Extraction and Purification of Protease from *Nigella sativa* for Its Potential Use in Celiac Disease

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**Author’s contribution**

The sole author designed, analysed, interpreted and prepared the manuscript.

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**ABSTRACT**

Celiac disease (CD) is a disease of the digestive system resulting from the intolerance of autoimmune system against gluten and gliadin proteins in wheat, barley, rye and some varieties of oats. In this work protease was isolated and partially purified from *Nigella Sativa* using ammonium sulphate, acetone and trichloroacetic acid. The purified protease was evaluated further for its possible use in celiac disease by its ability to hydrolyse both, gluten and gliadin. Data indicate that the highest purity of digestive protease enzymes was obtained by acetone 80% and 0.2M trichloroacetic acid (TCA). The enzyme purified by acetone exhibited the high value of Vmax (166.66 mg/min) and the low value of Km (0.133 mg/ml). When the obtained enzyme was tested to hydrolyse gluten and gliadin at two increasing concentrations of 1 and 2 mg/ml, almost similar efficacy was observed (28.46% and 32.02%; for gluten) and (22.7% and 16.84%; for gliadin), whereas Digestin, a positive control which contain Papain and Sanzyme 3500 and works by helping in the breakdown of proteins, produced higher efficacy at 2 mg/ml to hydrolyse gluten (38.67%) with 1 mg/ml (25.68%) whereas similar results were observed against gliadin at both concentrations.

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This study show the efficacy of protease obtained from *Nigella Sativa* as a natural and economical source for the hydrolysis of gluten and gliadin, which is considered the primary reason for celiac disease.

**Keywords:** Celiac disease; proteases; *Nigella sativa*; gluten; gliadin.

### 1. INTRODUCTION

Wheat is an important consumable food because of its characteristics of high nutritional values [1]. The wheat endosperm contains 8–15% protein, of which 80% is glutenins and gliadins [2]. It is well known that gliadin is toxic directly or indirectly through immune mediated reactions to small bowel mucosa in relatively small genetically predisposed population who develop celiac disease (CD) [3]. These patients need to eat food without gluten, i.e., they need to be on gluten free diet (GFD).

Celiac disease is a gluten sensitive disease [4]. Gluten is derived from wheat, barley, and rye [5]. The gluten protein is enriched in glutamine and proline and is poorly digested in the human upper gastrointestinal tract [6].

CD is characterised by severe immune damage to the intestinal mucosa, typically involves abdominal distension, weight loss, chronic diarrhoea, severe malnutrition and osteoporosis [7-9]. CD affects approximately 1% of the world population [10-12].

After ingestion of gluten, it is degraded to multiple segments. An immune dominant peptide of 33 amino acids (residues 57 to 89) identified from an α-gliadin fraction has functional properties attributable to many proline and glutamine residues [13]. Proline gives the peptide increased resistance to gastrointestinal proteolysis causing risk for CD [14,15].

Proteases can be produced from a wide range of organisms such as insects, bacteria, yeasts, molds, plants and animals [16,17]. The plant products have less toxic effects are good sources of novel therapeutic agents [18]. Thus, the researcher’s attention shifted to use herbal medicine in disease treatment. *Nigella sativa* Linn. belongs to family Ranulaceae [19].

The herb is widely known in different parts of the world and its seeds are used as spice [20]. Its Arabic name is “Al-Haba Al-Saudaa”. In the west it is known as “Black Cumin”. There is a Hadith of Prophet Muhammad (PBUH) that, “Black seed is treatment of every disease” [21].

The aim of this study is, therefore, to extract, purify and evaluate the ability of proteolytic enzyme from *Nigella Sativa* as a natural and economical source and use it as for hydrolyse gluten and gliadin as alternative treatment for celiac disease.

### 2. MATERIALS AND METHODS

#### 2.1 Materials

*Nigella sativa* and Digestin tablet were purchased from local market and gluten was obtained from Agriculture Research Center, Cairo, Egypt.

#### 2.2 Preparation of Crude Enzyme Extract from *Nigella sativa*

Four gram of *Nigella sativa* were taken in a mortar and ground into fine powder, then mixed with 50 ml of of homogenisation buffer (10 mM Tris HCl, pH 8.0). The homogenate was centrifuged at 8500 × g for 30 min at 4°C. The pellet was discarded and the supernatant (50 mL) was collected and used as the crude protease extract [22].

