Storage stability of minced beef supplemented with chickpea legumin at 4 °C as a potential substitute for nisin

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\textbf{ARTICLE INFO}

Keywords: Minced beef Legumin Nisin Antimicrobial Storage Oxidation inhibition

\textbf{ABSTRACT}

Chickpea legumin has a high molecular weight (300 KDa), consists of 5 subunits (25–45 KDa) and has a relatively high isoelectric point (pH 6.5). It could inhibit the growth of four bacteria strains (\textit{Staph. aureus}, \textit{P. aeruginosa}, \textit{E. coli} and \textit{B. subtilis}), grown in agar (MIC = 100–150 g/ml) and bring the liquid bacterial growth to their minimum levels after 24 h of incubation using 1 MIC. Transmission electron microscopy micrographs of \textit{Staph. aureus} (Gram positive) and \textit{P. aeruginosa} (Gram negative) treated by 150 μg/ml chickpea legumin (1 MIC) showed cellular swelling and cell membrane disintegration explaining the mode of action of this protein. Minced beef samples supplemented with 200 μg/g chickpea legumin extended the storage time to 14–15 days instead of 7–8 days in case of un-supplemented control and 10–11 days in case of nisin, reducing the total viable count by 2.6–2.9 log compared to control against a reduction of 1.6 log in case of nisin.

\section{1. Introduction}

Using natural antimicrobials in food preservation is gradually becoming more spread (Zohri \textit{et al.}, 2013). Nisin, which is a bacteriocin obtained from \textit{Lactococcus lactis} subsp. lactis (Hurst, 1967) is a good example of such substances that has been extensively used as a food preservative since the mid of that century (Khan & Oh, 2016).

Nisin is a small amphiphilic cationic peptide (3510 Da) having an isoelectric point above pH 8.5. It is an effective antimicrobial against a broad spectrum of Gram-positive bacteria and has been shown to adsorb to bacterial surfaces leading to their inhibition and finally to their death (Bower, McGuire, & Daeschel, 1995), through a multi-step process that destabilizes the phospholipid bilayer of the cell, creating transient pores (Tai, McGuire, & Neff, 2008).

Although it is an effective antimicrobial against a broad spectrum of Gram-positive bacteria, its activity may be deteriorated or lost in foods or medical preparations due to possible interactions with other food components or enzymatic degradation (Aasen \textit{et al.}, 2003; Krivorotova \textit{et al.}, 2016). Some interventions (e.g. encapsulation) were proposed to counteract this phenomenon (Xiao, Davidson, & Zhong, 2011).

The wide use of nisin in food preservation is based on its safety, i.e. preventing contamination without toxicity, it has become now a well-established food preservative (De Arauz, Jozala, Mazzola, & Penna, 2009). No toxicologically significant changes were apparent in both sexes of rats fed diet containing 1–5.0% nisin A for 90 days. Therefore, the no-observed-adverse-effect level (NOAEL) for nisin A was concluded to be a dietary level of 5.0% (Hagiwara \textit{et al.}, 2010).

However, a persister population of \textit{L. monocytogenes} has been recently reported as being able to survive at high concentration of nisin, probably related to less negative bacterial cell surface charges (Wu, Yu, & Flint, 2017). This discovery necessitates the search for new natural antimicrobials that may excel the action of nisin and thus possibly replace it.

Alternatively, food spoilage and poisoning, particularly by oxidative processes or by microorganism activity during production and storage are still the major concerns for both the food industry and consumers (Viuda-Martos \textit{et al.}, 2011). Ground beef is a perishable product that provides a favorable medium for the growth of both spoilage and food-borne microorganisms. Since synthetic preservatives in foods are regarded as ‘chemical’ additives and ‘unnatural’ and are rejected by many consumers, a new interest is growing in new and effective techniques to reduce cases of food-borne illnesses.

