Improved Antioxidant Activity of Low-Fat Herb-Fortified Cottage Cheese

Triana Setyawardani1*, Juni Sumarmono1, Hidayah Dwiyanti2, Heni Rizqiatı3, Naofal Dhia Arkan1, and Tasnim Hunin A. Mohamed4

1Faculty of Animal Science, Jenderal Soedirman University, Purwokerto, 53122, Indonesia
2Faculty of Agriculture, Jenderal Soedirman University, Purwokerto, 53122, Indonesia
3Faculty of Animal and Agricultural Sciences, Diponegoro University, Tembalang, Semarang, 50275, Indonesia
4Department of Animal Production, Faculty of Agriculture, Omdurman Islamic University, Khartoum, 14415, Sudan

ABSTRACT

This study aimed to evaluate the properties of low-fat cottage cheese with the addition of herbs. We conducted an experiment with nine treatments, namely P1: whole-milk cottage cheese; P2: low-fat cottage cheese; P3: low-fat cottage cheese + 20% bidara leaf extract; P4: low-fat cottage cheese + 20% bay leaf extract; P5: low-fat cottage cheese + 20% moringa oleifera leaf extract; P6: low-fat cottage cheese + 10% bidara leaf extract + 10% bay leaf extract; P7: low-fat cottage cheese + 10% bidara leaf extract + 10% moringa oleifera leaf extract; P8: low-fat cottage cheese + 10% bay leaf extract + 10% moringa oleifera leaf extract; and P9: low-fat cottage cheese + 6.67% bidara leaf extract + 6.67% bay leaf extract + 6.67% moringa oleifera leaf extract. The microbial profile of cheese showed that the total LAB count was retained at 6.23 log CFU/g. While herb-fortified cottage cheese contained a significantly higher level of antioxidants, the lowest cholesterol level was observed in cottage cheese fortified with 20% bidara leaf extract. The general fatty acids in cheese without herb addition were saturated fatty acids (51.94%) and unsaturated fatty acids (15.67%), with palmitic acid being the most predominant fatty acid (36.62%). In conclusion, herbs can improve antioxidant levels and retain total fatty acid in low-fat cottage cheese.

Keywords: Antioxidant, Cholesterol, Cottage, Herbs, Lactic acid bacteria

Introduction

Cottage cheese is a dairy product enriched in nutrients, particularly protein, and is common among consumers across age groups. Low-fat milk is one of the main components of cheese intended to reduce fat content. Cheese product development should consider incorporating herbs into the cheese-making process. Herbs are a source of antioxidants and antimicrobial agents that collectively improve product functionality. In addition to their antioxidant properties, herbs and spices contain natural preservatives (Souza et al., 2005). Bidara is an herbal plant whose leaves possess antioxidant and antimicrobial properties that may extend the shelf life of fortified products. In addition, bay leaves are a source of bioactive compounds as antimicrobials and, therefore, exhibit medicinal value (Kusuma et al., 2011) in addition to antioxidant properties (Hidayati et al., 2017).

Herbals and spices generate aroma and flavor and contribute to therapeutic characteristics such as antioxidant, anti-inflammatory, antidiabetic, antihypertensive, and antimicrobial properties. Furthermore, herbs and spices help to produce functional dairy products (El-Sayed and Youssef, 2019).

The antimicrobial activity of herbal extracts is beneficial as a natural preservative, including in dairy products. Herbs added to the cheese production process are expected to increase the product functionality with the antioxidant activity of herbs. Several studies have been conducted by adding herbs or spices, such as Kariessh cheese with turmeric and sage (Hasneen et al., 2020). In addition, Allium vineale L; Mendi (Chaerophyllum macropodum Boiss.), and Siyabo (Ferula rigidula DC.) in herby cheese (Kose and Ocak, 2020), as well as extracts of cinnamon, garlic, lemon grass, rosemary, sage, and oregano, were able to inhibit L monocytogenes in cheese (Tayel et al., 2015).

The antimicrobial activity of herbal extracts is beneficial as a natural preservative, including in the application of dairy products. The use of different percentages of herbs in cheese will affect these characteristics. The combination of low-fat raw materials and adding herbs produces distinctive cheese characteristics in terms of microbial profile, antioxidant activity, cholesterol, and fatty acids. The antimicrobial activity resulting
from using herbs affects the product's shelf life. Therefore, it is important to study the characteristics of cheese with the addition of herbs. The originality of this study lies in the production of low-fat cottage cheese with the addition of bidara leaf extract, bay leaf extract, and moringa oleifera leaf extract alone and in combination. This differs from previous studies that added rosemary leaves (Rosmarinus officinalis) to cheeses (Marinho et al., 2015).

