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The impact of Atmospheric Cold Plasma treatment on inactivation of lipase and lipoxygenase of wheat germs

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Abstract

Wheat germ is a by-product of milling process which contains large amount of nutrients. The shelf life of wheat germ could improve by inactivation of destructive endogenous enzymes especially lipase and lipoxygenase. In this work, the impact of atmospheric cold plasma treatment on the inactivation of lipase and lipoxygenase enzymes of wheat germ was studied.

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Dielectric barrier discharge plasma was utilized to treat wheat germs. The impact of treatment time and voltage of plasma on the inactivation of lipase and lipoxygenase were investigated as well. The higher voltage and treatment time led to higher inactivation, however, the inactivation of lipase and lipoxygenase was not significant after 25 min treatment time. The DPPH radical scavenging activity and total phenolic of treated samples did not change significantly compared to controlled samples. However lipase and lipoxygenase recovered some of their loss activity during the storage. The recovery of activity was higher for lipase compared to lipoxygenase. According to the overall results, the cold plasma could be introduced as a new potential to stabilize the wheat germ and extending its shelf-life.

Keywords: Wheat germ; Cold plasma; Lipolytic enzymes; Antioxidant activity

1. Introduction

Wheat germ (WG) is main by-product (2-3%) of wheat milling process. The germ contain valuable micro- and macro-nutrients including vitamin B and E, minerals, essential amino acids, dietary fibers, and functional phytochemicals such as flavonoids and sterols (Brandolini and Hidalgo 2012; Ghafoor et al. 2016). Due to its health-beneficial components, WG has a great potential as a food supplement, as well as a source for the preparation of various value added foods such as bread (Ge et al. 2000; Rizzello et al. 2010), cookies (Arshad et al. 2007), and high protein foods and beverage (Hassan et al. 2010).

On the other hand, the WG has a short shelf life due to high activity of endogenous enzymes especially lipase and lipoxygenase. Inactivation of these two enzymes is crucial for long-term storage of WG. Until now, various thermal processing methods have been used to inactivate WG

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endogenous enzymes. Studied thermal methods include direct heating of WG in autoclave or fluidized bed drying and indirect heating by use of drum dryers (Srivastava et al. 2007). The thermal treatments have low energy efficiency and can destroy the nutrient content of WG (Xu et al. 2012). Microwave irradiation also had been used to inactivate lipase and lipoxygenase enzymes of WG (Xu et al. 2016; Xu et al. 2013; Błaszczak et al. 2002). The non-uniform temperature distribution of microwave heating limits the wide application of this method (Li et al. 2016). Li et al. 2016 and Gili et al. 2017 used infrared radiation to inactivate lipase and lipoxygenase of WG and optimized the operating parameters to retain the antioxidant property of WG and its fatty acid content (Gili et al. 2017; Li et al. 2016). Infrared radiation required relatively high temperatures which may affect the vitamin and other nutrient contents of WG.

Atmospheric cold plasma (ACP) treatment can be considered as another alternative for inactivation of enzymes (Tolouie et al. 2017). Plasma is mainly consisting of positive and negative ions, radicals, electrons and also excited or ground state of atoms, which results from subjecting a gas to an energy source (Conrads and Schmidt 2000; Ramazzina et al. 2015). The gases utilized in plasma can be air or noble gases like helium and argon, or a mixture of different types of gases in an appropriate ratio. The resulted reactive species (RS) can react with enzymes in a food system and change the protein structures which lead to inactivation of enzymes (Tolouie et al., 2017).

Previous researches have proposed ACP as an effective method to inactive enzymes in various food systems (Segat et al. 2016; Fridman et al. 2008; Surowsky et al. 2013; Pankaj et al. 2013; Bußler et al. 2016). Fridman et al. (2008) studied the influence of ACP on trypsin activity, where the enzymatic activity of trypsin dropped to about zero by ACP treatment (Fridman et al. 2008). According to Surowsky et al. (2013), plasma treatment could reduce the polyphenol oxidase

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(PPO) and peroxidase (POD) activity by about 90 and 85% respectively (Surowsky et al. 2013). The activity of PPO and POD enzymes in freshly cut apples and potatoes was reduced by about 62% and 77% in freshly cut apples and potatoes, respectively (Bußler et al. 2016). Segat et al. (2016) evaluated the effect of ACP treatment on the alkaline phosphatase activity of milk, the residual activity was reported to be below 10% of initial extent following plasma exposure (Segat et al. 2016)

Our literature survey has shown that there is no work on the use of ACP for inactivation of lipase and lipoxygenase enzymes in WG. The aim of this work is (1) to investigate the impact of ACP on the lipase and lipoxygenase activity of WG; (2) to determine DPPH free radical scavenging activity and total phenolic content of treated WG; (3) to explore reversibility of inactivation of studied enzymes during storage.

2. Material and Methods

2.1. Wheat germ storage and characterization

WG flakes, which were separated from commercially available hard red winter wheat, were obtained from Zar flour Co. (Tehran, Iran). The collected germ was stored in an airtight two-layer (aluminum and polyethylene) package in a freezer at −20 ºC. Wheat germ was allowed to defrost at room temperature before treated with plasma. The approved method of American Association of Cereal Chemist was used to measure the moisture, fat, protein, fibre and ash content of germ (AACC 2003; Zhang et al. 2015a).