Natural alternatives of preservatives can be found in different plant sources, e.g.; plant basic proteins, spices and herbs (Arora & Kaur, 1999; Mahgoub, Osman, & Sitoxy, 2011; Mahgoub, Sitoxy, & Osman, 2013; Osman, Mahgoub, & Sitoxy, 2013, 2014; Sitoxy, Mahgoub, & Osman, 2011, 2012; Sitoxy, Mahgoub, Osman, El-Masy, & Al-Gaby, 2013). Soy bean protein isolate, is a common example of legume proteins, whose major components are glycinin (11S globulin) and β-conglycinin (7S globulin), which represent 34% and 27% of the whole protein, respectively.
respectively (Iwabuchi & Yamauchi, 1987). Chickpea globulins, mainly consist of legumin (11S globulin) and vicilin (7S globulin) (Deep Singh, Wani, Kaur, & Sogi, 2008). The similarity between soy 11S and chickpea 11S may be reflected on some antimicrobial activity of the latter one. Based on this hypothesis, the aim of the current work was to study the antibacterial activity of legumin from chickpea (11S) in vitro against pathogenic bacteria and in situ in minced beef meat under refrigeration conditions to test its efficiency as a substitute of nisin.

2. Materials and methods

2.1. Plant material

Chickpea (Cicer arietinum L.) seeds were purchased from the local market, Zagazig, Sharkia governorate, Egypt.

2.2. Meat samples

The fresh raw beef (21% Protein, 15% Fat), purchased from local market in Zagazig city, Egypt was finely minced in sanitized meat mincers and transferred to sterilized polyethylene sachets.

2.3. Microorganisms

Gram positive bacteria (Staphylococcus aureus and Bacillus subtilis) and Gram negative bacteria (Pseudomonas aeruginosa and Escherichia coli), were kindly obtained from the Laboratory of Microbiology, Department of Microbiology, Faculty of Science, Zagazig University, Egypt.

2.4. Legumin extraction from chickpea

Chickpea seeds were manually cleaned and ground using a Moulinex mixer (Type 716, France) at a maximum speed to pass through a 1 mm sieve. The powder was then defatted using chloroform: methanol (3:1 v/v) for 8 h. Solvent was evaporated and the dried-defatted meal was used to separate legumin (11S globulin) according to Liu et al., 2007.

2.5. Legumin characterisation

2.5.1. Native PAGE

Five milligrams of chickpea legumin were dissolved in a buffer (pH 6.8) containing 0.25M Tris base and 50% glycerol and analyzed by native PAGE according to Laemmli, 1970 in 3 and 12% acrylamide for the stacking and resolving gels, respectively.

2.5.2. SDS-PAGE

An amount of chickpea legumin (20 mg) was dispersed in 1 ml SDS 10% with 100 μl β-mercaptoethanol for 15 min with vortexing every 5 min. The extract was centrifuged at 11,000 g for 10 min. A mixture of 20 μl extract and 20 μl of SDS-loading sample buffer (SDS 4%, β-mercaptoethanol 3%, glycerol 20%, Tris HCl 50 mM pH 6.8 and bromophenol blue traces), was heated at 96 °C for 3 min and 10 μl aliquot (per lane) was electrophoresed by SDS-PAGE according to Laemmli, 1970.

2.5.3. Urea-PAGE

Chickpea legumin was dissolved (5 mg/ml) in a pH 6.8 buffer containing 0.25 M Tris-base, 50% glycerol and bromophenol blue traces and centrifuged at 15,800 g for 5 min at 20 °C. Supernatants were analyzed by Urea-PAGE (10 μl of protein/lane) in 3% and 10% stacking and resolving gels, respectively, according to Evans & Williams, 1980.

2.6. Antibacterial activity

2.6.1. Agar well-diffusion assay

Chickpea legumin was tested for the antimicrobial activity against Gram positive bacteria (Staph. aureus and B. subtilis) and Gram negative bacteria (P. aeruginosa and E. coli) by the conventional well-diffusion assay (Nanda & Saravanan, 2009). The pure cultures of bacterial strains were sub-cultured in Mueller Hinton broth (MHB) and incubated on a rotary shaker at 200 rpm at 37 °C (Staph. aureus, P. aeruginosa and E. coli) or 28 °C (B. subtilis) for 24 h. An aliquot (0.1 ml) of the last culture was transferred into 10 ml MHB and incubated at 37 °C (Staph. aureus, P. aeruginosa and E. coli) or 28 °C (B. subtilis) for 24 h, to reach a count of 1.05 × 10^8 CFU/ml. Each strain was uniformly spread onto Mueller Hinton Agar (MHA) plates (6-mm diameter) were made using gel puncture. Aliquots (40 μl) of legumin (50, 100, 150, 200 and 250 μg/ml) were transferred into each well. Negative control (sterilized distilled water) was similarly carried out. After incubation at 37 °C (Staph. aureus, P. aeruginosa and E. coli) or 28 °C (B. subtilis) for 24 h, the diameters (mm) of inhibition zones were measured giving the minimum inhibitory concentration (MIC).

