing mainly targets the surface of the food microorganism and damages its DNA by generating reactive oxygen and nitrogen species<sup>22,23</sup>.

Most existing research on CP has focused on microbial decontamination, with limited exploration of its effects on food quality attributes. Further research is needed to pinpoint the specific active particles and determine the optimal doses of CP. Additionally, studies should evaluate its effects on the nutritional attributes of different food products. The present study aims to assess the impact of CP treatment on aflatoxin range and other quality parameters in groundnuts while addressing the existing research gap. Additionally, it seeks to better understand CP's interaction with food components, supporting its potential use in large-scale, sustainable, and energy-efficient food processing, such as in groundnut to create functional foods.

### Materials and methods

# Procurement of raw materials, chemicals and equipment

Groundnuts (Kadiri 9 variety) were procured from the Regional Agricultural Research Station, Palem, Telangana, India. Healthy groundnut kernels with no signs of infection were used in the present study. The glassware and equipment utilised were from the MFPI-Quality Control Laboratory, Post Graduate and Research Centre, College of Community Science and Central Instrumentation Cell, Professor Jayashankar Telangana Agricultural University, Rajendranagar, Hyderabad.

#### Aflatoxin inoculation of groundnut

The groundnut samples underwent a surface disinfection to remove any potential microbial contamination. First, the samples were immersed in 90 per cent ethanol for 1 min, then rinsed with sterile water. Next, they were

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soaked in a 1 per cent sodium hypochlorite solution for 15 min. The samples were subsequently washed twice with sterile water to eliminate any leftover disinfectant residues. Lastly, the disinfected samples were dried in a hot air oven at 60°C. To facilitate the inoculation process, the disinfected groundnut samples were immersed in an artificial spore suspension of A. flavus. The samples were allowed to stand in the suspension to ensure uniform contact and inoculation. This step aimed to artificially introduce A. flavus into the groundnut samples, enabling the production of aflatoxins (AFB1). The inoculated samples were maintained under controlled conditions to promote AFB1 synthesis, targeting levels close to the prescribed maximum limits within 7-14 days (Figure 1). After the AFB1 formation period, the inoculated groundnut samples were carefully dried in a laminar airflow chamber to prevent further contamination. These samples were then subjected to subsequent plasma treatment for experimental analysis.

### Aflatoxins analysis

Aflatoxin B1 content in untreated and treated groundnut samples was quantified using the ELISA method. The analysis was carried out at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad<sup>24</sup>.

#### CP treatment

For each CP treatment, approximately 100 g of groundnut (Kadiri 9 variety) samples were evenly spread on a  $15 \times 15$  cm polyethylene terephthalate (PET) tray. An open-air multipin-plane plasma reactor (Ingenium Naturae Pvt Ltd, India) was used for the experiments. The reactor features 88 pins arranged in an  $11 \times 8$  grid with 20 mm spacing,



Figure 1. Groundnut samples artificially infected with *Aspergillus flavus*.

## **RESEARCH ARITCLES**

and the pin-to-plane distance is adjustable. It is powered by a high-voltage step-up transformer that generates up to 40 kV root mean square (RMS) voltage from a 220– 250 V, 50 Hz supply, with output voltage regulated through an electronic control panel. The samples were exposed to plasma for 1, 5, 10 and 15 min at three voltage settings: 20, 25 and 30 kV, as presented in Figure 2. Each treatment condition was repeated three times for reliability. An untreated groundnut sample was used as the control group for comparison, allowing for a thorough assessment of how CP treatment affects the aflatoxin levels and nutritional properties of the groundnuts.

#### Determination of proximate composition

The analysis of CP-treated and untreated groundnut samples was carried out using standard methods. Moisture content was determined using IS 1155:1968 (Reaffirmed 2022) method<sup>25</sup>. Protein content was estimated using the Association of analytical Chemists (AOAC) 992.23, 32.2.02. 2016c, Generic Combustion method with a Leco FP-528 Nitrogen Analyzer<sup>26</sup>. Fat content was measured by crude hexane extraction of the groundnut samples using the automatic Gerhardt Soxtherm extraction unit (AOAC 922.06-2016a)<sup>27</sup>. Crude fibre content was analysed by AOAC, 962.09-2016b method<sup>28</sup>. Carbohydrate content was calculated by subtracting the sum of ash, lipids and proteins from the total dry extract (AOAC, 1980)<sup>29</sup>.