**Materials and Methods**

**Cheese production**

Cheese production involves the coagulation of proteins in milk to form curds using rennets. Cottage cheese is made using full-cream milk and low-fat milk according to the treatment. Milk was obtained from the Unsoed ex-farm, which was fed forage, elephant grass, and concentrates. Milk was separated from the fat using a cream separator. According to the treatment, Full-cream and low-fat milk are the ingredients for making cheese. Milk was heated at 85°C for 15 min and then the temperature was lowered to 40°C. The herbal extract was added to the treated cheese; then a five-percent starter was added and incubated until a pH of 6.1 was reached. The next step was to add one mL of rennet and leave it until a perfect lump was formed. The cheese lumps were cut into pieces and left at 40°C to remove water. The cheese was then drained and pressed to obtain dense cheese curd.

The study used a completely randomized design with nine treatments following P1: whole-milk cottage cheese; P2: low-fat cottage cheese; P3: low-fat cottage cheese + 20% bidara leaf extract; P4: low-fat cottage cheese + 20% bay leaf extract; P5: low-fat cottage cheese + 20% moringa oleifera leaf extract; P6: low-fat cottage cheese + 10% bidara leaf extract + 10% bay leaf extract; P7: low-fat cottage cheese + 10% bidara leaf extract + 10% moringa oleifera leaf extract; P8: low-fat cottage cheese + 10% bay leaf extract + 10% moringa oleifera leaf extract; and P9: low-fat cottage cheese + 6.67% bidara leaf extract + 6.67% bay leaf extract + 6.67% moringa oleifera leaf extract.

Herb extract preparation started with collected herb bays, moringa oleifera, and bidara leaves, which were washed well, dried with a filter pro dehydrator, and powdered with an herb grinder. This herb powder was used for extraction. They were then extracted with hot distilled water using a soxhlet apparatus until a colourless solvent was obtained. The extracts obtained were filtered, concentrated, and allowed to dry until a constant weight was obtained.

**Microbiological analysis**

One gram of sample was mixed with nine mL of NaCl 0.98% and homogenized using a vortex (Velp Zx3 type, Italy) in a test tube as the first-stage dilution, followed by multilevel dilution. Total microbes and LAB were diluted up to 106 and 104 for total yeast. In the pour plate method, one milliliter of each microorganism was grown in different media, that is, PCA (Oxoid, UK) and MRSA (Merck) for the total LAB, and PDA (Oxoid, UK) for yeast. A petri dish containing the sample and media was incubated at 37-40°C for 24 h to gather total microbial and yeast count data. In contrast, LAB is incubated for 48 h (Setyawardani and Sumarmono, 2015).

**Determination of fatty acid**

The fatty acid content of cheese was determined following the procedures described by AOAC (2005). Gas chromatography analysis was based on the partition of fluid components between the moving phases in terms of gas and static phases, in the form of solids and fluids that do not easily vaporize, attached to the inert supporting materials. The phases were started by hydrolyzing the fat into fatty acids and then transforming it into an ester form that evaporates better. In this method, the transformation was conducted by methylation until the fatty acid ester methyl (FAME) was formed. Next, FAME was analyzed by gas chromatography. Each component was identified by comparing its retention time with that of the standard under the same analytical conditions. The retention time was calculated on a recorded paper as the distance from the line when a solvent peak occurred until the center of the treated component peak. Determination of the component content in the samples can be performed using either external or internal standard techniques. The area of each component was directly proportional to the total component in the sample. To minimize the error from the injection volume, sample preparation, dilution, and other factors, it is better to use the internal standard technique. In addition, the detector response and interaction among components in the sample matrices must be corrected when it passes the columns.