#### 2.3 Determination of Protein Content

The concentration of total protein, gluten and gliadin were measured by the Bradford method [23]. The Bradford assay relies on the binding of the dye is a rapid Comassie Brilliant Blue G-250 to protein. 10 µl of protein fraction was added to 300 µl of Bradford reagent, incubated for 5 min and measured at 595 nm.

#### 2.4 Partial Purification of Protease Enzymes

This was achieved by salting-out of the crude protein extract solution with ammonium sulphate, precipitation with acetone and TCA separately [24].

#### 2.4.1 Purification by ammonium sulfate

The crude proteins were sequentially precipitated from 50 mL of this crude extract by stepwise
addition of solid ammonium sulphate with stirring at a certain degree of saturation, followed by incubation on ice for at least 2 h and centrifugation at 10,000 x g at 4°C for 15 minutes. The pellet obtained after each centrifugation was resuspended in max. 10 mL of buffer containing 10 mM phosphate, pH 7. Those steps above were done for 50, 60, 70 and 80% of ammonium sulphate saturation, respectively. Aliquots of precipitated fractions were analysed for its protein concentration, and enzyme activity. As comparison, part of the crude extract was also directly subjected to the same analysis [25].

2.4.2 Purification by acetone

In the 50 mL of crude protease extract, an equal volume of ice-cold acetone (50 mL) was added in a dropwise manner with continuous stirring on ice. After the addition of the organic solvent is completed, the stirring was continued on ice for 10–20 min. The mixture was transferred to the chilled screw-cap polycarbonate centrifuge tubes and recovered the precipitated proteins by centrifugation at 10000 × g for 10 min at 4°C. The supernatants were discarded and the centrifuge tubes were inverted over filter paper for air drying [26]. Then, the pellets were suspended in 15 mL of 25 mM Tris-HCl buffer (pH 8.0) [27].

2.4.3 Purification by trichloroacetic acid (TCA)

The crude protein extract was treated with (0.2 M) TCA, centrifuged at 15,000 rpm for 15 min at 4°C. The precipitate dissolved in chilled acetone and again centrifuged at 14000 rpm for 5 min at 4°C and repeated twice to remove remaining TCA. The obtained pellet was dissolved in 0.1M phosphate buffer at pH 7 and used for protein and enzyme assay [28].

2.5 Assay of Protease Activity

The enzyme activity was determined by casein digestion assay as described by EL-Beltagy et al. [29]. One unit of the enzyme activity was the amount of enzyme that liberates sufficient trichloroacetic acid-soluble hydrolysis products so that the absorbance at 280 nm increased by 1.00 in 1 min. Tyrosine which absorbs ultraviolet radiation at 280 nm was used to make a standard curve. The specific activity was calculated as follows and reported as U/mg protein. Percentage of recovered activity was calculated by dividing of the total activity of an enzyme fraction over the total activity of a crude extract and multiplying the result by a hundred.

2.6 Determination of Enzyme Kinetics

Enzyme kinetics and Michaelis constants (Km and Vmax) are determined by studying the effect of substrate concentration on the enzyme activity. The effect of substrate concentration on the activity of partial purified protease enzymes extracted was carried out by using bovine serum albumin (BSA) as an enzyme substrate. Km and Vmax of the extracted enzyme were determined from the Lineweaver-Burk plot [30].

2.7 Extraction of Gliadin Fraction

Gliadin and glutenin were sequentially extracted from wheat flour following the method of Laura et al. [31]. Briefly, wheat flour was extracted twice using 0.3 M sodium iodide–7.5% 1-propanol. The supernatants of these extractions were pooled and precipitated for 48 h, at −20°C with four volumes of cold 0.1 M ammonium acetate. The resulting precipitate was the gliadin fraction, which was freeze-dried and stored at −20°C.

