Effects of herbal yogurt with fish collagen on bioactive peptides with angiotensin-I converting enzyme inhibitory activity

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Abstract

Illicium verum (IV; dried fruit), Psidium guajava (PG; dried leaves), and Curcuma longa (CL; dried rhizome) water extract and/or fish collagen were added into yogurt to determine their effects on acidification and the proteolysis of milk up to 21 days of storage at 4°C. The angiotensin-I converting enzyme (ACE) test was conducted to analyze the inhibitory activity of bioactive peptides produced during proteolytic activity on the ACE enzyme. The addition of fish collagen in PG- yogurt significantly decreased pH compared to control on day 7 of storage. The presence of IV, PG, and CL enhanced (p<0.05) OPA peptide amount during 7 & 14 days of storage. The highest ACE inhibitory activity was shown on day 7 for all herbal yogurt. Herbal yogurt either in the presence or absence of fish collagen may improve the manufacture and formulation of yogurt with anti-ACE activity.

Keywords: yogurt; herbal water extract; proteolysis; fish collagen; ACE inhibitory activity.

Practical Application: Herbal yogurt and fish collagen are potential approaches for the management of hypertension.

1 Introduction

Hypertension (high blood pressure) is a major risk factor for the development of cardiovascular diseases. This disease is one of the main causes of mortality in Western countries (Skoog & Gustafson, 2003; Shori & Baba, 2015) and is getting more common in affluent societies. It occurs when blood pressure stays elevated over time, which is a dangerous condition where the blood pressure is persistently higher than normal (Gobbetti et al., 2004). The increased pressure of hypertension puts a strain on the circulatory system which can ultimately lead to serious problems such as stroke, heart disease, and kidney failure (Gobbetti et al., 2004).

The renin-angiotensin-aldosterone system (RAS) is considered to be a hormonal regulator of blood pressure together with sodium, potassium and water balance (Muñoz-Durango et al., 2016). The angiotensin-I converting enzyme (ACE) has been classically associated with the RAS regulating peripheral blood pressure (Coates, 2003; Muñoz-Durango et al., 2016). Cleavage of angiotensinogen by renin produces angiotensin I which is subsequently hydrolyzed by angiotensin-I-converting enzyme (ACE) to angiotensin II (a potent vasoconstrictor) which can raise blood pressure (Chen et al., 2014). Besides, ACE also inactivates the vasodilator bradykinin (Chen et al., 2014). The angiotensins are peptides that act as vasoconstricting agents (causing blood vessels to narrow). Narrowing the diameter of the blood vessels sends up the blood pressure and will lead to hypertension.

In many modern societies, fermented dairy products make up a substantial proportion of the total daily food consumption (Ariyoshi, 1993; Donkor et al., 2005; Bütikofer et al., 2008; Lignitto et al., 2010). Furthermore, it has long been believed that consuming fermented milk products (i.e. yogurt) provides various health benefits (Shori, 2013; Shori et al., 2013; Baba et al., 2014; Shori & Baba, 2014; Muniandy et al., 2016; Alenisan et al., 2017; Shori et al., 2018). Yogurt products are the outcome of intense bacterial activity of the starter cultures, leading to production of lactic acid and biologically active compounds, adding nutritional and physiological value (Shori & Baba, 2012; Muniandy et al., 2017).

Bioactive peptides are peptides formed from food proteins that have not only nutritional value as a source of amino acids but are also able to exert a physiological effect on the human body (Vermeiren et al., 2004; Shori & Baba, 2015). Bioactive peptides are not active as long as they are still part of a dietary protein. When they are released from the protein they can exert their physiological effects Vermeiren et al. (2004). Milk proteins are the most important sources of bioactive peptides. Various peptides have been identified in recent years with anti-ACE activity (Bütikofer et al., 2008).

Illicium verum, Psidium guajava, and Curcuma longa are consumed by most people to treat disease and enhance general health and longevity (Jiménez-Escrig et al., 2001; De et al., 2001; Doudova et al., 2007; Nair & Chanda, 2007; Peng et al., 2016; Karlowicz-Bodalska et al., 2017; Mueen Ahmed et al., 2018). The water extract of these herbs are used in yogurt preparation. In addition, fish collagen has been used to decrease systolic blood pressure by inhibiting ACE (Zhu et al., 2010; Lee et al., 2014). In the present study, I. verum, P. guajava, and C. longa water extract and/or fish collagen were added into yogurt to determine their effects on acidification and the proteolysis of milk up to 21 days of storage at 4°C. Also, the inhibitory activity of bioactive peptides produced during proteolytic activity on the ACE enzyme was also conducted during 14 days of storage.
2 Materials and methods

2.1 Water herbal extract

*Ilicium verum* (IV; dried fruit), *Psidium guajava* (PG; dried leaves), and *Curcuma longa* (CL; dried rhizome) were purchased in dried form from local medicinal store. Five gram from each powder was homogenized in 50 ml dH₂O individually and incubated overnight in a water bath (70 °C). The mixture was centrifuged (15 minutes, 2000rpm and 4 °C) and the supernatant was used as herbal water extract (Shori & Baba, 2011).

