The effects of *Lycium barbarum* water extract and fish collagen on milk proteolysis and *in vitro* angiotensin I-converting enzyme inhibitory activity of yogurt

Amal Bakr Shori 1*  
Kong Siew Ming 2  
Ahmad Salihin Baba 2

1 King Abdulaziz University, Faculty of Science, Department of Biological Sciences, Jeddah, Saudi Arabia  
2 Biomolecular Research Group, Division of Biochemistry, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia

Abstract

Plain and *Lycium barbarum* yogurt were made in the presence and absence of fish collagen. Yogurt samples were analyzed for acidification, milk protein proteolysis, angiotensin I-converting enzyme (ACE) inhibitory activity, and sensory evaluation during refrigerated storage for up to 21 days. The o-phthaldialdehyde peptides amount of *L. barbarum* yogurt both in the presence and absence of fish collagen were significantly increased during 14 days of storage. SDS-PAGE showed improvement in whey proteins degradation of *L. barbarum* yogurt with/without fish collagen after 3 weeks of storage. *L. barbarum* yogurt in absence of fish collagen was acting as a great ACE inhibitor reached up to 85% on day 7 of storage. The incorporation of *L. barbarum* and/or fish collagen affected to a small extent the overall sensory characteristics of yogurt. Yogurt supplemented with *L. barbarum* and/or fish collagen may lead to the improvement in the production and formulation of yogurt differing in their anti-ACE activity.

Keywords: yogurt, *Lycium barbarum*, proteolysis, fish collagen, ACE inhibitory activity

1. Introduction

Hypertension is estimated to affect one-third of the western population [1]. It is a risk factor for cardiovascular disease and stroke. In view of the role of the diet in the prevention and treatment of the disease, efforts are being made to produce foods with antihypertensive activity. Despite the higher doses needed in comparison with antihypertensive drugs, the consumption of food products containing antihypertensive peptides has shown to significantly reduce the blood pressure of moderately hypertensive subjects [2]. Therefore, functional foods such as fermented dairy products with blood pressure-lowering properties have recently received considerable attention.

Fermented dairy foods such as yogurt have long been considered safe and nutritious. Yogurt has the property of containing live microorganisms such as those belonging to genus *Bifidobacterium*, *Lactobacillus*, and *Streptococcus* featuring sensory and nutritional qualities [3]. These products with probiotic bacteria when consumed in certain quantities, confer beneficial effects on health [4,5].

In the fermented dairy products, milk proteins serve as an important source of a range of bioactive peptides encrypted within the sequence of the native proteins and can thus be released by proteolysis [6]. From the bioactive components detected in dairy products, inhibitors of angiotensin I-converting enzyme (ACE), which has a central role in the regulation of blood pressure in mammals, have been studied extensively because of their potential use in the treatment of elevated blood pressure [7].

ACE is the enzyme associated with the renin-angiotensin system, which regulates the peripheral blood pressure [7]. The
enzyme can increase blood pressure by converting angiotensin I to the potent vasoconstrictor, angiotensin II. ACE can also catalyze the degradation of bradykinin, a vasodilatory peptide. Inhibition of ACE may therefore exert an antihypertensive effect through a decrease of angiotensin II and an increase of bradykinin [1,7].

*Lycium barbarum* is the taxonomic name for Chinese wolfberry, or more commonly known as goji or goji berry [8]. *L. barbarum* is regarded as a superfood as it is high nutrient dense and it has been used for healthy food products such as medicinal beverages and dietary soups [9]. It mainly contains betacarotene, beta-sitosterol, vitamins B1, B2, and C, linoleic acid, sesquiterpenoids (i.e., cyperone and solavetivone), tetraterpenoids (i.e., zeaxanthin and physalin), immunologically active polysaccharides, and betaine [8]. There are 18 amino acids in *L. barbarum*, eight of which are essential amino acids. Among the 18 amino acids, L-leucine is present in higher concentrations [8,10]. In addition, *L. barbarum* fruits are rich with phytochemical compounds that showed an important role in preventing and treating diseases such as helping strengthen immune system, inhibiting tumor cells, protective effects onin preventing and treating diseases such as helping strengthen immune system, inhibiting tumor cells, protective effects on