**Determination of cholesterol levels**

Cheese fat content was determined by the soxhlet method (Pombal et al., 2017). Weigh 10 mg of oil from the analysis of fat content into a 10 mL volumetric flask. Add a few mL of 2-propanol into a 10 mL volumetric flask containing the oil sample, then extract using ultrasonic for a few minutes; the cholesterol contained in the oil was dissolved in 2-propanol. Add 2-propanol into the flask until it reaches the limit. The next step is to filter the solution using a 0.45 µM Millipore membrane. The sample is ready for analysis using HPLC.

\[
\text{Cholesterol concentration (mg/dL) } = \frac{A \times C \times V}{W}
\]

Description:
- A = Sample peak area
- B = Standard peak area
- C = Standard concentration (mg/dL)
- V = Tera volume (10 mL)
- W = Sample weight
Determination of antioxidant activity

Antioxidant activity was determined based on the reference method (Payet et al., 2005). Two hundred milligrams of the sample were mixed with 5 mL of Methanol and vortexed. Then, 0.2 mL of the resulting extract was collected. The next step was adding 2.8 mL of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent and incubation at room temperature in the dark for 30 minutes. The sample was measured with a spectrophotometer of 517 nm. The same was done for a blank of 0.2 mL methanol + 2.8 mL of 0.1 mM DPPH reagent.

Formula: 
\[
\text{Inhibition} = \frac{A - B}{A} \times 100
\]

Description:
A = Abs blank
B = Abs sample

Phytochemical tests

Screening of the bay, moringa oleifera, and bidara leaf for various phytochemical constituents quantitative (solid) and qualitative (phytochemical compounds) tests were carried out using the reference method (Yadav et al., 2014).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) and a post hoc test of Duncan was performed with SPSS® 25.0 software.

Results and Discussion

Phytochemical compounds

Table 1 informs the compounds of the herbal leaf as the source of antioxidants for making low-fat cottage cheese. The phytochemical compounds of leaf extract incorporated in cheese making (Table 1) were examined with ethanol and water dilutions. All three leaves contain tannin and flavonoid, except bidara leaf extract with water dilution. The quantitative analysis to observe antioxidant activities using the DPPH assay showed that the antioxidant activities were >88% and the flavonoid was >2%. These herbs are a highly potential source of antioxidants for cheese. Antioxidants in natural compounds can inhibit lipid oxidation, especially in animal and fish products with high polyunsaturated fatty acids and cholesterol that are vital for health. The natural antioxidant is an alternative to synthetic antioxidants to prevent the formation of oxidative cholesterol products. Studies have shown the use of natural antioxidants to curb cholesterol oxidation (de Oliveira et al., 2018).

Microbiological profile

Natural antioxidants from the addition of natural extracts can increase bioactive molecules and maintain microflora in food products (Ritota and Manzi, 2020). The number of microbes in low-fat cottage cheese with the addition of herbs is shown in Figure 1.

The addition of herbal extracts into cheese-making affected the microbiological profile in the present study as follows. The total Plate Count (TPC) of cheese with the addition of herbal extracts has a range of 0.83–2.43 log CFU/g, but the highest TPC was observed in cheese without herbal extract addition. It is in line with a previous finding (Alexa et al., 2018) that fresh cheese (control) contained the highest TPC compared to the other treatment groups. The addition of herbal extract significantly (p<0.05) affected the TPC. Furthermore, herbal extract incorporated into cheese-making affected the total yeast. The lowest and highest total yeast was observed in P8 (1.58 log CFU/g) and P3, respectively. Regarding the total LAB of cheese, it was significantly (p<0.05) affected by the addition of herbal extract, the highest being 7.2 log CFU/g in P2 and P5. However, the addition of herbal leaf extract did not affect the growth of LAB in cheese because LAB could grow well (6.23–7.25 log CFU/g) regardless of the addition. One of the highest total LAB was observed in cheese incorporated with an herbal extract that did not show antimicrobial activities. Antimicrobial activities were tested with the well-diffusion method using Staphylococcus aureus ATCC 2592; Bacillus cereus ATCC13061; E Coli ATCC 8739; and Salmonella typhimurium ATCC 14028. The extract was diluted with water and the cheese sample did not show any antimicrobial activities. It was in line with (Kose and Ocak, 2020) who examined antimicrobial activities in traditional Herby cheese originating from Turkey.