2.2. Plasma Apparatus

The type of plasma apparatus used in this work is a dielectric barrier discharge (DBD) plasma using a modulated voltage and it consists of two electrodes which is shown in Fig. 1 (Ghomi

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Marzdashty et al. 2017). The upper electrode is made of copper and the lower electrode is a stainless steel in contact with quartz to create homogenous plasma at different discharge gap. The gap between two electrodes can be changed from 5 mm to 30 mm. A 6 kHz and 10 kV high voltage power supply was applied to the upper electrode and the lower electrode was connected to a 50 Hz and variable voltage up to 24 kV. The samples were placed in the sample holder between the two electrodes. After applying the both high voltages to the DBD, homogenous cold plasma will be generated in the sample holder. The temperature of this plasma was about 30 $^{\circ}$C. The shape of the sample holder and the defined amount of WG that have been loaded in resulted in sample layer thickness of 3±1 mm. The same sample thickness was used in all experiments.

2.3. Experimental design

2.3.1. Plasma treatment of WG

Three set of experiment was designed to provide adequate quantity of treated WG for measurement of lipase, lipoxygenase, DPPH radical scavenging activity and total phenolic content. In each set, identical amount of WG was weighed and placed in the sample holder of plasma. The clearance between electrode and sample holder was 2 cm. To study the impact of clearance between electrodes, a set of experiment were repeated using electrode clearance of 1.5 cm. Two voltages 20 and 24 kV of 50 HZ power supply and fixed 6 kHZ and 10 kV of second power supply for various holding time (5-35 min) were applied to maximize the enzymes inactivation. All treatments were carried out in triplicate.

2.4. Lipase activity measurement

Extraction of WG lipase was performed as described by Godoy et al, 2009 and Brunschwiler et al., 2013 with some modifications (Godoy et al. 2009; Brunschwiler et al. 2013). For lipase

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extraction one gram of WG was transferred into a 50 mL falcon tube. Then, 5 mL of phosphate buffer, 100 mM, pH 7.0, was added and the extraction procedure completed in a rotary shaker at 30 °C and 200 rpm for 20 min. Lipase activity was measured using p-nitrophenyl laurate as an enzymatic substrate as described by Pinsirodom and Parkin 2001 (Pinsirodom and Parkin 2001). The enzymatic activity was determined by the addition of 1 mL of extracted enzyme solution to 2.5 mL of 420 µM p-nitrophenol laurate substrate solution. The reaction was carried out at 30 °C and the absorbance was measured over time at 410 nm. One unit of lipase activity was defined as the enzyme amount that releases 1 µmol of p-nitrophenol per minute under assay conditions. Enzyme activity was expressed as units/gram of the initial dry solid medium.

2.5. Lipoxygenase activity measurement

We used described methods in previous publications to measure lipoxygenase activity (Surrey 1964; Wu et al. 1997; Chen et al. 2015; Carrera et al. 2007). Accordingly, one gram of WG was ground and extracted with 0.1 M potassium phosphate buffer pH 7.0. The slurry was collected and centrifuged at 9,000×g for 15 min at 4 °C. The prepared solution was filtered through a 0.20 µm filter, and the filtrate was used as the source of enzyme. To make substrate solution, 0.5 ml of tween 20 was dissolved in 10 ml of 0.1 M borate buffer at pH 9.0 and 0.5 ml of linoleic acid (≥99%) was added drop by drop. Then 1.3 ml of 1 N NaOH was added and the mixture once again agitated until a clear transparent solution was obtained. Then 90 ml of the borate buffer was added to the solution and the final volume made up to 200 ml with water. The reaction was initiated by combining 0.04 mL of substrate with 0.92 mL 0.1 M potassium phosphate buffer (pH 6.0) and adding 0.04 mL of the WG filtrate as an enzyme source. To measure the activity of lipoxygenase, the absorbance of mixture at 234 nm was compared with the absorbance of control samples as below:

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$$X\ (\%) = \frac{\Delta Abs234nm/\min sample}{\Delta Abs234\ nm/mincontrol} \times 100$$

Where X is the relative enzyme activity of lipoxygenase, ΔAbs234 nm/min sample is the increase in absorbance at 234 nm per minute per mg of the sample lipoxygenase tubes under assay conditions and ΔAbs234 nm/min control is the increase in absorbance at 234 nm per minute per mg of the control lipoxygenase tubes under assay conditions.

2.6. Determination of DPPH free radical scavenging activity

We used the procedure described by Zhu et al. (2011) to measure the DPPH radical scavenging activity of WG sample with some modifications (Zhu et al. 2011). In which, 2 mg of prepared samples was dissolved in the 2 ml of 70% ethanol. Then 2 mL of 0.1 mM DPPH in ethanolic solution was added to the previous solution. Then the mixture was shaken and incubated in the dark at ambient temperature for an hour. The control samples were prepared as well in the same way except replacing the antioxidant solution with the corresponding extract solvent. The absorbance of samples was read at 517 nm. The percentage of scavenging DPPH radical obtained by the subtracting of the absorbance of samples from the absorbance of control samples and dividing on the absorbance of control samples.

2.7. Total phenolic content

Analysis of total phenolic was performed according to the procedure described by Rizzello et al., 2010 and 2011 (Rizzello et al. 2010; Rizzello et al. 2011). Total phenolic content of WG was prepared by weighing 1 g of wheat germ and mixing with 10 ml of 80% methanol. The mixture was purged with nitrogen, mixed for 30 min and centrifuged at 6,000×g for 20 min. The extracts were transferred into culture tubes, purged with nitrogen and stored at 4 ºC before analysis. Gallic acid was used as the standard. The reaction mixture contained 20 µl of the WG extract, 100 µl of folin-ciocalteu reagent (Sigma Chemical Co.) and 1.58 ml of distilled water. After few

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min, 300 µl of saturated sodium carbonate solution was added to the reaction mixture. Incubation was allowed at 20 ºC for 2 h and the absorbance at 765 nm was recorded. The concentration of total phenolic content was calculated as gallic acid equivalent.

2.8. Investigation the reversibility of lipase and lipoxygenase inactivation in ACP treated WG during storage

ACP treated and controlled samples were stored in closed Petri dishes at room temperature. To explore the impact of light, samples were stored in a dark room as well. The activity of ACP treated samples and raw WG samples were measured during 30 days of storage.