2. Materials and Methods

2.1. *L. barbarum* preparation and storage

*L. barbarum* was purchased from a local Chinese medicinal shop. *L. barbarum* was first washed with running distilled water. Then, the fruit left to dry to constant weight in an oven (Memmert) at 45 °C. Dried fruit was then grounded into powder form. However, *L. barbarum* is unable to form powder because of its sticky content (like raisins). Last, the grounded fruit was placed in an airtight container and then stored in the refrigerator for future used.

2.2. Water extraction of *L. barbarum*

Dried grounded *L. barbarum* (10 g) was homogenized with 100 mL sterile distilled water (1:10; with a concentration of 0.1 g/mL) by using a homogenizer. The mixture was left incubated overnight in a water bath (70 °C) to extract bioactive compounds [19]. The *L. barbarum* solution was then centrifuged (15 Min, 1,000g, and 4 °C; Eppendorf, Germany; 5804 R) and the supernatant obtained (~90%) was used as *L. barbarum* water extract in the experiment.

2.3. Preparation of yogurt samples

Yogurt was prepared as described by Shori and Baba [20] with some modifications. (The starter culture used in this study contained no *Lactobacillus rhamnosus*, *Bifidobacterium infantis*, and *Bifidobacterium longum*.) Four types of yogurt were made: plain yogurt (control), *L. barbarum* yogurt, plain yogurt + FC (control), and *L. barbarum* yogurt + FC. Figure 1 shows the process scheme of preparing 100 g of *L. barbarum* and plain yogurt with/without fish collagen. Pasteurized full cream milk (90 mL; Dutch Lady’s® with lactose content = 4.42 g/100 mL; total solids = 12.51%; moisture content = 87.49%; ash content = 0.51%; and fat content = 3.40%) was mixed with 10 mL of *L. barbarum* water extract or sterile distilled water for *L. barbarum* and plain yogurt, respectively. For fish collagen yogurt, 2.5 g fish collagen was added into the milk. One milliliters (1%) of the starter culture mix consisting of *Lactobacillus acidophilus* LA-5, *Bifidobacterium bifidum* Bb-12, *Lactobacillus casei* LC-01, *Streptococcus thermophilus* Th-4, and *Lactobacillus bulgaricus* in the ratio of 4:4:1:1:1 (Chris-Hansen, Denmark). All samples were incubated at 41 °C until the pH was reduced to 4.5 after 4 H. The pH was measured every hour

### Highlights

- OPA peptides were increased in LB-yogurt both in the presence and absence of FC.
- SDS-PAGE improved whey proteins degradation of LB yogurt with/without FC.
- LB yogurt in absence of FC had a great ACE inhibitor.
- FC enhanced free amino acids production in both plain- and LB yogurt.
FIG. 1  The process scheme of preparing 100 g of L. barbarum (LB) and plain (P) yogurt (Y) in the presence and the absence of fish collage (FC).

by using a pH meter. Yogurt samples were stored at 4 °C in the refrigerator.

2.4. The pH and total titratable acidity
The pH and total titratable acidity (TTA) of plain and L. barbarum yogurt both in the presence and absence of fish collagen were determined as described by Shori [5] at 0, 7, and 21 days of storage at 4 °C.

2.5. Preparation of yogurt water extract
Yogurt sample (5 g) was homogenized with 1.25 mL of sterile distilled water [19]. The pH was acidified to 4.0 with HCl using a pH meter (Mettler Toledo 320) before heated in a water bath (45 °C; Haake Model SWD 20) for 10 Min. The precipitated protein was spun down (4,000 g, at 4 °C; Eppendorf, Germany; 5804 R) for 20 Min to ensure a clear water extract. NaOH was added to adjust the pH of supernatant to pH 7.0. The supernatant was stored at −20 °C.