Cheese fortified with 20% Moringa oleifera leaf extract contained a similar level of LAB to low-fat non-herbal-fortified cheese (P2), but not significantly different LAB in P6, P7, P8, and P9. The LAB was not affected by the addition of the combination of bidara, bay, and moringa oleifera leaf extract. Cheese is rich in tartaric acid, lactic acid, and malic acid, which could be used as preservatives in cheese fermentation. Because organic acids diffused in bacterial cell membranes,

Table 1. Compounds of herbal leaf extract

<table>
<thead>
<tr>
<th>Phytochemical compounds</th>
<th>Ethanol extract</th>
<th>Water extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bay leaf</td>
<td>Moringa oleifera</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Quantitative/solid

<table>
<thead>
<tr>
<th></th>
<th>Bidara</th>
<th>Moringa oleifera</th>
<th>Bay leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPH</td>
<td>90.96</td>
<td>90.54</td>
<td>88.24</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>2.16</td>
<td>2.01</td>
<td>2.33</td>
</tr>
</tbody>
</table>
cholesterol oxidation natural research cholesterol acids, activities Antioxidant making useful as molds antibacterial synergies pH, dissociated in the cytoplasm, reduced intracellular pH, and led to cessation of growth or cell death, the synergies of organic acids could produce antibacterial properties to inhibit contamination by molds and yeasts (Kisadere et al., 2018). The LAB is a gram-positive bacteria that produces lactic acid as a primary fermentation end product; they are useful as starter cultures to aid in the coagulation of milk proteins during the process of cheese making (Blaya et al., 2018).

Antioxidant activity

Natural compounds that contain antioxidant activities can prevent or protect from fat oxidation in animal-based products, including milk, which is easily oxidized due to the high unsaturated fatty acids, such as polyunsaturated fatty acids and cholesterol (de Oliveira et al., 2018). A number of research have been undertaken to incorporate natural antioxidants into food as a way to prevent oxidation in the manufacturing and storing processes while reducing the intake of oxidative cholesterol (COPs) (Barriuso et al., 2015; Barriuso et al., 2015).

Figure 2 showed that incorporating herbal leaf extract could significantly (p<0.05) increase the antioxidant level of cheese compared to non-fortified cheese. The use of herbal leaves provides a good source of antioxidants (Tapsell et al., 2006). Meanwhile, spices or extracts are incorporated into milk products as carriers of nutraceutical compounds that can improve the functional values of dairy-based products (El-Sayed and Youssef, 2019). Antioxidant-bearing chemicals would increase the antioxidant level in cheese. It is observed in cheese with a 2.3 and 4 g/100 ratio, which can increase the total probiotic, phenol, and antioxidant. The results showed that the antioxidant levels in P5, P6, and P7 are the highest of all treatments. It demonstrates that cheese fortified with mono herbal extracts could retain the antioxidant activities better than the other treatments. The antioxidant activity of bidara leaf extract is classified into highly strong antioxidant activity (Hendrawati et al., 2020). Bay leaf has been shown to possess various biological activities, such as antioxidant activity (Brinza et al., 2021). Morinda oleifera leaves are rich in flavonoids and phenolic

Triana Setyawardani et al. Improved Antioxidant Activity of Low-Fat Herb-Fortified Cottage Cheese

Figure 1. Effect of adding herbs to cottage cheese on the number of microorganisms. P1: whole-milk cottage cheese; P2: low-fat cottage cheese; P3: low-fat cottage cheese + 20% bidara leaf extract; P4: low-fat cottage cheese + 20% bay leaf extract; P5: low-fat cottage cheese + 20% moringa oleifera leaf extract; P6: Low-fat cottage cheese + 10% bidara leaf extract + 10% bay leaf extract; P7: low-fat cottage cheese + 10% bidara leaf extract + 10% moringa oleifera leaf extract; P8: low-fat cottage cheese + 10% bay leaf extract + 10% moringa oleifera leaf extract; and P9: low-fat cottage cheese + 6.67% bidara leaf extract + 6.67% bay leaf extract + 6.67% moringa oleifera leaf extract.

Figure 2. The antioxidant level of cheese fortified with the extract of bidarka leaf, moringa oleifera leaf, bay leaf, and the combined leaves. P1: whole-milk cottage cheese; P2: low-fat cottage cheese; P3: low-fat cottage cheese + 20% bidara leaf extract; P4: low-fat cottage cheese + 20% bay leaf extract; P5: low-fat cottage cheese + 10% moringa oleifera leaf extract; P6: Low-fat cottage cheese + 10% bidara leaf extract + 10% bay leaf extract; P7: low-fat cottage cheese + 10% bidara leaf extract + 10% moringa oleifera leaf extract; P8: low-fat cottage cheese + 10% bay leaf extract + 10% moringa oleifera leaf extract; and P9: low-fat cottage cheese + 6.67% bidara leaf extract + 6.67% bay leaf extract + 6.67% moringa oleifera leaf extract.
Compounds with high antioxidant and anti-inflammatory activities (Xu et al., 2019). These three herbs contain antioxidant activity. Thus, the addition of mono-herbal extracts of bidarka, bay, and moringa oleifera leaf increases the antioxidant activity of low-fat cottage cheese.