2.6.2. Bacterial growth curve

Legumin (at its MIC values) was added to 10 ml brain heart infusion broth media containing 100 μg/ml of Gram positive or Gram negative bacteria (10^8 CFU/ml) and examined for their growth as compared to control (without adding any substance). All treatments were incubated at 37 °C for different time periods (0, 6, 12, 18 and 24 h) before measuring the turbidity (A600) according to Murray et al., 1995.

2.6.3. Transmission electron microscopy examination

Each bacterial type (Staph. aureus and P. aeruginosa) was grown in tryptone soy broth supplemented with legumin (at 1MIC = 150 μg/ml) and incubated at the optimum temperature for 24 h before examining with TEM (Transmission electron microscopy) as described by (Sitohy et al., 2013).

2.7. Storage of minced beef supplemented with legumin

Minced beef samples (100 g) were placed in stomacher bags and homogenized in a stomacher for 2 min at room temperature. Following homogenization, legumin (100, 150 and 200 μg/g) was added to the samples, except the negative control. The positive control included nisin (200 μg/g). Nisin (2.5%) from Lactococcus lactis was purchased from Santa Cruz Biotechnology, Inc. 2145 Delaware Avenue, Santa Cruz, California 95,060 U.S.A. All stomacher bags with samples from all treatments were wrapped and stored under aerobic conditions at 4 °C for 15 days. Antioxidant and microbiological analysis of samples were carried out at different intervals (0–15 days).

2.7.1. Physicochemical analysis of meat sample

Analyses of moisture, protein, and lipids content of minced beef samples were carried out according to Manzi, Marconi, Aguzzi, & Pizzoferrato, 2004.

2.7.2. Lipid peroxidation assay

Lipid peroxidation was measured in the minced beef supplemented with legumin (100, 150 and 200 μg/g) according to Niehaus & Samuelsson, 1968; Qvleke et al., 2013 after different storage intervals (0–15 days) at 4 °C. Five gram of each meat sample was homogenized. A volume of 10% w/v homogenate was prepared in 0.05 Mol/L phosphate buffer (pH 7) and centrifuged at 12,000 × g for 60 min at 4 °C. An aliquot (100 μl) of the supernatant was reacted with 2000 μl of (1:1:1 ratio) TBA–TCA–HCl reagent (thiobarbituric acid 0.37%, 15% trichloroacetic acid and 0.25 N HCl) and placed in a boiling water bath for 30 min before allowing to cool. The optical density of the formed color was assessed at 535 nm using JENWAY 6405 UV/visible.
spectrophotometer (UK) against a reagent blank. Percentage inhibition was calculated using the equation:

\[
\text{Lipid oxidation inhibition (\%)} = \left[1 - \frac{\text{absorbance of sample}}{\text{absorbance of control}}\right] \times 100
\]

2.7.3. Microbial analysis

Microbial analysis of minced beef supplemented with legumin (100, 150 and 200 μg/g) was assessed as compared to nisin (200 μg/g) after different intervals of preservation (0–15 days) at 4 °C following the procedures outlined in (Association, 1992). The samples (10 g) were transferred aseptically to a stomacher bag containing 90 ml of peptone saline diluent (1.0 g peptone and 8.5 g sodium chloride in 1 L of
Table 1  
Antibacterial activity of chickpea legumin at different concentrations (50–250 µg/ml) against Gram+ (Staph. aureus and B. subtilis) and Gram− (P. aeruginosa and E. coli) bacteria using agar well diffusion assays.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Inhibition zone diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 µg/ml</td>
</tr>
<tr>
<td><strong>Gram positive bacteria</strong></td>
<td></td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>34 ± 0.3 [38%]</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>24 ± 0.21 [27%]</td>
</tr>
<tr>
<td><strong>Gram negative bacteria</strong></td>
<td></td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>28 ± 0.2 [31%]</td>
</tr>
<tr>
<td>E. coli</td>
<td>16 ± 0.11 [18%]</td>
</tr>
</tbody>
</table>

The values between brackets are the relative inhibition zone as related to the total surface area of Petri dish.