#### Determination of minerals

The mineral composition, including calcium (Ca), iron (Fe), and zinc (Zn), of control and CP-treated red chillies was analysed following the AOAC official methods<sup>30</sup>.

#### Determination of bioactive component analysis

The bioactive compounds in both CP-treated and untreated groundnuts were assessed for total phenolic content  $(TPC)^{31}$  and total flavonoid content  $(TFC)^{32,33}$ .

### Determination of anti-nutritional factors

Anti-nutritional factors, including phytates<sup>34</sup>, oxalates<sup>35</sup> and tannins<sup>36</sup>, were evaluated in both control and CP-treated groundnut samples, adhering to the respective established protocols.

#### Statistical analysis

The data were analysed using Analysis of Variance (ANOVA) in SPSS version 23 (SPSS, IBM, Chicago, USA), with mean differences identified using the Duncan Multiple Range Test. The results are expressed as the

mean  $\pm$  standard deviation of three replicate measurements. Statistical significance between treatments was determined at a 95% confidence level (P < 0.05).

#### **Results and discussion**

### Effect of CP treatment on aflatoxin levels

Figure 3 graphically illustrates the effect of CP treatment on AFB1 levels in groundnuts, evaluated under different voltage conditions (20, 25 and 30 kV) and exposure times (1, 5, 10 and 15 min). The untreated control sample exhibited the highest AFB1 concentration  $(12.00 \pm 0.10 \,\mu\text{g/kg})$ , highlighting the significant presence of aflatoxin in groundnuts in the absence of CP treatment. As voltage increased from 20 to 30 kV and duration extended from 1 to 15 min, a progressive decline in AFB1 content was observed. The most significant (P < 0.05) reduction was achieved with 15 min of CP treatment, where at 20 kV the AFB1 content decreased to  $3.02 \pm 0.04 \,\mu\text{g/kg}$ , at 25 kV it further dropped to  $2.63 \pm 0.49 \,\mu\text{g/kg}$ , and at 30 kV it reached its lowest value of  $2.16 \pm 0.06 \,\mu\text{g/kg}$ , representing 82.1% decrease in comparison with the control groundnut sample.

Figure 2. Open-air multipin-plane plasma reactor.

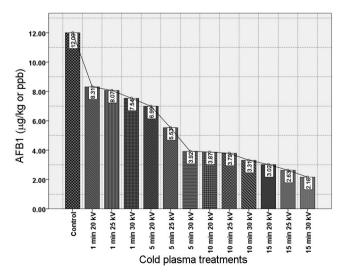


Figure 3. Effect of cold plasma (CP) treatment on aflatoxin levels.

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The outcomes align with the findings of Devi *et al.*<sup>6</sup>, who demonstrated that plasma treatment effectively reduces aflatoxin B1 (AFB1) levels. Their study showed a reduction of over 70% in AFB1 content in groundnut samples treated at 40 W for 15 min, and more than a 90% reduction with plasma treatment at 60 W for 12 min. These results are consistent with studies by Makari *et al.*<sup>37</sup> and Sen *et al.*<sup>38</sup>, who observed similar reductions in AFB1 levels in pistachios and hazelnuts respectively, with longer CP treatment durations and higher voltage levels.