2.6. Proteolysis analysis of milk proteins
2.6.1. O-phthaldialdehyde assay
O-Phthaldialdehyde (OPA) peptides concentration of yogurt samples were analyzed during 0, 7, and 14 days of storage [21]. Yogurt extract (30 μL) was added directly into 1 mL OPA reagent (25 mL of 1,000 mM sodium tetraborate, 2.5 mL of 20% (w/w) SDS, 40 mg OPA dissolved in 1 mL of methanol and 100 μL of β-mercaptoethanol top up with dH2O until final volume is 50 mL) in a 1.5 mL quartz cuvette. The solution was mixed briefly by inversion and left to stand at room temperature for 2 Min. The absorbance was then determined at 340 nm and the OPA peptides concentration was estimated against the tryptone standard curve [21].

2.6.2. Cd-ninhydrin method
Free amino acids of yogurt samples were analyzed during 0, 7, and 21 days of storage [22]. A 10 g yogurt was mixed with 40 mL distilled water and incubated at 40 °C for 1 H before centrifuged at 6,000 rpm for 30 Min. Water-soluble nitrogen extract of yogurt without fish collagen (200 μL) and with fish collagen (50 μL; since fish collagen contains a high concentration of amino acids) was diluted to 1 mL with distilled water [19]. The samples were mixed with 2 mL of Cd-ninhydrin reagent (0.8 g ninhydrin was dissolved in the mixture of 80 mL 99.5% ethanol and 10 mL acetic acid, followed by the addition of 1 g CdCl2 dissolved in 1 mL of distilled water). The mixture was heated at 84 °C for 5 Min and subsequently cooled to room temperature. Absorbance was read at 507 nm and the absorbance values were converted to total free amino acids against leucine standard curve. Leucine (2 mM) was serially diluted with distilled water to obtain the concentrations of 0.01, 0.02, 0.04, 0.08, 0.1, 0.3, 0.5, and 1 mM.

2.6.3. SDS-PAGE
2.6.3.1. Yogurt caseins extraction—Yogurt (10 g) was added to 20 mL of 1 M ammonium-acetate buffer pH 4.3 [23]. The mixture was then homogenized for 1 Min using a homogenizer. Homogenate was held at 4 °C for 20 Min and was then centrifuged at 6,000 rpm for 15 Min. The supernatant was discarded to remove fat and the pellet (casein) was resuspended in 25 mL of ammonium-acetate 1 mM pH 4.3. The mixture was then centrifuged at 6,000 rpm for 15 Min at 4 °C. This procedure of adding 25 mL ammonium-acetate 1 mM and centrifugation was repeated twice. The casein (pellet) was then washed with acetone to remove the fat and the protein was allowed to precipitate. The upper layer of acetone was removed using pipettes and the casein was left to dry at room temperature. The dried casein was then ground into a powder and was kept in dry condition in the fridge (8 °C) until required for further analysis.

2.6.3.2. SDS-PAGE analyze—SDS-PAGE of yogurt samples were analyzed during 0, 7, and 21 days of storage [23]. Casein powder (50 mg) from each yogurt was suspended in a mixture of 1 mL EDTA–Tris pH 8.00, 350 μL SDS 10% and 50 μL β-mercaptoethanol, then mixed for 30 Sec and was placed into the boiling water for 5 Min. Twenty-five microliters aliquot of mixture was mixed with 200 μL sample buffer (0.20 g of SDS, 1 mL of glycerol, 1.25 mL of 0.5M Tris–HCl pH 6.8, and 0.5 mL of β-mercaptoethanol in a total volume of 10ml distilled water, containing a 0.01% bromophenol blue which
store at room temperature). Aliquot of 6.5 µL was taken out and loaded into the wells. Detected protein bands were identified using the standard markers of α-casein, β-casein, κ-casein, α-lactalbumin, β-lactoglobulin, and bovine serum albumin (BSA).