3. Results and discussion

3.1. Chemical characterization

The Native-PAGE of chickpea legumin (11S globulin) indicates one major band with a molecular weight approximately 300 kDa, confirming its chemical identity (Fig. 1). This may partially agree with the results of Sitohy et al., 2013 showing two major subunits; 11S globulin (360 kDa) and 7S globulin (200 kDa) of that protein. Likewise, native-PAGE of soybean protein exhibited two major subunits (molecular weights of 350 and 180 kDa) corresponding to 11S globulin and 7S globulin, respectively (Sathe, 1991).

SDS-PAGE of chickpea legumin (Fig. 1) separated this high molecular weight band into 5 bands corresponding to smaller molecular weights (25–45 kDa) in accordance with (Sánchez-Vioque, Clemente, Vioque, Bautista, & Millán, 1999) reporting molecular weight range of 24.5–46.5 kDa. It also agrees with Amaral, Ferreira, Neves, & Demonte, 2014 who reported a molecular weight range 18–41 kDa for these subunits. The variations in the minimal molecular weight may be due to variant effect.

Urea-PAGE chickpea (Fig. 1) indicated similar anode to cathode migration for chickpea legumin (lane 4) and soy glycinin (lane 2), evidently faster than that of the corresponding chickpea protein isolate (lane 3) or soy protein isolate (lane 1) referring to similar high positive charge and basicity of chickpea legumin and soy glycinin. The migration of a mixture of chickpea legumin and soybean glycinin produced a band in the same location of each component (lane 5), confirming this similarity which also produced nearly two similar pH-solubility curves with two close isoelectric points of chickpea legumin and soybean glycinin; 6.5 and 6.4, respectively. So, it is expected that the functional properties of chickpea legumin may be similar to that of soybean glycinin.

3.2. Antibacterial activity of chickpea legumin

3.2.1. In-vitro antibacterial activity

The data in Table 1 show the antibacterial activity using agar well diffusion assay. The four investigated bacteria (2 Gram-positive and 2 Gram-negative) showed nearly similar susceptibility to chickpea legumin without clear distinction between the two sections of bacteria. The maximum susceptibility of Gram-positive bacteria was recorded for Staph. aureus and the Gram-negative bacteria of P. aeruginosa; 57 and 51% inhibition, respectively. Minimum inhibition (29%) was observed with the other two bacteria (B. subtilis and E. coli). This nearly equal range of bacterial inhibition shows equal potency of chickpea protein against both Gram-positive and Gram-negative bacteria and broad specificity. In the same token, the values of MIC were in the range of 100–150 µg/ml without distinction between the two bacterial sections.

The data in Fig. 2 represent the growth curves of 4 bacterial strains (2 Gram-positive and 2 Gram-negative) in the presence of chickpea legumin and nisin (1 MIC each) during 24 h incubation at 37 °C (in case of Staph. aureus, P. aeruginosa and E. coli) or 28 °C (B. subtilis). It is observed that the growth curves of the four studied bacterial reached their minimum levels after 24 h of incubation at their optimal temperatures. However, limited growth of the four bacteria was observed after 6–12 h of incubation (~17-12%), respectively, representing the number of bacteria which could escape the action of the chickpea legumin. This antibacterial action is evidently strong since the negative control showed maximum levels of bacterial growth after 24 h of incubation at the optimum temperatures, while the viable bacterial cells in the treated samples was around only 7% in case of Staph. aureus and P. aeruginosa and 10–12% in case of B. subtilis and E. coli respectively.

3.2.2. Electron microscope micrographs

Transmission electron microscopy (TEM) micrographs of Staph. aureus (Gram positive) and P. aeruginosa (Gram-negative), treated with...
150 μg/ml chickpea legumin and compared to control are shown in Fig. 3. The general overview at the lower magnification extent (20,000×) shows the swelling of the bacteria treated with chickpea legumin (1 MIC), without distinction between the Gram-negative or Gram-positive bacteria, referring to the status of most bacterial cells after absorbing much liquid from the media. Using the high magnification power (80,000×) and focalizing one bacterial cell, revealed the disintegration of the bacterial cell membrane and the disappearance of its clear boundaries, probably indicating the first acting step in the antibacterial process. This action of chickpea legumin (11S) agrees with the action of soybean glycinin (11S), previously reported by Sitohy et al., 2011. This similarity of action between these two fractions is evidently due to similar chemical composition and functionality as referred to in the previous sections. It is also similar to the action reported for nisin (Solomakos, Gvaris, Koidis, & Botsoglou, 2008). So this result clarifies the mechanism of action legumin supporting its nomination to be used as a substitute of nisin in some food preservative applications.
may render this material much susceptible to the bacterial contamination.