2.8 Gluten and Gliadin Hydrolysis

Hundred milliliters of a 2% gluten or gliadin suspension was prepared by phosphate-citrate buffer pH 6.9 and then 1mg of protease enzymes was added to the protein solution. The mixture was incubated at 31°C for 2h with shaking. After incubation, the reaction mixture was immediately heated for 30s at 100°C to inactivate the protease enzymes. The enzymatically treated gluten and gliadin solutions obtained after centrifugation at 10,000 rpm for 15 min at 4°C were subjected to further investigation [32].

2.9 Determination of Free Amino Acids

The samples (0.2 ml) and standard glycine (0.1 – 0.6 ml) were taken in a series of test tubes and the volume was made up to 1.0 ml with distilled water. Then, 5 ml Ninhydrin reagent was added and tubes were vigorously shaken. Then, tubes were kept in boiling water bath for 10 min for colour development. The tubes were cooled in running water at room temperature. After that, 4 ml distilled water was added. A reagent blank was prepared by taking only reagents and make up the volume with distilled water. The absorbance was recorded at 570 nm in spectrophotometer [33]. The free amino acids
were calculated as per the below formula and expressed as mg. g⁻¹.

Free Amino Acids (mg. g⁻¹) = Graph Factor /Aliquot (ml) x 1/Dilution factor x 1/ 1000

2.10 Statistical Analysis

The data were presented as means ± SD from three replicates. Data were subjected to one-way ANOVA. The means of different treatments were compared using Duncan’s multiple range tests at p ≤ 0.05. Statistical analyses were performed using SPSS statistical software (IBM SPSS Statistics, version 20) [34].

3. RESULTS AND DISCUSSION

3.1 Fractional Precipitation of Protease Enzymes Extracts

3.1.1 Salting out with ammonium sulphate

Data presented in Table (1) describes the precipitation of the enzyme extract with different concentrations of ammonium sulphate. The crude enzyme extract from Nigella sativa contained 0.40 mg protein. The 80% precipitated fraction show high specific activity accounted by 6857 U/mg protein and 1.79 purification fold followed by 60% precipitation which exhibits 6000 U/mg protein. These results are in agreement with Kumar et al., [35] and El-Safey and Abdul-Raouf [36] who purified the crude extracellular alkaline proteases by fractional precipitation with ammonium sulfate. On the other hand, several studies reported the alkaline proteases purified by using 60% ammonium sulfate [37-39].

3.1.2 Fractionation with acetone

Data recorded in Table 2 indicates the precipitation of protease enzymes extracted from Nigella sativa with acetone. The precipitated fractions obtained by acetone at concentrations of 40, 60 and 80% exhibited total activity of protease enzymes accounted by 302, 380 and 505 U, respectively compared with crude enzyme extract 1530 U. Whereas, the specific activity of enzyme at precipitation with 60 and 80 % acetone fractions was higher than that of fraction at 40%. On the hand side, high specific activity 10100 U/mg was recorded for the enzyme extract of Nigella sativa at 80% saturation with 2.64 purification fold. These results are in line with Thangam and Rajkumar [40] who found that the maximum specific activity of protease enzymes occurs with purification by acetone at 80% concentration.

Table 1. Purification of protease enzymes extracted from Nigella sativa by ammonium sulphate

<table>
<thead>
<tr>
<th>(NH₄)₂SO₄ Saturation (%)</th>
<th>Total protein (mg)</th>
<th>Recovered protein (%)</th>
<th>Total activity (U)</th>
<th>Recovered activity (%)</th>
<th>Specific activity (U/mg protein)</th>
<th>Purification fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.40±0.007</td>
<td>100.00</td>
<td>1530±3.591</td>
<td>100.00</td>
<td>3825±150.32</td>
<td>1.00</td>
</tr>
<tr>
<td>40</td>
<td>0.09±0.004</td>
<td>22.5</td>
<td>350±0.930</td>
<td>25.92</td>
<td>3888±319.68</td>
<td>1.01</td>
</tr>
<tr>
<td>60</td>
<td>0.06±0.004</td>
<td>15</td>
<td>360±0.598</td>
<td>23.52</td>
<td>6000±399.71</td>
<td>1.56</td>
</tr>
<tr>
<td>80</td>
<td>0.07±0.002</td>
<td>17.5</td>
<td>480±0.610</td>
<td>31.37</td>
<td>6857±410.2</td>
<td>1.79</td>
</tr>
</tbody>
</table>

Data represent the means ± SE calculated from three replicates. *P > 0.05, **P ≤ 0.01, ***P ≤ 0.001, show comparison of respective parameters at ammonium sulphate 40, 60 and 80% versus Control (0% ammonium sulphate) (One-way ANOVA followed by Tukey test).