2.9. Statistical Analysis

All the experiments were made in triplicate. Data were assessed on the statistical significance of $p<0.05$. Variance analysis was performed with Minitab statistical software version 14.0. When Duncan's test was used for multiple comparisons. The non-linear regressions of data have been realized with Origin software (OriginLab Corporation).

3. Results and Discussion

3.1 Characterization of WG

The moisture, ash, protein, fat and fiber content of WG used in our study were presented in Table 1. The results are in agreement with those reported by Brandolini and Hidalgo, 2012 (Brandolini and Hidalgo 2012).

3.2 Effect of ACP treatment on WG lipase and lipoxygenase inactivation

Figure 2 presents the inactivation of WG lipase at two different voltages including 20 and 24 kV. As it could be seen, the activity of lipase decreased rapidly after 5 min treatment. The residual activity of lipase after 5 min treatment at 20 and 24 kV were 39.97% and 36.45%, respectively.

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Lipase activity was reduced continuously during 25 min and reached to 27.11% and 25.03% of initial amounts at voltage 20 and 24 kV, respectively. The enzyme inactivation rate was not the same at two applied voltage. According to the experimental results, the inactivation rate was higher at 24 kV versus inactivation rate at 20 kV. No significant inactivation of lipase was observed for treatments longer than 25 min suggesting an optimum treatment time of 25 min (Figure 2). According to two-way ANOVA analysis, time and voltage are significant factors for the lipase inactivation ($p<0.05$). However, the higher voltage is more favourable for inactivation of enzyme.

Due to the low temperature of the process (25 ^{o}C), the most probable cause for enzyme inactivation was attributed to the formation of reactive species in plasma and their reactions with protein structure. The reaction of free radicals with enzyme can cause changing in the protein conformation and structure mainly secondary structure in term of a loss of α-helical structure (Surowsky et al. 2013) and modification of some amino acids side chains of the enzyme (Pankaj et al. 2013).

Despite of rapid inactivation of lipase at a short period of treatment (5 min), lipoxygenase activity was only reduced by 2.2% and 7.7% at voltage of 20 and 24 kV, respectively (Figure 3). Further inactivation of lipoxygenase happened by increasing the treatment time and after 25 min, the residual activity of lipoxygenase were recorded equal to 55.18% and 49.98% at voltages of 20 and 24 kV, respectively. Similar to lipase inactivation, treatments longer than 25 min did not change the residual activity of lipoxygenase significantly (Figure 2). The lower rate of inactivation of lipoxygenase compared to lipase inactivation can be attributed to the different secondary structure of these two enzymes which made plasma to be more selective for inactivation of lipase. Effect of time and voltage were significant ($p<0.05$) in inactivation of

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lipoxygenase similar to lipase inactivation. Pankaj et al. (2013) studied the effect of treatment time and voltage of the ACP plasma process on the activity of tomato peroxidase (Pankaj et al. 2013). They observed that enzymatic activity decreased with increasing treatment time from 1 to 5 min and voltage from 30 to 50 kV. The complete inactivation of enzyme was achieved at 30 kV within 6 min treatment time. Increasing voltage to 50 kV decreased the required time for inactivation to 3 min (Pankaj et al., 2013).

The deactivation of enzymes can be described using exponential model as A=B exp(-kt) where A, B, k, and t are enzyme activity, constant, specific deactivation rate and time, respectively (Aymard and Belarbi, 2000). A regression was carried out to fit exponential equations for inactivation of both enzymes by ACP as a function of time (5-25 min). Eq.1-4 predicts the residual activity of lipase and lipoxygenase in WG, respectively.

Lipase activity $_{(20\,kV)}$ (%) = 46.40 exp(-0.029t) R^2=0.96 Eq.1

Lipase activity $_{(24\,kV)}$ (%) = 42.41 exp(-0.030t) R^2=0.95 Eq.2

Lipoxygenase activity $_{(20\,kV)}$ (%) =113.1 exp(-0.028t) R^2=0.93 Eq.3

Lipoxygenase activity $_{(24\,kV)}$ (%) = 107.8 exp(-0.031t) R^2=0.96 Eq.4

In which, t is treatment time (5-25 min). Eq.1 and 3 represent the results of samples treated at voltages of 20 kV and Eq. 2 and 4 are for voltage of 24 kV. As could be seen, the deactivation rates (k) increases by increasing the voltage. The deactivation rates for lipase and lipoxygenase are 0.029 and 0.028 at 20 kV and they increased to 0.030 and 0.031 at 24 kV, respectively.

To explore the impact of electrode clearance on the inactivation of enzymes, the clearance was reduced from 2 to 1.5 cm while voltage was kept at 24 kV. According to the experimental results, the inactivation of both enzymes showed no significant change when the electrode clearance reduced (Table 3). The changes in activities were only about 1.0 to 2.5% when

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electrode clearance decreased from 2 to 1.5 cm. This difference may come from experimental variations. This is a great advantageous of DBD plasma system used in this work which the impact of electrode clearance was not significant.

There was a significant difference in inactivation of lipase and lipoxygenase by ACP in our study. On the other hand, Li et al. 2016's work found that the infrared radiation inactivated both enzymes about the same (Li et al. 2016). They reported that infrared radiation for 20 min at temperature of 90^{o}C reduced the residual activity of lipase and lipoxygenase of WG to 18.02% and 19.21%, respectively. Srivastava et al., 2007 (Srivastava et al. 2007) studied the stabilization of WG using different heat treatment methods including direct steaming, drum drying and fluidized bed dryings. Complete inactivation of lipase was achieved in direct steaming (15 min, 120^{o}C) and drums drying (3-5 min, 125^{o}C) methods. On the other hand, fluidized bed dryings (1 min, 240^{o}C) inactivated lipase by 85%. Inactivation of enzymes in heat treatment methods is a function of temperature and time. Lower temperature of fluidized bed drying and shorter exposure time caused less inactivation of enzyme compared to drum drying and direct steaming. Similar to our study results, lipoxygenase was less inactivated compared to lipase in all three heat treatment methods and the residual activity of lipoxygenase was 8 to 22% depends on the utilized method. Such findings demonstrated that lipoxygenase structure has higher resistance to all the mentioned treatment methods except infrared radiation treatment.