2.2 Preparation of yogurt

Two groups of yogurt were prepared, plain and herbal yogurt with or without fish collagen. For herbal yogurt preparation, fresh and pasteurized full-cream cows’ milk (900 mL) was heated until 41 °C in a 1 L beaker. A small volume of (100 mL) milk was placed into a clean and sterilized beaker and 10 ml starter culture (Chris-Hansen, Denmark) was added. The starter culture was stirred thoroughly and inoculated to milk and then 100 mL herbal water extract was added. Full creamed milk (20 g; Dutch Lady’s) was added to correct the milk solid content and the mixture was mix thoroughly. For fish collagen-yogurt, 2.5g fish collagen was added into the milk. For plain yogurt, 100 mL dH₂O was added instead of herbal water extract. All yogurts were incubated at 41 °C in an incubator until the pH was reduced to 4.5 next the yogurts were stored at 4 °C (Shori & Baba, 2012).

2.3 The pH and Total Titratable Acidity (TTA)

The acidification of yogurt during 21 days of storage was evaluated by measuring pH and TTA using methods described by Shori & Baba (2014).

2.4 Preparation of yogurt water extract

Yogurt water extract was prepared as described by Shori & Baba (2014).

2.5 O-phthalaldehyde (OPA) assay

OPA assay is a very high sensitivity detection of proteins, peptides, and amino acids which was performed as described by Shori & Baba (2011).

2.6 ACE inhibitory activity

The ACE inhibitory activity was carried out as described by Shori & Baba (2011).

2.7 Statistical analysis

Data were presented as mean ± SE (n=3). One way analysis of variance and Duncan’s post hoc test for mean comparison were performed by SPSS 14.0. The criterion for statistical significance was p < 0.05.

### 3 Results and discussion

3.1 Effects of herbal extract with/without fish collagen on the pH and total titratable acidity of yogurt

There was no significant difference in pH values of all herbal yogurt as compared to control over 21 days of storage (Table 1). However, PG- yogurt showed higher pH (4.4 ± 0.6; p<0.05) than plain- yogurt (4.2 ± 0.2) on day 7 of storage. In the same way, the addition of fish collagen to all types of herbal yogurt showed no significant effect (p>0.05) on pH values compared to plain yogurt during the storage (Table 1). Nevertheless, the addition of fish collagen in PG- yogurt significantly decreased pH to 4.2 ± 0.1; p<0.05 compared to control (4.4 ± 0.4) on day 7 of storage. In addition, CL- yogurt + FC showed higher (p<0.05) pH than control on day 21 (Table 1).

TTA of fresh plain yogurt was 0.89 ± 0.5%. The addition of IV, PG, and CL in yogurt decreased (p<0.05) TTA to 0.70%, 0.79%, and 0.74%; respectively. Refrigerated storage of yogurt to 21 days had no significant effect on TTA of all herbal yogurt compared to plain yogurt (Table 2). Furthermore, the addition of fish collagen showed no effects on TTA (p>0.05) of fresh herbal yogurt samples compared to control (Table 2). CL- yogurt + FC showed higher TTA% (0.98 ± 0.04%; p<0.05) than control (0.84 ± 0.1%) on 7 days of storage. Extended refrigerated storage to 3 weeks significantly reduced (p<0.05) TTA% in PG-, and CL- yogurt compared to control (Table 2).