Table 2. Purification of protease enzymes extracted from Nigella sativa by acetone.

<table>
<thead>
<tr>
<th>Acetone (%)</th>
<th>Total protein (mg)</th>
<th>Recovered protein (%)</th>
<th>Total activity (U)</th>
<th>Recovered activity (%)</th>
<th>Specific activity (U/mg protein)</th>
<th>Purification fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.40±0.007</td>
<td>100.00</td>
<td>1530±3.591</td>
<td>100.00</td>
<td>3825±150.592</td>
<td>1.00</td>
</tr>
<tr>
<td>40</td>
<td>0.06±0.002</td>
<td>15</td>
<td>302±1.761</td>
<td>19.73</td>
<td>5033±299.784</td>
<td>1.31</td>
</tr>
<tr>
<td>60</td>
<td>0.06±0.003</td>
<td>15</td>
<td>380±1.190</td>
<td>24.83</td>
<td>6333±299.981</td>
<td>1.65</td>
</tr>
<tr>
<td>80</td>
<td>0.05± 0.005</td>
<td>12.5</td>
<td>505±1.931</td>
<td>33.00</td>
<td>10100±832.884</td>
<td>2.64</td>
</tr>
</tbody>
</table>

Data represent the means ± SE calculated from three replicates. *P > 0.05, **P ≤ 0.01, ***P ≤ 0.001, show comparison of respective parameters at acetone 40, 60 and 80% versus Control (0% acetone) (One-way ANOVA followed by Tukey test).
3.1.3 Purification by trichloroacetic acid

Results shown in Table 3 indicate the purification of protease enzymes extracted from *Nigella sativa* by 0.2M of TCA. The specific activity of the extracted protease was 7257 U/mg protein with 1.89 purification fold. These results are similar with Shrawan et al. [28] who found that the protease enzymes extracted with 0.1 M of phosphate buffer at pH 7 and then precipitated with 0.2 M of TCA show good recovery of enzyme activity and reported that TCA does not cause denaturation of protease enzymes.

From the previous results it can be concluded that, 80% acetone, 0.2 M of TCA and 80% ammonium sulfate were the sequence order can be used to purify protease enzymes extracted from *Nigella sativa*. Also, the purification of the extracted protease by 80% acetone was giving the higher specific activity (10100 U/mg proteins) with 2.64 purification fold compared to all another treatment. These results are in conformity with Thengam & Rajkumar, [40] and Omran, [41] who reported that 80% acetone is the best agent to purify alkaline proteases extracted from *Alcaligenes faecalis* and *Thermoactinomyces* sp because precipitation by acetone does not affect the enzymatic activity of proteases and does not cause denaturation of protease enzymes.

<table>
<thead>
<tr>
<th>TCA (M)</th>
<th>Total protein (mg)</th>
<th>Recovered protein (%)</th>
<th>Total activity (U)</th>
<th>Recovered activity (%)</th>
<th>Specific activity (U/mg protein)</th>
<th>Purification fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.40± 0.007</td>
<td>100.00</td>
<td>1530±3.591</td>
<td>100.00</td>
<td>3825±159.592</td>
<td>1.00</td>
</tr>
<tr>
<td>0.2</td>
<td>0.07*± 0.002</td>
<td>17.5</td>
<td>508*±2.081</td>
<td>33.20</td>
<td>7257*±286.109</td>
<td>1.89</td>
</tr>
</tbody>
</table>

Data represent the means ± SE calculated from three replicates. *P < 0.001, show comparison of respective parameters at TCA 0.2M versus Control (0M TCA) followed by Tukey test.

![Graph](image)

**Fig. 1.** Lineweaver- Burk plot show kinetic constants (Km and Vmax) of protease enzyme extracted from *Nigella sativa* and purified by 80% acetone.