3.3 Effect of ACP treatment on DPPH free radical scavenging activity and total phenolic in WG

Table 3 and 4 present DPPH free radical scavenging activity and total phenolic content of WG during ACP treatment. Since the higher inactivation of WG enzymes was achieved at voltage of 24 kV, these set of experiments were performed at voltage of 24 kV. DPPH free radical scavenging activity of WG did not change significantly during plasma treatment and it stayed at

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range of 46.31 to 47.17% (Table 3). The DPPH free radical scavenging activity of raw WG was 47.03%. These results were in agreement with Ramazzina et al.'s results that DBD treatment did not change the antioxidant activity of kiwifruit (Ramazzina et al. 2015). Furthermore, Amini and Ghoranneviss, 2016 found that cold plasma treatment had no effect on the antioxidant capacity of fresh walnuts (Amini and Ghoranneviss 2016). This is a great advantage of ACP treatment method that the antioxidant capacity as a sensitive bioactive component of treated samples stays unchanged. However, this result is strongly dependent to plasma condition such as the applied voltage and the treatment time as well as food matrix. Pankaj et al, 2017 reported that both DPPH free radical scavenging activity and total phenolic content of grape juice reduced after high voltage (80 kV)-ACP treatment (Pankaj et al. 2017).

The total phenolic concentration of WG used in our work was 4.06 mg GAE/g (Table 4). Experimental results showed that cold plasma treatment did not have significant impact on phenolics concentration of WG ($p>0.05$). Results of this work was in agreement with previous publications (Amini and Ghoranneviss 2016; Matan et al. 2015). Amini and Ghoranneviss, 2016 investigated the impact of plasma jet treatment on the walnuts and there was no change in total phenolic content after 11 min treatment. Matan et al., 2015 showed that total phenolics of dragon fruit did not change during the treatment of plasma jet. Despite of these three studies results, Garofulic et al., 2015 and Herceg et al., 2016 found that total phenolics content increased during argon plasma jet treatment of cherry and pomegranate juice which might be due to the difference in food matrix and its reactions with resultant plasma species (Garofulić et al. 2015; Herceg et al. 2016).

3.4 Impact of storage time on the recovery of lipase and lipoxygenase activity

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Figure 4 and 5 present the lipase and lipoxygenase activity of 25 min cold plasma treated samples at 24 kV as optimum conditions for target enzymes inactivation during 30 days of storage, respectively. It was seen that lipase and lipoxygenase regained some of their lost activity in treated WG samples during the storage (Figure 4 and 5). These results showed that ACP treatment could not permanently destroy lipase and lipoxygenase enzymes. Similar to finding of this work, Li et al., 2016 concluded that infrared treated WG samples recovered part of their lipase and lipoxygenase activity during storage (Li et al. 2016). In Li et al. work, the recovery of lipase and lipoxygenase activity was about 5-10% (Li et al., 2016). In this work, the impact of light was not significant in enzyme activity recovery (Figure 4 and 5). The changes in activity of lipase and lipoxygenase for dark and light storage were only 0.02 and 0.70 U/g for lipase and lipoxygenase, respectively (Table 5 and 6).

The lipase activity was 0.60 U/g immediately after 25 min ACP treatment and increased to 1.78 U/g after 30 days of storage at room temperature (Table 5). This increase is equivalent to the recovery of 1.18 U/g enzyme activities. On the other hand, the lipase activity of untreated samples increased from 2.39 to 4.96 U/g after 30 days of storage (Table 5) which is equivalent to about 2.57 U/g increase in activity. The lipase activity was much higher for untreated samples compared to ACP treated samples after 30 days of storage. In other word, comparing lipase activity of 25- min ACP treated WG (1.78 U/g) with lipase activity of untreated samples (4.96 U/g) after 30 days of storage, it is clear that ACP treated samples have significantly lower lipase activity compared to raw samples.

Lipoxygenase activity of 25 min ACP treated samples shifted from 8.10 to 14.62 U/g after 30 days of storage (Table 6). While the lipoxygenase activity of untreated samples increased from

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16.20 to 32.6 U/g during 30 days storage. Similar to lipase enzyme, increase in lipoxygenase activity during storage was much higher for raw samples compared to ACP treated samples.

After one month of storage, the activity of both enzymes in treated samples was significantly lower than the untreated samples before and after the storage period (Figure 4 and 5). These findings show that ACP treatment can effectively increase the shelf life of WG.

Eq. 5-8 describe lipase and lipoxygenase activities recovery in WG samples during 30 days of storage.

Residual lipase activity of untreated $WG_{(24)}$ (U/g) = 4.69/(1 + 10^((11.63-t)*0.17))

R^2=0.98 Eq. 5

Residual lipase activity of ACP treated $WG_{(24)}$ (U/g) = 1.78/(1 + 10^((9.32-t)*0.17))

R^2=0.96 Eq. 6

Residual lipoxygenase activity of untreated $WG_{(24)}$ (U/g) = 32.65/(1+ 10^((14.95-t)*0.22))

R^2=0.97 Eq. 7

Residual lipoxygenase activity of ACP treated $WG_{(24)}$ (U/g) = 14.8/(1 + 10^((12.56-t)* 0.31))

R^2=0.95 Eq. 8

Which t is the storage time (days).