3.3.2. Microbiological assay of minced beef during storage at 4 °C

Total viable count, psychrotrophic bacterial count and coliform bacterial counts were estimated in minced beef during storage for 15 days at 4 °C either in its un-supplemented case or after supplementation with nisin at different levels (100, 150 and 200 μg/g) as compared to nisin (200 μg/g) (Fig. 4). Generally, it is observed that the three bacterial counts increased with the time of storage in all samples. However, reducing effects on these counts can be observed by legumin and nisin supplementation. This reducing action was always greater in case of legumin than nisin alongside the whole period of storage (15 days at 4 °C) and was most conspicuous in case of total viable bacteria. It was also concentration-dependent at all the time points of storage (3–15 days).

The negative control reached a level of 5.0 and 6.5 logs after 6 and 9 days of storage at 4 °C, indicating that the secured time period of minced beef storage may be between 7 and 8 days, while the samples supplemented with 200 μg/g chickpea legumin maintained the level of the total viable count at 5.4 and 6.3 logs after 12 and 15 days, i.e. increasing the secured time of storage to about 14–15 days. The samples supplemented with nisin kept the viable total count at 5.1 and 6.7 logs after 9 and 12 days of storage at 4 °C, respectively, indicating that the secured time of storage to be about 10–11 days of storage. In conclusion, supplementation with chickpea legumin can increase the safe storage period of minced beef by about 7 days compared to control and by about 4 days compared to nisin. The higher preserving action of legumin over nisin is probably due to its higher antibacterial action; reducing the total bacterial count by about 2.6–2.9 compared to only 1.6 log in case of the latter. In conclusion, chickpea legumin can be effectively used as a good natural preservative and better substitute of nisin.

Globally, this result may excel other approaches of using natural compounds in the preservation of minced beef. The recommended maximal level of the studied material (200 μg/ml) is much lower than the recommended level of nisin (normally > 500 μg/ml) or other natural product, e.g. essential oils which is about 0.6% (6000 μg/ml) (Solomakos et al., 2008). Using a high level of the natural preservative agent may pose some interfering effects on the food components including the organoleptic properties apart from the additional economic costs.

3.3.3. Lipid oxidation and protein degradation the stored minced beef

Lipid oxidation inhibition (%) in minced beef as supplemented with chickpea legumin at different concentrations (100, 150 and 200 μg/g) was determined at different periods (0–15 day) of storage at 4 °C and compared to nisin (200 μg/g) and negative control (NC). Both chickpea legumin and nisin could inhibit this process during the whole period of storage. The inhibiting action of chickpea legumin was concentration dependent (Fig. 5). The data manifested increasing lipid oxidation in the negative control samples with the time of storage reaching a maximum of 92% after 15 days of storage at 4 °C. The highest legumin concentrations (150–200 μg/g), could keep the level of maximum oxidation down to 78–79% against 80% in case of nisin (200 μg/g). This higher antioxidant effect of chickpea legumin over nisin may be due to its greater antibacterial action or to its direct antioxidant action in accordance with Osman et al., 2014.

The SDS-PAGE of the control minced beef samples (Fig. 5 B) shows considerable protein degradation particularly of the bands corresponding to myosin heavy chain (MHC), with storage time. Supplementation with chickpea legumin could effectively counteract this process in a concentration-dependant manner. The protecting effect of Chickpea legumin seems more evident than that of nisin (200 μg/g) even at lower concentrations (100–150 μg/g). This protecting effect on MHC applies also to lesser extents to other protein components, i.e.;
paramyosin (PM) and actin. In conclusion, it can be stated that chickpea legumin could protect meat proteins from the degradation incurred by the storage conditions surpassing the action of nisin.

3.3.4. The pH and metmyoglobin

The data in Table 2 indicate that the control minced samples (non-supplemented) revealed considerable decreases in the pH with storage, while the samples supplemented with either nisin or chickpea legumin could remarkably limit this change. This may be evidently attributed to the inhibited microbial growth and the associated reduced hydrolysis of both lipids and proteins.