According to Misra et al.<sup>39</sup> and Rahnavard et al.<sup>40</sup> reactive oxygen and nitrogen species are key factors in aflatoxin degradation, primarily by damaging DNA within chromosomes during CP treatment. Two main degradation pathways occur when AFB1 is exposed to CP. The first pathway involves the generation of hydroxyl (OH<sup>•</sup>), hydrogen (H<sup>•</sup>), and formyl (CHO<sup>•</sup>) radicals from strong oxidising agents produced by plasma, which then bind to AFB1. The formation of hydroxyl radicals (OH<sup>•</sup>) is further enhanced by the relative humidity and surface moisture content of groundnuts, which are highly reactive and capable of damaging DNA molecules, mitochondria, and other cellular components. The second pathway involves epoxidation by H<sub>2</sub>O radicals, forming a 9.8-epoxide structure that blocks the harmful effects of aflatoxins. These reactive species target the C8=C9 double bond in the furan ring of AFB1, leading to the opening of the terminal furan ring and the disruption of the lactone ring, which ultimately generates less harmful compounds.

As per FSSAI standards, the allowable limit for aflatoxins in groundnuts is set at 15  $\mu$ g/kg for oilseeds meant for further processing and 10  $\mu$ g/kg for ready-to-eat products. Similarly, Codex standards set a limit of 15  $\mu$ g/kg for groundnuts. These regulations aim to control aflatoxin contamination due to its carcinogenic potential, particularly in high-risk commodities like groundnuts<sup>41</sup>. The results of this study indicate that CP technology holds significant potential as an effective method for decontaminating groundnuts contaminated with aflatoxins. By effectively reducing or eliminating harmful aflatoxins, this technology enhances food safety, mitigates risks in the supply chain, and reduces post-harvest losses, ultimately benefiting farmers and consumers through safer food products.

# *Effects of CP treatment on proximate composition of groundnuts*

The proximate composition of CP-treated and untreated groundnut samples is presented in Figure 4. The analysis illustrated a significant (P < 0.05) reduction in the moisture content of groundnuts, decreasing from  $7.13 \pm 0.06$  g% in the control sample to  $6.31 \pm 0.06$  g% following CP treatment at 30 kV for 15 min. A similar trend was observed in Ahangari *et al.*<sup>42</sup>, where the moisture content was slightly reduced from 3.38% to 2.42% following CP treatment

(50 W for 20 min). The reduction in moisture content may be due to the breakdown of water molecules into simpler oxygen-free radicals generated during the CP treatment process<sup>43</sup>.

Conversely, the fat, protein, and fibre contents showed a significant increase (P < 0.05) with longer durations and higher voltages of CP treatment. The highest fat content, measured at  $42.37 \pm 0.03$  g%, was observed after 15 min of CP treatment at 30 kV, representing a significant (P <(0.05) increment than untreated samples. The improvement in fat content after CP treatments can be ascribed to the breakdown of crude fats into simpler compounds, which enhances the overall fat content<sup>44,45</sup>. The highest protein content was observed after 15 min of treatment, with values of  $28.56 \pm 0.04$  g%,  $28.82 \pm 0.05$  g%, and  $28.96 \pm$ 0.02 g% for the applied voltages of 20, 25 and 30 kV respectively. Sarangapani et al.<sup>46</sup>, reported similar findings, observing a significant variation (P < 0.05) in the protein content of black gram samples treated with plasma. The changes in protein content were attributed to the direct effect of hydroxyl radicals and atomic oxygen on surface proteins and other proteinaceous matter<sup>45</sup>. The fibre content of the untreated (control) groundnut sample was  $7.48 \pm$ 0.01 g%, and it significantly (P < 0.05) varied among the CP-treated samples, ranging from  $7.59 \pm 0.02$  g% to  $7.98 \pm 0.01$  g%. According to Madathil *et al.*<sup>47</sup>, CP treatment may enhance crude fibre content by causing structural modifications to cell walls. The reactive species produced during CP treatment, including oxygen and nitrogen radicals, can degrade cell wall components, making them more accessible for fibre analysis<sup>47</sup>.

In contrast, the carbohydrate content gradually decreased from  $21.19 \pm 0.04$  g% to  $18.43 \pm 0.04$  g% as the duration and voltage of CP treatment increased. A similar reduction in carbohydrate content was observed by Thirumdas *et al.*<sup>48</sup>, when CP was applied to basmati rice. The

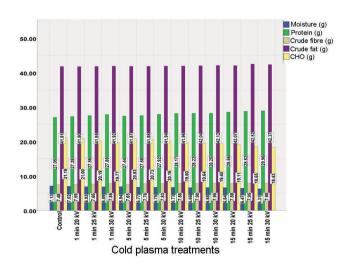


Figure 4. Effect of CP treatment on proximate composition of groundnuts.