To explore the impact of ACP treatment time on the recovery of enzyme activity during storage, the residual activity for treated samples of 5 to 25 min were obtained during 30 days of storage and results were presented in Table 5 and 6. Samples with lower ACP treatment time regenerated more of their lipase and lipoxygenase enzyme activity compared to samples with higher ACP treatment time. For example, for 5 min treated sample, the lipase activity increased from 0.87 to 2.62 U/g after 15 days of storage (Table 5). This increase was equivalent to 1.75 U/g. On the other hand, the lipase activity for 25 min treated samples increased from 0.60 to 1.75 U/g,

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equivalent to an increase of 1.15 U/g. The same trends were seen for lipoxygenase (Table 6). The lipoxygenase activity of 5 min treated sample increased from 14.9 to 24.86 U/g, equivalent to an increase of 9.96 U/g. For 25 min treated sample, the increase in activity of lipoxygenase was 6.13 U/g after 15 days. The regeneration rate of both enzymes in plasma treated germs decreased significantly during the second 15 days of the storage period. Their corresponding activity of lipase and lipoxygenase in plasma treated samples were 1.78 and 14.62 U/g, respectively after 30 days of storage. These findings concluded that enzymes in samples with lower treatment time (or higher residual enzyme activity) regenerated more of their activity during storage.

The impact of light was not significant in enzyme activity recovery (Table 5 and 6).

The increase in the activity of lipase and lipoxygenase had been linked to water content and water activity of WG samples (Li et al. 2016; Labuza and Dugan Jr 1971). Li et al. 2016 concluded that lipase and lipoxygenase could regenerate their activity after adsorption of water during storage. As ACP treatment was carried at relatively lower temperature (25 $^{\circ}$C) compared to infrared radiations temperature (90 $^{\circ}$C), it was expected that the water content did not change significantly during ACP treatment. The moisture content of samples after ACP treatment was 6.98±0.31% showing a significant amount of water present in treated WG. Presence of water in samples could accelerate the recovery of enzyme activities. Another mechanism can be expected from the variation in the structural properties of plasma treated enzymes. Zhang et al., 2015 reported that regeneration rate of DBD-treated lactate dehydrogenase were increased after 24 h storage (Zhang et al. 2015b). They suggested that reversible changes in the functional domains of enzyme which affected by plasma reactive species involved in the recovery of activity.

4. Conclusion

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To enhance the shelf life of WG, it is essential to control the lipase and lipoxygenase activities. Result of this work concluded that ACP can inactivate these enzymes and increased the shelf life of WG. It was found that 25 min ACP treatment resulted in reduction of lipase and lipoxygenase activity of WG to 25.03% and 49.98% of initial extent, respectively. Increasing time and voltage can enhance the inactivation of enzymes. However, treatments more than 25 min did not increase the inactivation of enzyme significantly. In this work, an optimum time of 25 min and a voltage of 24 kV were obtained to maximize the enzyme inactivation. Though, cold plasma treatment could not permanently inactivate lipase and lipoxygenase. Despite of the recovery of enzymes activity during the storage, the enzyme activity of ACP treated samples was much lower than the untreated WG after 30 days of storage at room temperature. This result concluded that ACP treatment can enhance the shelf life of WG. ACP treatment was carried at low plasma temperature and relatively short period of time of 25 min. Due to low temperature the ACP treatment did not impact DPPH free radical scavenging activity and total phenolic content of WG. This is a great advantageous of ACP treatment that it could preserve WG antioxidant capacity while it increased WG shelf life by reducing the activity of lipase and lipoxygenase. Considering the great potential of wheat germ as a food supplement and a worthy source for preparation of value added innovative foods, any approach to extend shelf-life is welcome. Our results demonstrated the potential of cold plasma to inactivate endogenous lipase and lipoxygenase enzymes and increase WG stability. However, more nutritional and safety concerns should be addressed before recommending the industrial application of cold plasma for wheat germ stabilization.

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Figure captions

Figure 1. Schematic of experimental setup

Figure 2. Effect of cold plasma treatment on inactivation of lipase in WG

Figure 3. Effect of cold plasma treatment on inactivation of lipoxygenase in WG

Figure 4. The effect of storage time on the recovery of lipase activity in a) wheat germ (WG), b) 25 minutes-ACP treated WG, c) 25 minutes-ACP treated WG protected against light.

Figure 5. The effect of storage time on the recovery of lipoxygenase activity in a) wheat germ (WG), b) 25 minutes -ACP treated WG, c) 25 minutes-ACP treated WG protected against light.

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Table 1. The composition of wheat germ

Moisture (%)	Fat (%)	Protein (%)	Fibre (%)	Ash Content (%)
8.63±0.04	7.82±0.41	25.96±0.39	1.63±0.19	3.88±0.09

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Table 2. The impact of electrode clearance on the lipase and lipoxygenase inactivation

Enzyme activity	Electrode clearance (cm)	Treatment time (min)					
		0	5	10	15	20	25
Lipoxygenase	**2**	16.20±0.55	14.90±0.23	12.60±0.35	11.76±0.43	10.28±0.04	8.10±0.60
Lipoxygenase	**1.5**	16.20±0.55	15.20±0.12	12.48±0.28	12.04±0.25	10.40±0.20	8.40±0.18
Lipase	**2**	2.39±0.10	0.87±0.05	0.81±0.06	0.76±0.08	0.69±0.02	0.60±0.05
Lipase	**1.5**	2.39±0.10	0.84±0.08	0.79±0.03	0.78±0.06	0.72±0.02	0.63±0.07

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Table 3. The effect of plasma on DPPH free radical scavenging activity