3.2 Kinetics of Partially Purified Protease Enzymes

Enzyme kinetics and Michaelis constants (Km and Vmax) determines the effect of substrate concentration on the enzyme activity. Low Km means only a small amount of substrate is needed to saturate the enzyme, indicating a high affinity of the enzyme for a substrate [42]. Data presented in Fig. 1 show the effect of substrate (bovine serum albumin, BSA) concentration on the activity of partial purified protease enzymes extracted from *Nigella sativa*. The purified protease by 80% acetone exhibited high value of Vmax (166.66 mg/min) and the low value of Km (0.133 mg/ml) and specific activity (10100 U/mg protein).

3.3 Gluten Hydrolysis by Protease Enzyme

Data presented in Table 4 indicated that extracted protease from *Nigella sativa* and purified by 80% acetone gave the higher percentage of gluten hydrolysis (28.64%) and (32.02%) at respective enzyme concentrations of 1 and 2mg/ml. Comparatively, the percentages of hydrolysed gluten obtained by Digestin were 25.68% and 38.67% at 1 and 2 mg/ml concentrations respectively.
Table 4. Gluten hydrolysis with different protease concentration

<table>
<thead>
<tr>
<th>Protease Sources</th>
<th>Enzyme Conc.</th>
<th>Protein remaining (mg/ml)</th>
<th>Protein remaining (%)</th>
<th>Protein hydrolysed (mg/ml)</th>
<th>Protein hydrolysed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gluten (Control)</td>
<td>0 mg/ml</td>
<td>3.957±0.016</td>
<td>100</td>
<td>0.005±0.0001</td>
<td>0</td>
</tr>
<tr>
<td>Nigella sativa purified by 80% acetone</td>
<td>1 mg/ml</td>
<td>2.831±0.019</td>
<td>71.54</td>
<td>1.126±0.010</td>
<td>28.46</td>
</tr>
<tr>
<td>by 80% acetone</td>
<td>2 mg/ml</td>
<td>2.690±0.087</td>
<td>67.98</td>
<td>1.267±0.095</td>
<td>32.02</td>
</tr>
<tr>
<td>Digestin</td>
<td>1 mg/ml</td>
<td>2.941±0.029</td>
<td>74.32</td>
<td>1.016±0.034</td>
<td>25.68</td>
</tr>
<tr>
<td></td>
<td>2 mg/ml</td>
<td>2.427±0.010</td>
<td>61.33</td>
<td>1.500±0.009</td>
<td>38.67</td>
</tr>
</tbody>
</table>

Data represent the means ± SE calculated from three replicates. *P ≤ 0.001, show comparison of Nigella sativa and Digestin at different concentrations (1 and 2 mg/ml) with Gluten (Control) followed by Tukey test.

3.4 Gliadin Hydrolysis with Protease Enzymes

Protease enzymes extracted from Nigella sativa was also examined to hydrolyze gliadin which extracted from gluten. Gliadin is considered as the primary reason for celiac disease. Gliadin is a polypeptide consists of 33 amino acids with known sequence rich of proline and glutamine [43]. Proline gives the peptide increased resistance to gastrointestinal proteolysis causing risk for CD [37]. In healthy peoples, this polypeptide does not permeate into their intact intestine and excrete in an undigested form. Data presented in Tables 5 demonstrated that, the extracted protease resulted in higher percentage of gliadin hydrolysis (22.7%) at 1 mg/ml and (16.84%) at 2 mg/ml of enzyme concentration. While, the percentages of hydrolysed gliadin by Digestin were 22 and 22.5% at 1 and 2 mg/ml concentrations respectively.