CP treatments				
Voltage (kV)	Time (min)	Ca (mg/kg)	Fe (mg/kg)	Zn (mg/kg)
Control		$394.69 \pm 0.01^{m}$	$33.84\pm0.05^{\circ}$	$20.59\pm0.02^{a}$
20	1	$355.78 \pm 0.01^{\rm h}$	$35.23\pm0.04^{\text{d}}$	$20.75\pm0.04^{\text{b}}$
25		$324.26\pm0.06^{\text{d}}$	$43.86\pm0.04^{\rm g}$	$21.52\pm0.05^{\circ}$
30		$314.77 \pm 0.06^{a}$	$44.34\pm0.04^{\rm h}$	$21.59\pm0.09^{\circ}$
20	5	$360.43 \pm 0.05^{j}$	$39.14\pm0.06^{\rm f}$	$22.42\pm0.02^{d}$
25		$348.22 \pm 0.06^{g}$	$43.92\pm0.05^{\rm g}$	$26.82\pm0.10^{\rm f}$
30		$346.20 \pm 0.05^{\rm f}$	$44.69\pm0.02^{\rm j}$	$28.09\pm0.09^{\rm g}$
20	10	$384.60 \pm 0.09^{1}$	$44.58 \pm 0.06^{i}$	$24.95 \pm 0.03^{\circ}$
25		$358.81\pm0.05^{\rm i}$	$45.49 \pm 0.05^{1}$	$28.81\pm0.02^{\rm h}$
30		$319.07 \pm 0.01^{b}$	$44.94 \pm 0.03^{k}$	$33.18\pm0.09^{\rm j}$
20	15	$374.70 \pm 0.02^{k}$	$38.28 \pm 0.02^{\circ}$	$39.80 \pm 0.08^{1}$
25		$343.94 \pm 0.06^{\circ}$	$23.27\pm0.08^{\rm b}$	$39.41\pm0.05^{\rm k}$
30		$323.88 \pm 0.02^{\circ}$	$21.83\pm0.04^{\rm a}$	$31.61\pm0.05^{\rm i}$
Grand mean		349.94	38.72	27.65
SE of mean		3.92	1.27	1.04
CD		0.08	0.07	0.10
CV%		0.01	0.11	0.22

 Table 1. Effects of cold plasma (CP) treatment on the mineral content of groundnut samples

Note: Values are expressed as mean  $\pm$  standard deviation of three determinations.

Values with similar superscripts within columns are statistically similar at 0.05% level. SE, Standard error of the mean; CD, Critical difference; CV, Coefficient of variation.

decrease in carbohydrate content can be linked to the depolymerisation of starch that occurs during CP treatment. Additionally, the loss of moisture from the surface of food products during this process may have further contributed

# Effects of CP treatment on mineral content of groundnuts

to the observed reduction in carbohydrate levels<sup>49</sup>.