High antioxidant activities are due partly to the highest content of Morinda oleifera leaf extract with 23% of protein (unpublished data) than bidara leaves and bay leaves. This trend was in line with a study of cottage cheese added with different spices to determine the antioxidant activities of the products (Josipović et al., 2015). The study reported an interaction between phenolic molecules and protein interactions (pH, temperature, phenolic structure, molecule mass, amino acid composition) that explains the differences in Phyto phenolic contents that contribute to the antioxidant activities of cheese.

The use of natural antioxidants is superior to synthetic antioxidants. Antioxidants derived from natural spice extract can increase the taste and nutrition and protect the product from fat oxidation (Kumar et al., 2015; Rasei et al., 2016).

The lowest cholesterol was observed in low-fat cottage cheese fortified with 20% bidara leaf extract (P3), which was not significantly different from P4 and P5. The added 20% extract had the least effect compared to other treatments, especially P5, which contained the highest antioxidant activities.

Two mechanisms of antioxidants in preventing fat oxidation are first, the primary antioxidants donate electrons or hydrogen in the radicals formed during the initiation and propagation phase of fat oxidation, thus turning the products into a stable thermodynamic state or forming complex fat-oxidants that can react with other radicals (Kumar et al., 2015). Secondly, the secondary antioxidants reduce the oxidation rates through the different active mechanisms to inhibit chain reactions to prevent continuous abstraction of the hydrogen from the substrate (Shahidi and Ambigaipalan, 2015).

Phenolic are the main bioactive compounds in plants whose products play a role as antioxidants. The components of phenolic compounds contain potential redox that allows phenolic compounds to serve as hydrogen donors, oxygen-reducing agents, and iron ions in the food matrix (Viji et al., 2015); therefore, the mechanism of phenolic compounds can control the pro-oxidant concentration and deactivate free radicals, and thus inhibiting and preventing cholesterol oxidation (Medina-Meza and Barnaba, 2013).

Table 2 shows that the highest percentage of fatty acids are palmitic acids (36.82%), followed by Oleic acids (17.49%), stearic acids (11.06%), and myristic acids (10.88%) and lauric acids (9.94%). Similarly, the highest SFA in cow milk cheese is Palmitic acid (C16:0), myristic acid (C14:0), and stearic acid (C18:0). The percentage of fatty acids composition is caused by many factors: season of the year and climatic conditions, breed of animals and their feeding diets (Topnikova et al., 2019). It was in line with the previous findings that the dominant fatty acids in fresh milk are palmitic, stearic, and myristic acids (Sumarmono and Sulistiyowati, 2015; Setyawardani et al., 2016; Paszczyk and Łuczyńska, 2020). Low-fat cheese without herbal extract fortification contained lower C7-C12 fatty acids than the other treatments.

The figure above shows that the percentage of short-, medium-, and long-chained fatty acids are 2.77%, 14.43%, and 83.17%, respectively. It is apparent that long-chained fatty acids have the highest proportion and the means of total fatty acids are 67.70%, saturated fatty acids (SFA) are 51.94%, and UFA is 15.76%. The SFA in this study is lower than that reported by (Prandini et al., 2007), namely 65.23 - 68.52%, and the fatty acids PUFA were 3.48-4.17%. High levels of saturated fatty acids in milk and dairy products are often associated with the development of diseases such as cardiovascular, type 2 Diabetes Mellitus, obesity, and cancer (Paszczyn and Łuczyńska, 2020). While the long-chained fatty acids and short-chained fatty acids across treatments are not significantly different (p>0.05), medium-chained fatty acids are lower and significantly different (p<0.05) in low-fat cheese without herbal extract fortification. Fatty acid composition affects the fat yield, which is determined by carbon chains, the number of double bonds, and cis or trans geometric configurations (Markiewicz-Kęszycka et al., 2013). The low level of short-chained total fatty acids (C4-C6) of cheese fortified with herbal extracts is a typical characteristic of fatty acids derived from milk

Table 2. Mean of total cholesterol and fatty acids of cottage cheese with or without herbal fortification