Treatment time (min)	**0**	**5**	**10**	**15**	**20**	**25**
ΔV: 24kV	47. 03 ± 0.19	47.01± 0.05	46.82 ± 0. 23	46.56± 0.12	46.38± 0.15	46.31± 0.23
ΔV: 20kV	47.06 ± 0.01	47.17± 0.19	47.03± 0.22	46.79± 0.15	46.84± 0.19	46.81± 0.22

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Table 4. The effect of plasma on total phenolic content (mg GAE/g)

Treatment time (min)	**0**	**5**	**10**	**15**	**20**	**25**
ΔV: 24kV	4.06 ± 0.01	4.06±0.03	4.07±0.02	4.06±0.02	4.08±0.00	4.07±0.01
ΔV: 20kV	4.06 ± 0.00	4.06± 0.02	4.06± 0.01	4.07± 0.01	4.06± 0.03	4.07± 0.01

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Table 5. Activity of lipase (U/g) in raw and ACP treated wheat germ during storage

Storage (days)	Storage conditions	Treatment time (min)					
		0	**5**	**10**	**15**	**20**	**25**
0	-	2.39±0.10[b]	0.87±0.05[b]	0.81±0.06[d]	0.76±0.08[d]	0.69±0.02[d]	0.60±0.05[d]
15	**Dark**	4.05±0.15[a]	2.62±0.12[b]	2.23±0.15[b]	2.09±0.09[c]	1.99±0.10[c]	1.75±0.01[c]
	Light	4.17±0.17[a]	2.63±0.11[b]	2.22±0.25[b]	2.11±0.14[c]	2.01±0.11[c]	1.75±0.13[c]

* Means followed by different superscript letters are significantly different ($P<0.05$).

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Table 6. Activity of lipoxygenase (U/g) in raw and ACP treated wheat germ during storage

Storage (days)	Storage conditions	Treatment time (min)					
		0	**5**	**10**	**15**	**20**	**25**
0	-	16.20±0.55^{c}	14.90±0.23^{c}	12.60±0.35^{d}	11.76±0.43^{d}	10.28±0.04^{d}	8.1±0.6^{f}
15	**Dark**	25.10±0.07^{a}	24.3±0.15^{a}	20.98±0.28^{b}	19.24±0.35^{b}	17.30±0.2^{b}	13.70±0.29^{c}
	Light	25.30±0.2^{a}	24.86±0.30^{a}	21.39±0.56^{b}	19.95±0.45^{b}	17.75±0.67^{b}	14.23±0.47^{c}

* Means followed by different superscript letters are significantly different (P<0.05).

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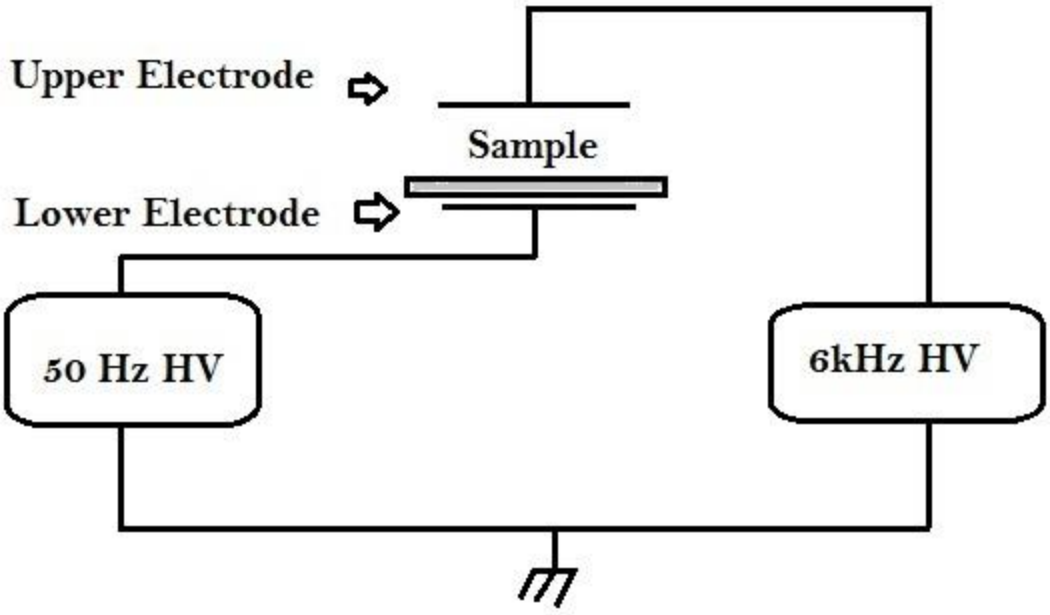

Fig. 1

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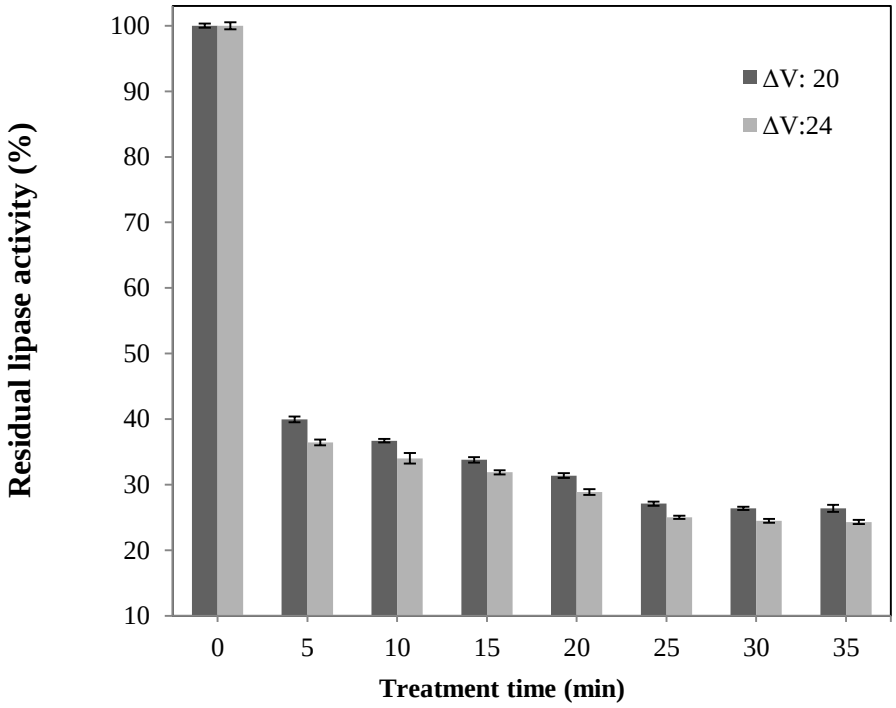