The impact of CP treatment on mineral content in groundnuts varied with voltage and treatment time (Table 1). The calcium (Ca), iron (Fe), and zinc (Zn) content of the untreated groundnut samples were found to be  $394.69 \pm$ 0.01 mg/kg,  $33.84 \pm 0.05$  mg/kg and  $20.59 \pm 0.02$  mg/kg respectively. The findings revealed a gradual decline in calcium content in groundnut samples as the duration and voltage of CP treatment increased. The lowest calcium content  $(314.77 \pm 0.06 \text{ mg/kg})$  was observed at 30 kV after 1 min, showing statistically significant differences (P < 0.05). At 20 kV, Fe content of groundnut samples increased from  $35.23 \pm 0.04$  mg/kg at 1 min to  $39.14 \pm$ 0.06 mg/kg at 5 min, reaching a peak of  $44.58 \pm$ 0.06 mg/kg at 10 min. However, a sharp decline was observed at 15 min, with Fe content dropping significantly (P < 0.05) to 23.27 ± 0.08 mg/kg at 25 kV and 21.83 ± 0.04 mg/kg at 30 kV, highlighting the adverse effects of prolonged exposure at these voltage levels. Similarly, the zinc content in the samples showed a gradual improvement with an increase in CP treatment time compared to the untreated sample. The highest zinc content,  $39.80 \pm$ 0.08 mg/kg, was achieved after 15 min of treatment at 20 kV, highlighting the effectiveness of this specific combination of treatment duration and voltage. However, at 25 kV and 30 kV, a significant (P < 0.05) decline in Zn content was observed, with values decreasing to  $39.41 \pm 0.05 \text{ mg/kg}$  and  $31.61 \pm 0.05 \text{ mg/kg}$  respectively. This suggests that prolonged exposure to CP, especially at higher voltage levels, can have an adverse impact on iron and zinc content. Therefore, it is crucial to carefully optimise the parameters of CP treatment.

The findings of the present study align with those reported by Charu et al.<sup>50</sup>, who similarly observed an increase in iron content in samples subjected to CP treatment. The initial increase in Fe content suggests enhanced surface availability or concentration, while extended exposure, particularly at higher voltages, leads to substantial degradation. However, they reported a decrease in Ca content in pearl millet samples, highlighting the variable effects of CP treatment on different minerals. The ionisation and reactive species generated during CP treatment may alter certain calcium compounds, either by converting them into less soluble forms or by modifying their chemical structures. This, in turn, results in a decrease in their detectable concentrations<sup>50</sup>. Additionally, the results align with the research by Lotfy et al.<sup>51</sup>, who noted a slight increase in Zn content in palm dates following CP treatment attributed to the process of mineralisation.

# *Effects of CP treatment on bioactive components and antioxidant activity of groundnuts*

Table 2 displays the changes in TPC, TFC, and antioxidant activity of CP-treated groundnut samples, depending on various voltage and treatment time.

*Total phenolic content:* The initial TPC in the groundnut samples was  $126.69 \pm 0.01 \text{ mg}/100 \text{ g}$ , which increased to  $142.05 \pm 0.04 \text{ mg}/100 \text{ g}$ ,  $147.73 \pm 0.05 \text{ mg}/100 \text{ g}$  and

CP treatments			
Voltage (kV)	Time (min)	Total phenolic content (mg/100 g)	Total flavonoid content (mg of QE/100 g)
Control		$126.69 \pm 0.01^{a}$	$804.02 \pm 0.01^{a}$
20	1	$127.88 \pm 0.03^{\rm b}$	$822.07 \pm 0.04^{\mathrm{b}}$
25		$136.71 \pm 0.06^{d}$	$878.16 \pm 0.06^{\circ}$
30		$137.18 \pm 0.01^{\rm f}$	$906.24 \pm 0.05^{\circ}$
20	5	$132.44 \pm 0.04^{\circ}$	$882.08 \pm 0.03^{d}$
25		$136.96 \pm 0.02^{\circ}$	$922.11 \pm 0.06^{\rm f}$
30		$138.07 \pm 0.01^{ m g}$	$936.10 \pm 0.02^{h}$
20	10	$142.05 \pm 0.04^{\mathrm{j}}$	$954.08 \pm 0.01^{j}$
25		$147.73 \pm 0.05^{\rm k}$	$968.21 \pm 0.09^{k}$
30		$151.07 \pm 0.01^{\rm m}$	$973.04 \pm 0.04^{1}$
20	15	$148.29 \pm 0.02^{1}$	$984.05 \pm 0.03^{\rm m}$
25		$140.64 \pm 0.09^{i}$	$948.18 \pm 0.05^{i}$
30		$138.57 \pm 0.07^{\rm h}$	$927.06 \pm 0.02^{g}$
Grand mean		138.79	915.80
SE of mean		1.14	8.73
CD		0.06 0.07	
CV%		0.03	0.005

Table 2. Effects of CP treatment on bioactive compounds in groundnut samples

Note: Values are expressed as mean  $\pm$  standard deviation of three determinations.