2.7. ACE inhibitory activity
2.7.1. Preparation of ACE reagent
The ACE reagent was prepared as follows: sodium chloride (NaCl; 2.34 g) was dissolved in approximately 80 mL of sterile distilled water and the volume was made up to 100 mL in a volumetric flask [6]. Tris solution was prepared by mixing 0.607 g of Tris in 50 mL of sterile distilled water and the pH was adjusted to 8.3. The volume was made up to 100 mL. Both solutions (NaCl and Tris) were mixed thoroughly and Furanacryloyl-Phe-Gly-Gly (FAPGG; 25 mg) was mixed in the 62.6 mL of the solution. The dissolved FAPGG (ACE reagent) was aliquoted into 500 µL Eppendorf tubes. The aliquots were stored at −20 °C until required for analysis.

2.7.2. Anti-ACE activities of yogurt water extract
Yogurt water extract (300 µL) was mixed with ACE reagent (500 µL) in a cuvette [6]. The mixture was incubated in a water bath (37 °C) for 2 Min. The mixture was then taken out from the water bath and 300 µL of rabbit lung enzyme (Sigma-Aldrich Chemical Co., USA; 1 g rabbit lung enzyme powder in 10 mL buffer consisting of 50 mmol/L Tris–HCl with 400 mmol/L NaCl, pH 8.3) was added and the mixture was mixed thoroughly. The enzymatic reaction was allowed to take place in the water bath (37 °C) for a total period of 20 Min. The absorbance was measured at 340 nm, which was compared to a control (300 µL of the buffer instead of the extract). The ACE inhibition activity was calculated as follows:

\[
\text{ACE inhibition} \% = \left( \frac{\text{Absorbance of control} - \text{Absorbance of extracts}}{\text{Absorbance of control}} \right) \times 100.
\]

2.8. Sensory evaluation
Sensory evaluations of yogurt during 0, 7, and 21 days were conducted using an inexpert panel (n = 12). The panelists had no experience in judging yogurt. Samples were presented to panelists in ±200 g per cup. The panelists were required to evaluate all the samples on a 10 point scale. There are two types of scoring according to intensity and liking. Sensory parameters included characteristics of watery, grainy, lumpy, firmness, sourness, sweetness, and bitter aspects were scored according to the intensity (1–4 = low, 5–7 = moderate, 8–10 = high). The overall aroma, appearance, consistency, and taste were scored according to the liking where 1–2 = unacceptable, 3–4 = poor, 5–6 = fair, 7–8 = good, and 9–10 = excellent.

2.9. Statistical analysis
SPSS 14.0 was used for the statistical analysis. Data were expressed as mean ± SE (n = 3). One-way analysis of variance and Duncan’s post hoc test for mean comparison were performed. The criterion for statistical significance was P < 0.05.

3. Results and discussions
3.1. Effects of L. barbarum and fish collagen on postacidification activity in yogurt
There were no significant differences (P > 0.05) in pH and TTA% between both types of yogurt in the presence and absence of fish collagen compared with their respective controls (Figs. 2 and 3). The pH was reduced to 4.24 and 4.32 for plain and L. barbarum yogurt respectively and to 4.34 and 4.40 for plain and L. barbarum yogurt with fish collagen respectively at day 21 storage. Plain- and L. barbarum yogurt in the presence
of fish collagen showed higher (P < 0.05) TTA% than in the absence at 7 days of storage (Fig. 3).

The yogurt starter cultures including L. bulgaricus and S. thermophilus are slow acid producers [24] but active even at refrigerated temperature and still can produce small amounts of lactic acid by fermentation of lactose, which results in noticeable pH decrease and total lactic acids increase [25]. Postacidification due to β-galactosidase is still active at 0–5 °C during storage. In this case, pH may decrease to less than 4.2, resulting in whey separation and affecting also the LAB viability. However, all yogurt samples except plain yogurt showed a pH higher than 4.2 at the end of refrigerated storage. The activity to enhance acid production by the addition of L. barbarum and/or fish collagen in yogurt during storage is also confirmed by several other studies [5,6,19,26].