Higher pH values in 7 days old PG- yogurt and 21 days old CL- yogurt + FC than their respective controls, suggesting a low rate of production of organic acids in these yogurt. On the other hand, fish collagen had positive effect on the reduction of pH in 7 days old PG- yogurt while showed negative effect on TTA% of 21 days PG-, and CL- yogurt. This observation can be explained by the differences in the number of viable microbes which have direct influence on the rate of acid formation and thus pH reduction during storage (Vandera et al., 2019). Several studies reported that flavoring materials added to yogurt have little effect on the survival of lactic acid bacteria (Venizelou et al., 2000; Najgebauer-Lejko, ...
3.2 Effects of herbal extract with/without fish collagen on the OPA peptides amount of yogurt

Figure 1 shows the OPA peptide amount in plain and herbal yogurt during 2 weeks of storage. All plain, IV and CL yogurt showed almost similar amount of OPA peptide (23 µg/g) at 0 day. However, the presence of PG in fresh yogurt increased (p<0.05) OPA peptide content to 32.70 ± 1.07 µg/g. Refrigerated storage of yogurt resulted in enhanced (p<0.05) the content of OPA peptide in all herbal yogurt as compared to control during 14 days (Figure 1). In addition, the highest OPA peptide value was shown for all yogurt on day 7 of storage where CL-yogurt registered 98.33 µg/g while both IV- and PG- showed similar OPA peptide content (50 µg/g).

The OPA peptide amount was tremendously enhanced (p<0.05) by the addition of IV and PG with fish collagen in yogurt compared to control over all storage period (Figure 2). OPA peptide content was varied in the range between 210- 360 µg/g and 284.3-442.7 µg/g for IV+ FC- and PG+ FC- yogurt respectively while plain+ FC yogurt ranged between 181.2- 275.3 µg/g with the highest result was shown on 7 days. On the other hand, the addition of CL+ FC in yogurt decreased (p<0.05) the content of OPA peptide which ranged from 51.6 to 69.4 µg/g compared to control (Figure 2).

Yogurt contain bioactive peptides which was formed as a result of milk fermentation (Shori et al., 2013). The OPA assay is a reliable method in the determination of proteolysis in milk protein (Church et al., 1983). OPA measures the concentration of peptides and free amino acids (Church et al., 1983). The proteolytic activity of lactic acid bacteria is influence the concentration of bioactive peptides directly with higher proteolytic activity resulting in higher concentration of peptides (Shori & Baba, 2014). It was shown that the proteolytic activity in all yogurt samples during storage had a bell-shaped curve (Figure 1 and 2). All the samples showed the highest concentration of peptides on day 7 of storage. High peptide concentration was due to the proteolytic activity of yogurt starter culture (Shori & Baba, 2014). Furthermore, the addition of CL extract into yogurt enhanced significantly the proteolytic activity of LAB in yogurt, however fish collagen in CL- yogurt showed a negative impact on milk proteolysis. Further study is needed to examine the influence of CL extract with/without fish collagen on the LAB viability in yogurt during the storage period. All yogurt in the presence of IV, PG, and CL improved the milk proteolysis during 7 and 14 days of storage. Previous studies reported that the inclusion of herbal extracts in yogurt (i.e. garlic, cinnamon, neem, goji berry, peppermint, dill, and basil) enhanced the proteolytic activity of LAB in yogurt (Amirdivani & Baba, 2011; Shori et al., 2012; Shori et al., 2013; Shori & Baba, 2013, 2014; Baba et al., 2014). It is obvious that the peptide concentration in all fish collagen yogurt samples is much higher compared to yogurt samples without fish collagen (Figure 1 and 2). Therefore, it could be concluded that fish collagen can further increase the proteolytic activity of LAB in yogurt. This is in agreement with previous studies that showed fish collagen and Allium sativum increased

Table 2. Changes total titratable acidity (TTA %) of yogurt in the presence of Illicium verum, Psidium guajava, and Curcuma longa water extracts with/without fish collagen during 21 days of storage at 4 °C.

<table>
<thead>
<tr>
<th>Sample</th>
<th>TTA (LAE %)</th>
<th>Day7</th>
<th>Day21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>P-Y</td>
<td>0.89±0.01^a</td>
<td>0.82±0.00^b</td>
<td>0.84±0.00^b</td>
</tr>
<tr>
<td>IV-Y</td>
<td>0.74±0.00^b</td>
<td>0.77±0.12^b</td>
<td>0.8±0.01^b</td>
</tr>
<tr>
<td>PG-Y</td>
<td>0.79±0.00^b</td>
<td>0.8±0.04^b</td>
<td>0.88±0.01^b</td>
</tr>
<tr>
<td>CL-Y</td>
<td>0.74±0.03^b</td>
<td>0.81±0.03^b</td>
<td>0.85±0.03^b</td>
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<tr>
<td>P-Y + FC</td>
<td>0.88±0.01^a</td>
<td>0.84±0.08^b</td>
<td>1.1±0.00^c</td>
</tr>
<tr>
<td>IV-Y + FC</td>
<td>0.9±0.01^a</td>
<td>0.9±0.03^b</td>
<td>1±0.01^c</td>
</tr>
<tr>
<td>PG-Y + FC</td>
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<td>0.92±0.01^b</td>
<td>0.95±0.02^c</td>
</tr>
<tr>
<td>CL-Y + FC</td>
<td>0.91±0.03^c</td>
<td>0.98±0.04^c</td>
<td>0.99±0.01^c</td>
</tr>
</tbody>
</table>