Fig. 2

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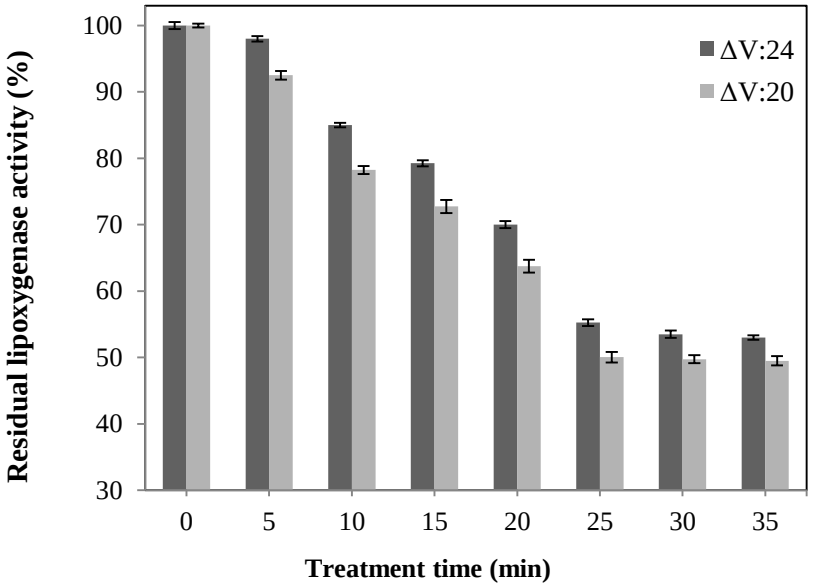

Fig. 3

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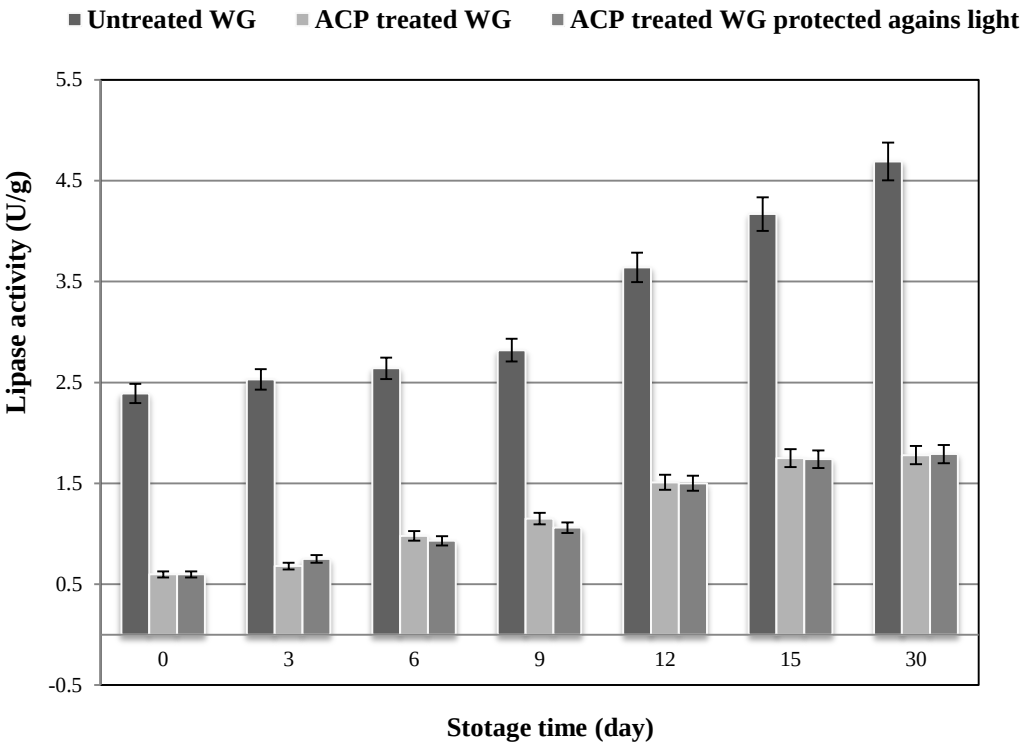