### 3.2. Effects of L. barbarum and fish collagen on OPA peptides content and free amino acids of yogurt

The OPA peptides amount of L. barbarum yogurt both in the presence and absence of fish collagen were significantly higher (P < 0.05) than their respective controls (plain yogurt with/without fish collagen) during 14 days of storage (Table 1). However, there was no significant difference in OPA peptides between fresh L. barbarum yogurt + fish collagen (182.5 ± 2.50 µg/g; 0 day) and plain yogurt + fish collagen (181.2 ± 4.50 µg/g). All yogurt samples showed the highest content of OPA peptides after a week of storage (Table 1). Furthermore, there were no significant differences (P > 0.05) in total free amino acids between L. barbarum yogurt with/without fish collagen and their respective controls (plain yogurt with/without fish collagen; Table 1) during 21 days of storage. Plain and L. barbarum yogurt with fish collagen showed higher (P < 0.05) OPA peptide concentrations and free amino acids than plain and L. barbarum yogurt without fish collagen (Table 1).

The production of yogurt is a complex process involving many physical and chemical changes including proteolysis, which involves the progressive hydrolysis of the caseins to polypeptides, peptides, and amino acids [27]. The results showed a significant (P < 0.05) improvement in the amount of OPA peptides and free amino acid in the presence of fish collagen. The ability of LAB to grow to high cell densities in milk is dependent on a proteolytic system that can liberate essential amino acids from casein-derived peptides [28]. In the present study, this may be connected with higher growth of LAB during fermentation and storage because fish collagen yogurts showed a significant increase in TTA% during 21 days of storage compared with without fish collagen (Fig. 3). In addition, fish collagen could be a rich source of the available peptide for yogurt bacteria. Similarly, L. barbarum contains 18 amino acids which are maybe essential for LAB growth [29].

### 3.3. Effects of L. barbarum and fish collagen on milk protein proteolysis of yogurt by SDS-PAGE

The intensity of the bands on the SDS-polyacrylamide gel (Fig. 4) reflected the concentrations of protein on the respective bands. Most of the casein components appeared at all-time points except BSA. The intensity of all bands for plain and L. barbarum yogurt tends to remain unchanged for caseins and whey protein during the storage (Fig. 4A). However, β-lactoglobulin and α-lactalbumin showed small degradation in fresh plain yogurt (0 day) and 21 days old L. barbarum yogurt. Similarly, the addition of fish collagen did not change the caseins bands intensity of plain and L. barbarum yogurt during storage (Fig. 4B). L. barbarum yogurt with fish collagen showed higher band intensity of α-lactalbumin and β-lactoglobulin (whey protein) than control at 0 and 7 days of storage. However, extended storage to 21 days resulted in more whey protein degradation with a reduction in the band intensity (Fig. 4B).

The soluble nitrogen fractions generally increased during storage, corresponding to the continued breakdown of casein and large peptides into small peptides and amino acids by the action of starter culture enzymes [30]. The previous study reported that caseins are the main source of amino acids for lactobacillus spp. [31]. The proteinase makes the first step in
casein breakdown producing a great number of oligopeptides [32]. These changes in casein micelle size could be related to the interaction of denatured whey proteins with serum or colloidal phase components in the milk. In the present study, the unchanged in the degradation of caseins in yogurt samples both in the presence and absence of fish collagen is in line with those observed by earlier studies in fermented milk [24,33,34]. Baba et al. [6] found that the addition of *L. barbarum* into yogurt enhanced significantly the viability and microbial growth in yogurt during 0 and 6 days of storage. However, lower degradation of whey protein in *L. barbarum* yogurt (0 day) could be suggested an interaction between the phenolic compounds and milk protein which is in agreement with other studies [26,35]. In addition, yogurt treated with *L. barbarum* and fish collagen had higher intensity of α-lactalbumin and β-lactoglobulin than control during 1 week of storage. It is possible that *L. barbarum* had the effect to reduce the proteolysis of whey protein by LAB. This is in disagreement with Shori et al. [19] who found that the addition of *A. sativum* and fish collagen in yogurt enhanced the degradation of whey protein during fermentation and storage. Future studies are needed to determine the viability of LAB in yogurt samples during refrigerated storage.