Table 5. Gliadin hydrolysis with different protease concentration

<table>
<thead>
<tr>
<th>Protease sources</th>
<th>Enzyme Conc.</th>
<th>Protein remaining (mg/ml)</th>
<th>Protein remaining (%)</th>
<th>Protein hydrolysed (mg/ml)</th>
<th>Protein hydrolysed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gluten (Control)</td>
<td>0 mg/ml</td>
<td>2.97±0.014</td>
<td>100</td>
<td>0.001±0.0001</td>
<td>0</td>
</tr>
<tr>
<td>Nigella sativa purified by 80% acetone</td>
<td>1 mg/ml</td>
<td>2.296±0.123</td>
<td>77.30</td>
<td>0.478±0.123</td>
<td>22.7</td>
</tr>
<tr>
<td>80% acetone</td>
<td>2 mg/ml</td>
<td>2.470±0.010</td>
<td>83.16</td>
<td>0.506±0.010</td>
<td>16.84</td>
</tr>
<tr>
<td>Digestin</td>
<td>1 mg/ml</td>
<td>2.315±0.001</td>
<td>77.94</td>
<td>0.402±0.001</td>
<td>22.06</td>
</tr>
<tr>
<td></td>
<td>2 mg/ml</td>
<td>2.302±0.027</td>
<td>77.50</td>
<td>1.104±0.027</td>
<td>22.5</td>
</tr>
</tbody>
</table>

Data represent the means ± SE calculated from three replicates. *P ≤ 0.001, show comparison of Nigella sativa and Digestin at different concentrations (1 and 2 mg/ml) with Gliadin (Control) followed by Tukey test.

3.5 Free Amino Acids Concentration after Gluten and Gliadin Hydrolysis

The results Table 6, revealed that, the free amino acids produced from hydrolysis of gluten were 0.68% and 0.74% at enzyme concentrations of 1 and 2 mg/ml while 0.66% and 0.70% free amino acids were obtained with Digestin at 1 and 2 mg/ml respectively.

Table 6. Free amino acids concentration from hydrolysed gluten

<table>
<thead>
<tr>
<th>Protease Sources</th>
<th>Enzyme Conc.</th>
<th>Free amino acids (mg/ml)</th>
<th>Free amino acids (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gluten (Control)</td>
<td>0 mg/ml</td>
<td>0.001±0.0005</td>
<td>0</td>
</tr>
<tr>
<td>Nigella sativa purified by 80% acetone</td>
<td>1 mg/ml</td>
<td>0.0271±0.001</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>2 mg/ml</td>
<td>0.0293±0.001</td>
<td>0.74</td>
</tr>
<tr>
<td>Digestin</td>
<td>1 mg/ml</td>
<td>0.0261±0.001</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>2 mg/ml</td>
<td>0.0274±0.001</td>
<td>0.70</td>
</tr>
</tbody>
</table>

Data represent the means ± SE calculated from three replicates. *P ≤ 0.001, show comparison of Nigella sativa and Digestin at different concentrations (1 and 2 mg/ml) with Gluten (Control) followed by Tukey test.
Table 7. Free amino acid concentration in hydrolysed gliadin

<table>
<thead>
<tr>
<th>Protease sources</th>
<th>Enzyme Conc.</th>
<th>Free amino acids (mg/ml)</th>
<th>Free amino acids (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gliadin (Control)</td>
<td>0 mg/ml</td>
<td>0.002±0.0007</td>
<td>0</td>
</tr>
<tr>
<td><em>Nigella sativa</em> purified by 80% acetone</td>
<td>1 mg/ml</td>
<td>0.0251±0.001</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>2 mg/ml</td>
<td>0.0235±0.003</td>
<td>0.79</td>
</tr>
<tr>
<td>Digestin</td>
<td>1 mg/ml</td>
<td>0.0263±0.002</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>2 mg/ml</td>
<td>0.0139±0.002</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Data represent the means ± SE calculated from three replicates. "P ≤ 0.01, "P ≤ 0.001, show comparison of *Nigella sativa* and Digestin at different concentrations (1 and 2 mg/ml) with Gliadin (Control) followed by Tukey test.

Data presented in Table 7 illustrated that the extracted protease enzymes from *N. sativa* give higher concentration of free amino acids from hydrolysed gliadin (0.84%) at 1 mg/ml as compared to 2 mg/ml (0.79%). On the other hands, the free amino acids concentrations from hydrolysed gliadin were 0.88% and 0.46% by using Digestin at 1 and 2 mg/ml, respectively.

4. CONCLUSION

This study show that the extracted and purified proteases from *Nigella sativa* exhibited high efficacy to hydrolyse gluten and gliadin, and thus can be developed as potential candidate to treat celiac disease.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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