Values with similar superscripts within columns are statistically similar at 0.05% level.

SE, Standard error of the mean; CD, Critical difference; CV, Coefficient of variation.

 $151.07 \pm 0.01 \text{ mg}/100 \text{ g}$  at 20, 25 and 30 kV respectively. after 10 min of CP treatment. However, following a 15 min treatment, the 30 kV CP treatment still resulted in the highest phenolic content, though it decreased to  $138.57 \pm$ 0.07 mg/100 g, which was lower than the 10 min value. The results indicate that CP treatments have a significant (P < 0.05) impact on the TPC of groundnut compared to the untreated sample. Similar observations were made by Xiang et al.<sup>52</sup> and Hou et al.<sup>53</sup>, who documented significant increases (P < 0.05) in TPC in apple juice and blueberries respectively. The initial rise could be linked to the disruption of covalent bonds caused by the energy produced during CP treatment. This process initiated several chemical reactions that resulted in the breakdown of cell membranes, thereby improving the availability of phenolic compounds<sup>54</sup>. Contrasting findings by Ranjitha Gracy et al.55 and Fernandes et al.56 noted a decline in TPC in tomatoes and acerola juice respectively, with extended CP exposure and higher voltages. This reduction is attributed to the formation of reactive species, such as ozone, during prolonged treatments. Ozone reacts with the aromatic rings of phenolic compounds, leading to their degradation into hydroxylated and quinone derivatives<sup>57</sup>.

Total flavonoid content: The control sample exhibited a TFC of  $804.02 \pm 0.01 \text{ mg}$  of QE/100 g. At 20 kV, the content improved to  $954.08 \pm 0.01 \text{ mg}$  of QE/100 g, while at 25 kV it reached  $968.21 \pm 0.09 \text{ mg}$  of QE/100 g. The highest TFC was recorded at 30 kV for 10 min, measuring  $973.04 \pm 0.04 \text{ mg}$  of QE/100 g. The increase in TFC observed in this study is consistent with findings on the chemical composition of plasma-treated blueberries. The increase in TFC can be attributed to CP treatment, which

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helps break down the cell walls and membranes, making the flavonoids more accessible. The high-energy plasma disrupts the physical structure of the cells, facilitating the release of flavonoids into the surrounding matrix<sup>46</sup>. After 15 min of treatment, a decline in flavonoid content was observed across all voltage levels. This reduction may be attributed to prolonged exposure and higher voltages generating an increased amount of reactive species, particularly ozone, which can degrade flavonoid compounds<sup>45,58</sup>.

Overall, while short-term CP treatments enhance the TPC and TFC values in groundnut, extended exposure, particularly at higher voltages, appears to diminish bioactive component and antioxidant activity. This highlights the need to optimise treatment parameters to ensure the maximum retention of bioactive compounds and antioxidant properties in groundnut products.

# *Effects of CP treatment on anti-nutritional factors of groundnuts*

Anti-nutritional factors such as phytates, oxalates, and tannins naturally develop in plants as a defence against environmental challenges. However, their excessive presence can reduce nutrient absorption by forming insoluble complexes with minerals like calcium, magnesium, zinc, and iron, as well as interfering with enzymes involved in protein digestion<sup>59–61</sup>. Employing effective food processing methods, such as CP treatment, can help mitigate these effects. CP treatment modifies the structure of these compounds, reducing their enzyme inhibition and protein precipitation activities, thereby enhancing nutrient bio-availability<sup>45</sup>.