The transformation of myoglobin from the reduced form (oxymyoglobin) into the oxidized form (metmyoglobin) presented in Table 2, shows an increasing level of metmyoglobin in the control samples reaching 6 times the level at zero time after 15 days of cold storage, referring to an accelerated oxidation process. Supplementing the minced beef with chickpea legumin decelerated this process in a concentration dependent manner, at the same time point, the level of metmyoglobin in the samples supplemented with chickpea legumin at 100, 150 and 200 μg/g was only 4.5, 4.0, 2.7 times of the zero time level, respectively. Nisin (200 μg/g) could maintain metmyoglobin level at 4 times the level of zero time against 2.7 in case of legumin (200 μg/g) at after 15 days of cold storage.

Table 2

<table>
<thead>
<tr>
<th>Storage time (day)</th>
<th>Control (μg/g)</th>
<th>Legumin (μg/g)</th>
<th>Nisin (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0</td>
<td>100</td>
<td>150</td>
</tr>
<tr>
<td>Metmyoglobin %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>11.2</td>
<td>11.2</td>
<td>11.2</td>
</tr>
<tr>
<td>3</td>
<td>33.6</td>
<td>26.3</td>
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<td>15</td>
<td>67.8</td>
<td>50.6</td>
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<td>3</td>
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<tr>
<td>12</td>
<td>6.74</td>
<td>6.16</td>
<td>6.11</td>
</tr>
<tr>
<td>15</td>
<td>6.91</td>
<td>6.18</td>
<td>6.18</td>
</tr>
</tbody>
</table>

3.3.5. Sensorial properties of the stored minced beef

It is observed in Fig. 6 that all sensorial properties have deteriorated with the advance of the storage time. Color has been particularly deteriorated in the negative control samples with storage to reach its minimum after 15 days, representing only about 14% of the zero point value. Supplementing the minced beef with chickpea legumin has slowed down this deteriorating process in a concentration-dependent manner. The highest concentration (200 μg/g) could keep this color trait at about 57% of the zero time level, i.e. better than nisin (200 μg/g) which kept this trait at about 29% after 15 days of cold storage. Color remained totally unchanged (100% color keeping) in the samples treated with 200 μg/g chickpea legumin after 6 days of cold storage against only 71% color keeping in case of samples supplemented with the same concentration of nisin.

The odor keeping quality deteriorated in all samples with the time of storage, especially in the negative control samples. Supplementation with chickpea legumin lessened the rate of deterioration in a concentration-dependent manner. Samples supplement with the high concentration of legumin (200 μg/g) maintained most of the odor keeping quality (92%) after 6 days of cold storage against only 77% in case of the nisin (200 μg/g). At the end of storage (15 days), the first samples maintained 46% of the odor quality against 31% in the latter samples confirming the privilege of supplementation with legumin over nisin in maintaining the odor quality. The appearance and overall acceptability of the stored minced beef followed the same trend in response to supplementation with chickpea legumin and nisin.

4. Conclusions

Chickpea legumin (11S globulin) is characterized by a high molecular weight (300 KDa) and 5 subunits (25–45 KDa) and a rather alkaline nature with a relatively high isoelectric point (pH 6.5), qualifying for antibacterial activity. It has been proved active against four bacteria strains (Staph. aureus, P. aeruginosa and E. coli and B. subtilis) without distinction between Gram positive and Gram negative bacteria. It antibacterial activity, which is higher than nisin, occurs mainly occurring through its effect on the bacterial cell membranes, leading to cellular swelling and cell membrane disintegration. Supplementing the minced beef with chickpea legumin can extend its secured storage life span to be about 14–15 days instead of 7–8 days in case of negative control (un-supplemented samples) or 10–11 in case of nisin, reducing the total viable count by 2.6–2.9 log compared to the negative control against only 1.6 log reduction in case of nisin. So, this product can be efficiently used in minced beef preservation instead of nisin which is facing also the problem of nisin-resistant bacteria.
Fig. 6. Sensory quality of minced beef during 15 days cold storage (0, 3, 6, 9, 12 and 15 days) as supplemented with chickpea legumin (Leg) at different concentrations (100, 150 & 200μg/g) and compared to negative control (without supplementation) or positive control (supplemented with 200μg/g nisin).

Conflicts of interest

The authors declare the absence of conflict of interest between any of the author with any external party regarding the subject or results of this research.

Acknowledgements

This research was funded by Zagazig University and Cairo University.

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