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cholesterol</th>
<th>Total fatty acids</th>
<th>Unsaturated fatty acid</th>
<th>Saturated fatty acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>1.11±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.26±1.51</td>
<td>13.97±0.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.29±1.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>P2</td>
<td>1.13±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>67.00±2.76</td>
<td>18.01±1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49.00±1.80&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>P3</td>
<td>0.99±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>68.18±2.39</td>
<td>14.06±1.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>54.12±1.50&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>P4</td>
<td>1.03±0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>69.73±2.22</td>
<td>27.67±1.29&lt;sup&gt;d&lt;/sup&gt;</td>
<td>42.06±1.00&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>P5</td>
<td>1.01±0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>67.71±2.96</td>
<td>11.97±3.95&lt;sup&gt;e&lt;/sup&gt;</td>
<td>55.75±3.32&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>P6</td>
<td>1.06±0.00&lt;sup&gt;f&lt;/sup&gt;</td>
<td>68.87±3.43</td>
<td>14.69±0.89&lt;sup&gt;f&lt;/sup&gt;</td>
<td>54.18±2.59&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>P7</td>
<td>1.52±0.03&lt;sup&gt;g&lt;/sup&gt;</td>
<td>65.64±1.99</td>
<td>14.18±0.89&lt;sup&gt;g&lt;/sup&gt;</td>
<td>51.46±1.09&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>P8</td>
<td>1.29±0.07&lt;sup&gt;h&lt;/sup&gt;</td>
<td>66.91±2.72</td>
<td>12.94±0.49&lt;sup&gt;h&lt;/sup&gt;</td>
<td>54.81±0.49&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>P9</td>
<td>1.62±0.04&lt;sup&gt;i&lt;/sup&gt;</td>
<td>69.14±0.68</td>
<td>14.33±0.42&lt;sup&gt;i&lt;/sup&gt;</td>
<td>54.81±0.49&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The mean value followed by different letters showed a significant difference at the 5% level (p<0.05).

P1: whole-milk cottage cheese; P2: low-fat cottage cheese; P3: low-fat cottage cheese + 20% bidara leaf extract; P4: low-fat cottage cheese + 20% bay leaf extract; P5: low-fat cottage cheese + 20% moringa oleifera leaf extract; P6: Low-fat cottage cheese + 10% bidara leaf extract + 10% bay leaf extract; P7: low-fat cottage cheese + 10% bidara leaf extract + 10% moringa oleifera leaf extract; P8: low-fat cottage cheese + 10% bay leaf extract + 10% moringa oleifera leaf extract; and P9: low-fat cottage cheese + 6.67% bidara leaf extract + 6.67% bay leaf extract + 6.67% moringa oleifera leaf extract.
and dairy products. Adding herbal extracts to cheese did not affect the number of short-, medium-, and long-chain fatty acids. The number of fatty acid chains was not affected by adding herbal extracts to low-fat cheese. Many factors such as the composition of milk, in terms of fat content and its fatty acid composition, depend on dietary (composition and availability), animal (breed, lactation stage, body condition), and environmental (especially cold and heat stress) factors. Dietary factors that affect milk fat and cheese yield (Nudda et al., 2014). Previous studies on the addition of palm kernel reported that the fatty acid profile experienced an increase in lauric acid (C12) and tridecanoic acids (C13) that do not impose health benefits while not affecting the atherogenicity index (Oliveira et al., 2015).

Conclusion

Herbs *moringa oleifera* leaf extract (20%), bidara leaf extract (10%) + bay leaf extract (10%), and bidara leaf extract (10%) + *moringa oleifera* leaf extract (10%) incorporated into low-fat cottage cheese making have increased the antioxidant level and retain the number of total LAB in cheese compared to non-herbal-fortified cheese.

Conflict of interest

The authors declare that they have no conflicts of interest.

Funding statement

This study was funded by Applied Research Scheme from the Ministry of Education, Culture, Research, and Technology, Indonesia, in 2021 with contract number T/1440/UN23.18/PT.01.01/2021.

Acknowledgement

The authors gratefully acknowledge support from Jenderal Soedirman University and the Indonesian Ministry for Research and Higher Education through Applied Research Grant.

Author’s contribution

This article was written by the corresponding Triana Setyawardani who contributed to the introduction and research materials and methods. Juni Sumarmono has contributed to the implementation of each research method. Hidayah Dwiyanti contributed to the discussion. Naofal Dhia Arkan contributed to the tabulation of research results, and Tasnim Hunin A. Mohamed contributed to the research data analysis.

References


Setyawardani, T. and J. Sumarmono. 2015. Chemical and microbiological