Fig. 4

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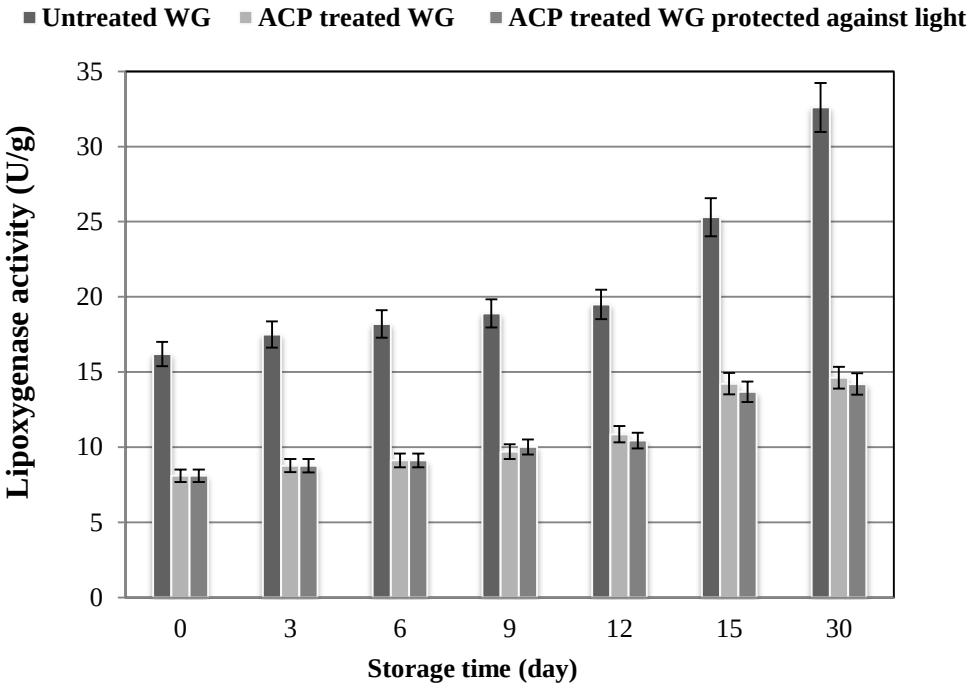

Fig.5

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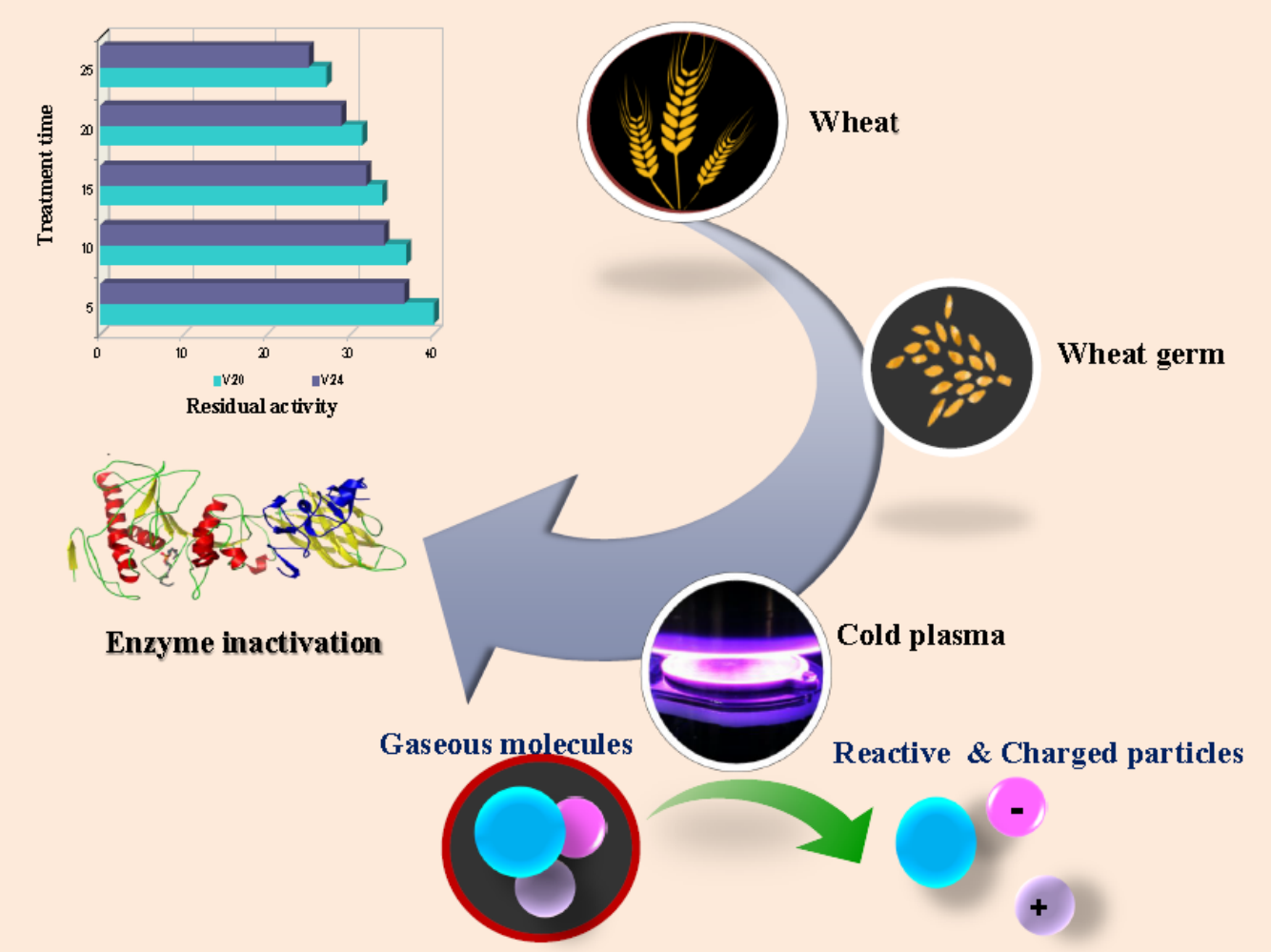
Graphical abstract



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Highlights:

- Wheat germ was treated with ACP to inactivate lipase and lipoxygenase.
- Enzymatic activity of lipase and lipoxygenase was reduced after ACP treatment.
- DPPH free radical scavenging activity and total phenolics of wheat germ did not change significantly after plasma exposure.
- Lipase and lipoxygenase regenerated some of their activity during storage.

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