# RESEARCH ARITCLES

*Phytates:* In the present study, the phytate content of groundnuts was assessed by analysing their phytic acid levels (Figure 5). The results revealed a significant decrease (P < 0.05) in phytate content as both the treatment time and voltage increased during CP processing. Initially, the phytate content in the groundnut samples was  $544.36 \pm 0.03$  mg/g. After 15 min of CP treatment, it reduced to  $499.04 \pm 0.04$  mg/g at 20 kV,  $486.67 \pm 0.02$  mg/g at 25 kV, and  $482.95 \pm 0.03$  mg/g at 30 kV. A similar decrease in phytate content with increased CP treatment time and voltage was noted in the studies by Sarkar *et al.*<sup>45</sup>, and Dharini *et al.*<sup>62</sup>. The decrease in phytate content in CP-treated samples may be linked to the breakdown of glycosidic bonds caused by the interaction with reactive species.

*Oxalates:* In the present study, the oxalate content, expressed as oxalic acid, was determined to be  $74.90 \pm 0.04$  mg/g in

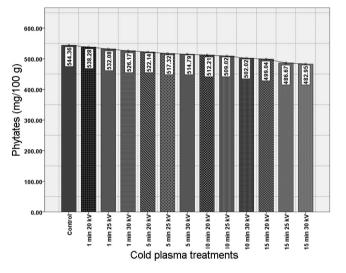


Figure 5. Effect of CP treatment on phytate content.

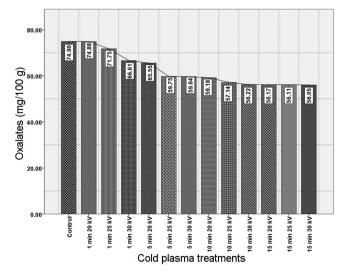


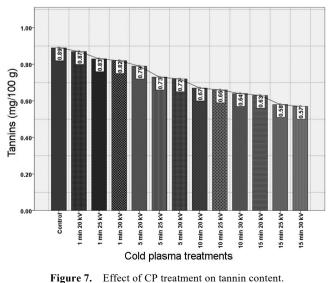
Figure 6. Effect of CP treatment on oxalate content.

the control sample (Figure 6). With an increased treatment time of 1 min, the oxalate content gradually decreased to 74.88  $\pm$  0.02 mg/g at 20 kV and 56.22  $\pm$  0.05 mg/g at 30 kV after 10 min. The lowest oxalate level, 56.05  $\pm$ 0.03 mg/g, was recorded after 15 min of treatment at 30 kV, showing a significant (P < 0.05) reduction compared to the untreated groundnut samples. Dharini *et al.*<sup>62</sup>, noted a similar significant reduction (P < 0.05) in the oxalate content of plasma-treated raw sesame milk samples when compared to the untreated ones. The reduction in oxalate content could be due to the interaction between reactive species and oxalate.

*Tannins:* Figure 7 shows the tannin content in both the untreated groundnut sample and those subjected to CP treatment. The tannin content in the control sample was  $0.89 \pm 0.08 \text{ mg/g}$ . After 15 min of CP treatment, the lowest tannin levels were observed at 20 kV ( $0.63 \pm 0.02 \text{ mg/g}$ ), 25 kV ( $0.58 \pm 0.01 \text{ mg/g}$ ), and 30 kV ( $0.57 \pm 0.02 \text{ mg/g}$ ), showing a significant (P < 0.05) reduction compared to the untreated groundnut samples. The findings of the present study are consistent with those of Charu *et al.*<sup>50</sup>, who observed a significant reduction in tannin content as both voltage and treatment duration increased during CP treatment of pearl millet and barnyard millet, compared to untreated controls. This reduction in tannin content may be due to the cleavage of glycosidic bonds, facilitated by reactive oxygen species during the CP treatment<sup>45</sup>.

### Conclusion

CP treatment is a highly effective and sustainable approach to improving the nutritional quality of groundnuts. It significantly reduces aflatoxin B1 levels, enhances bioactive components and increases essential minerals like iron and zinc. The treatment also reduces anti-nutritional



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factors, improving mineral bioavailability and protein digestibility. Compared to traditional methods, CP provides superior results by decontaminating toxins while preserving groundnut quality. This innovative technology has the potential to minimise losses caused by fungal contamination, boost the export competitiveness of Indian groundnuts, and increase profitability. Further research is needed to optimise processing parameters for large-scale applications in the food industry.

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