^a-c Means with different superscript within a column are significantly different (p<0.05).
Data are presented as mean ± s.e.m; n=3. P= plain; Y= yogurt; IV= Illicium verum; PG= Psidium guajava; CL= Curcuma longa; FC= fish collagen. LAE= lactic acid equivalent.
ACE inhibitory activity of plant yogurt

3.3 Effects of herbal extract with/without fish collagen on the ACE inhibitory activity of yogurt

Fresh plain yogurt showed ACE inhibitory activity of 40.15 ± 8.65% (Figure 3). However, the addition of IV, PG, and CL improved significantly (p<0.05) ACE inhibitory activity of yogurt to 52.42 ± 4.84%, 59.07± 0.60%, and 50.20 ± 13.10%; respectively. The highest ACE inhibitory activity was shown on day 7 for IV- yogurt (88.30 ± 0.40%) followed by PG-, CL-, and plain- yogurt (81.85 ± 2.02% and 79.80 ± 12.10%; 68.35 ± 2.65%; respectively). Extended refrigerated storage to 14 days decreased (p<0.05) ACE inhibitory activity with no difference (p>0.05) between plain, IV- and CL- yogurt. In addition, PG- yogurt showed higher (p<0.05) ACE inhibitory activity than control (Figure 3).

The presence of fish collagen in all fresh yogurt showed no significant differences (p>0.05) in ACE inhibitory activity (~ 52%) except for CL- yogurt that showed inhibition value of 67.30 ± 4.05% (p<0.05; Figure 4). All herbal yogurt with fish collagen displayed higher (p<0.05) ACE inhibitory activity than control at 7 days of storage and this followed by significant reduction (p<0.05) at 14 day (Figure 4). However, both PG- and CL- yogurt + FC showed higher ACE inhibitory activity (58.27 ±6.65% and 61.50 ± 6.25%; respectively) than control (51.60 ± 10.10%).

The angiotensin-converting enzyme (ACE) test was conducted to analyze the inhibitory activity of bioactive peptides produced by yogurt bacteria during proteolytic activity on the ACE enzyme (Ariyoshi, 1993; Donkor et al., 2005). Several studies have shown that, the bioactive peptides derived from milk proteins have been proven to possess ACE inhibitory properties (Bütikofer et al., 2008; Shori & Baba, 2015; Rahimi et al., 2016). Therefore, the purpose of this experiment was to determine whether the addition of different herbal extract and/or fish collagen may influence the milk proteolysis of yogurt which may indirectly affect the ACE inhibitory properties. The present study find a positive correlation between the ACE inhibitory activity and proteolytic activity of plain and herbal yogurt in the absence of fish collagen (R² = 0.6) but not in the presence (R² = 0.03; data not shown). All herbal yogurt samples in the presence and absence of fish collagen observed the highest ACE inhibitory activity at day 7 of storage (Figure 3 and 4), that achievement was strongly influenced by the highest proteolytic activity pattern (Figure 1 and 2). In addition, *P. guajava* and *C. longa* have been a powerful ACE inhibitor as it contains a phytochemical compounds with ACE inhibitory property (Sebastian & Blesson, 2012; Lekshmi et al., 2014). To the best of the authors knowledge, there have been no reports on the effect of *I. verum* extracts on the ACE inhibitory activity. The addition of fish collagen into herbal yogurt had little effect on the ACE inhibitory activity.

4 Conclusion

The addition of fish collagen in PG-, and CL-yogurt enhanced the reduction of pH and the elevation of TTA%; respectively on day 7 of storage. Fresh PG yogurt showed higher OPA peptide content than control whereas all herbal-yogurt samples enhanced OPA peptide amount during 7 & 14 days of storage. However, fish collagen had negative effect on OPA peptide content in CL- yogurt but not in other herbal yogurt. The highest ACE inhibitory activity was shown on day 7 for all herbal yogurt both in the presence and absence of fish collagen. Extended further refrigerated storage to 14 days caused significant reduction in ACE inhibitory activity for all yogurt samples. All herbal yogurt samples with or without fish collagen may improve the manufacture and formulation of yogurt with anti-ACE activity.

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References


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