3.4. Effects of *L. barbarum* and fish collagen on ACE inhibitory activity of yogurt

The presence of *L. barbarum* extract increased ($P < 0.05$) ACE inhibitory activity in yogurt from 53.63% to 84.88% compared with control (40.12–68.35%) after 1 week of storage (Fig. 5). However, these values followed by significantly decreased to 63.91% and 50.81% for *L. barbarum* and plain yogurt, respectively, on 14 days. The presence of fish collagen in both types of yogurt showed no significant differences in ACE inhibitory activity which ranged between 52% and 56% during 14 days of storage.

The ACE test determines the ability of the bioactive peptide that has been produced through the proteolytic activity to decrease the production of angiotensin II which causes the vasoconstriction of the blood vessel [36]. In the present study, *L. barbarum* and plain yogurt were showed the highest ACE inhibitory activity at 7 days of storage which correlated with high peptide concentrations (Table 1). On the other hand, the presence of fish collagen in yogurt did not significantly affect the ACE inhibitory activity compared to the absence. This is in agreement with Shori et al. [19] who found that the addition of fish collagen into yogurt had no significant
<table>
<thead>
<tr>
<th>Attributes</th>
<th>Samples</th>
<th>0 day</th>
<th>7 day</th>
<th>21 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall aroma</td>
<td>7.00±</td>
<td>7.33±</td>
<td>7.39±</td>
<td>7.44±</td>
</tr>
<tr>
<td></td>
<td>0.35a</td>
<td>0.44a</td>
<td>0.43a</td>
<td>0.15a</td>
</tr>
<tr>
<td>Overall appearance</td>
<td>6.83±</td>
<td>6.78±</td>
<td>7.00±</td>
<td>6.89±</td>
</tr>
<tr>
<td></td>
<td>0.35a</td>
<td>0.36a</td>
<td>0.10a</td>
<td>0.11a</td>
</tr>
<tr>
<td>Watery</td>
<td>4.28±</td>
<td>4.94±</td>
<td>5.50±</td>
<td>5.61±</td>
</tr>
<tr>
<td></td>
<td>0.48a</td>
<td>0.73a</td>
<td>1.34a</td>
<td>0.56a</td>
</tr>
<tr>
<td>Overall consistency</td>
<td>6.11±</td>
<td>5.56±</td>
<td>6.72±</td>
<td>6.50±</td>
</tr>
<tr>
<td></td>
<td>0.55a</td>
<td>0.68a</td>
<td>0.22a</td>
<td>0.25a</td>
</tr>
<tr>
<td>Grainy</td>
<td>3.17±</td>
<td>3.94±</td>
<td>3.50±</td>
<td>2.66±</td>
</tr>
<tr>
<td></td>
<td>0.25a</td>
<td>0.39a</td>
<td>0.59a</td>
<td>0.33a</td>
</tr>
<tr>
<td>Lumpy</td>
<td>3.11±</td>
<td>3.45±</td>
<td>2.33±</td>
<td>2.17±</td>
</tr>
<tr>
<td></td>
<td>0.20a</td>
<td>0.15b</td>
<td>0.10a</td>
<td>0.17a</td>
</tr>
<tr>
<td>Firmness</td>
<td>5.45±</td>
<td>4.56±</td>
<td>5.66±</td>
<td>5.00±</td>
</tr>
<tr>
<td></td>
<td>0.50a</td>
<td>0.39a</td>
<td>0.26a</td>
<td>0.44a</td>
</tr>
<tr>
<td>Overall taste</td>
<td>6.22±</td>
<td>6.44±</td>
<td>6.50±</td>
<td>6.17±</td>
</tr>
<tr>
<td></td>
<td>0.36a</td>
<td>0.24a</td>
<td>0.39a</td>
<td>0.38a</td>
</tr>
<tr>
<td>Soursness</td>
<td>5.78±</td>
<td>5.22±</td>
<td>5.22±</td>
<td>5.28±</td>
</tr>
<tr>
<td></td>
<td>0.20a</td>
<td>0.49a</td>
<td>0.49a</td>
<td>0.31a</td>
</tr>
<tr>
<td>Sweetness</td>
<td>0.50±</td>
<td>1.33±</td>
<td>0.50±</td>
<td>0.89±</td>
</tr>
<tr>
<td></td>
<td>0.01a</td>
<td>0.42a</td>
<td>0.34a</td>
<td>0.47a</td>
</tr>
<tr>
<td>Bitterness</td>
<td>0.61±</td>
<td>0.50±</td>
<td>0.44±</td>
<td>0.61±</td>
</tr>
<tr>
<td></td>
<td>0.31a</td>
<td>0.29a</td>
<td>0.46a</td>
<td>0.15a</td>
</tr>
</tbody>
</table>

Data are means (n = 12) ± SEM.

a,b means with different superscript in the column differ significantly (P < 0.05).
P, plain; Y, yogurt; LB, L. barbarum; FC, fish collagen.
effects on ACE inhibitory activity compared to without fish collagen during storage. This is suggesting that there was no association between a high amount of peptides/amino acids in yogurt and high ACE-I inhibitory activity which depended on the composition, size, and sequence of amino acids [37,38,39].

3.5. Effects of L. barbarum and fish collagen on sensory evaluation of yogurt

The means scores of organoleptic assessment of both plain and L. barbarum yogurt in the absence or presence of fish collagen during three weeks (0, 7, and 21 days) of storage at 4 °C are presented in Table 2. In general, the organoleptic properties of L. barbarum yogurt were considered unchanged (P > 0.05) compared with plain yogurt because the scores for overall aroma, overall appearance, overall consistency, and overall taste were not significantly different. However, fresh plain yogurt showed the lowest water content (4.28 ± 0.48% low intensity) among other samples (moderate intensity) during 21 days of storage (Table 2). The addition of fish collagen did not contribute to significant changes in the sensory evaluation of plain and L. barbarum yogurt. However, 7 days old L. barbarum yogurt with fish collagen showed the highest overall taste liking score among the other storage L. barbarum yogurt both in the presence and absence of fish collagen. Similarly, fresh and 7 days old yogurt with fish collagen registered higher overall taste liking score compared with without fish collagen. In addition, 7 days old plain yogurt with fish collagen showed a moderate intensity of grainy compared to other samples that showed low intensity (Table 2).

The sensory properties of yogurt offer quality control criteria [40]. Although the texture and appearance of yogurt are important quality characteristics, the flavor of the product is generally considered the most critical and important indicator of consumer acceptance [41]. In the present study, results showed that the preference of yogurt samples was not influenced by the addition of fish collagen and L. barbarum to the yogurt. The consumer panel did not detect any significant differences in the appearance, aroma, mouthfeel, and flavor between barbaram yogurt either in the presence or absence of fish collagen. The addition of fish collagen improved the sensory characteristics of all yogurts.

4. Conclusions

Incorporation of L. barbarum into yogurt both in the presence and absence of fish collagen did not substantially alter the acidification properties of yogurt compared to respective control. However, the addition of fish collagen helped to increased significantly the production of lactic acids during storage. In addition, L. barbarum yogurt with/without fish collagen enhanced OPA peptides concentrations in yogurt all over storage periods while degradation of whey proteins improved after 3 weeks of storage. L. barbarum yogurt in the absence of fish collagen was acting as a great ACE inhibitor but not in the presence. The incorporation of L. barbarum and/or fish collagen affected to a small extent the overall sensory characteristics of yogurt over its shelf life. Further study on L. barbarum with/without fish collagen can be carried out to identify the active constituents of yogurt which are responsible for the reduction in ACE inhibitory activity.

5. Acknowledgements

This project was funded by Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under grant No. D-047-247-1441. The authors, therefore, gratefully acknowledge the DSR technical and financial support.

The authors declare no conflict of interest.

6. References