Cheese whey as potential resource for antimicrobial edible film and active packaging production

Isfari Dinika, Gemilang L. Utama*
Universitas Padjadjaran, Bandung, Indonesia

* e-mail: g.l.utama@unpad.ac.id

Received March 01, 2018; Accepted in revised form June 10, 2019; Published October 03, 2019

Abstract: Many cheese manufacturers still have not utilized cheese whey that damages to the environment as it is directly been drained into waters. Cheese whey can be used as active packaging material to prolong the shelf-life of food products. Fermented cheese whey contains bioactive peptides which are able to improve the functional properties of cheese whey as an antimicrobial agent. The combination of cheese whey with polysaccharides, lipid, and other additional ingredients can improve the physical characteristics of the active packaging in the form of edible film. Around 20–45% of plasticizer will expose the film formed. Cheese whey with agro-industrial waste starch-based formulation can be used as an alternative way to produce an antimicrobial edible film as an active packaging. The film has shown acceptable physical characteristics and high antimicrobial activity, which makes it possible to extend the shelf life of food products. An advanced process, for example, the use of transglutaminase enzyme and Candida tropicalis mutant, is also effective. The result of that is the formation of the essential compound which can improve the active packaging quality. The utilisation of cheese whey and agro-industrial waste based on starch contributes significantly to the environmental conservation.

Keywords: Whey, protein, shelf-life, packaging, antimicrobial, edible film, fermentation, environment


INTRODUCTION

Cheese production process has significant impact on the environment. One of damaging factor is the disposal of cheese by-product. Cheese making process produces large amounts of by-product called cheese whey, which is almost 90% of used milk [1]. It implies if one batch of cheese production uses 100 L of milk, 80–90 L of cheese whey will be produced [2]. Although it is wasted, 30% of cheese whey still has been utilised as animal feed and fertiliser, while the rest has thrown away to the rivers or seas [3]. Cheese whey is able to damage the environment due to its characteristics. Cheese whey has high biochemical oxygen demand (BOD) and chemical oxygen demand (COD), which is more than 35000 and 60000 ppm, respectively [4]. Thus, 4000 L of whey from the cheese industry can damage the environment to the same extent as faecal waste from 1900 humans [5].

On the other hand, whey has a valuable chemical composition and contains 55% of total nutrients in milk [6]. Whey contains (w/w): 93.7% of water, 0.1–0.5% fat, 0.8% protein, 4.9% lactose, 0.5–0.8% ash, and 0.1–0.4% lactic acid [2]. Functionally, the beneficial effect of whey on the human health is due to immunoglobulin and glycoprotein, such as lactoferrin and transferrin, as well as enzymes – lysozyme and lactoperoxidase. All of these components contribute to human immunity and have an antimicrobial activity against allergy reaction [7, 8].

Cheese whey has been utilised in various ways. About 70% of whey is processed into whey powder that can be used in pastry, ice cream, sweets, glazes, sugar dressing, jams, and melted cheese industry [3, 9]. Whey is used as a food ingredient because of its gelling, emulsifying, antimicrobial properties, good solubility, viscosity, nutritional value, as well as the ability to reduce allergenicity [2, 10]. Unfortunately, it is difficult to utilise whey for cheese manufactures [11]. One of the causes is high cost drying process of whey. Therefore, the search of alternative whey processing is of great interest.

Organic compounds of whey are a potential biomass to be utilised as bio-energy. Bioethanol can be made from whey through fermentation by using Kluyveromyces fragilis var marxianus [11–13]. Lactose,
whose content in whey is 4.5–5%, acts as a carbon source for ethanol fermentation. The fermentation also can result in various bioproducts, such as ethanol, biogas (methane), organic acids (acetic, propionic, lactic, citric, and gluconic), amino acids (glutamic, lysine, and threonine), vitamins (B₁₂ and B₃), polysaccharides (gum, dextran, and gellan), lipids, enzymes (polygalacturonase), and others (calcium magnesium acetate, butanol, and glycerol) [11].

The fermentation of whey leads to other compounds which have high functional use, such as bioactive peptides. Native whey has minor bioactive compounds such as lysozyme, lactoperoxidase and lactoferrin that are reported to have antimicrobial activity towards pathogenic bacteria [7]. Fermentation causes protein hydrolysis – by a microorganism which releases bioactive peptides from protein molecules or by a digestive enzyme, such as proteases [14, 15].

Cheese whey can also be utilised as biodegradable packaging material, such as edible film. Such a film is safe to consumers and environmentally friendly. It is also expected to extend the shelf life of food products because it protects them from gases, such as oxygen, carbon dioxide, and ethylene, as well as from water loss [16–18].

The use of the edible film as a food packaging material is expected to reduce plastic waste. Annually, Indonesia produces 3.22 million metric tons of plastic waste. It is the second largest plastic waster after China that produces 8.82 million metric tons. The use of plastic has rapidly increased since the development of plastic commercialisation in the 1930s and 1940s. It reached 288 million metric tons of global plastic resin production in 2012 [19].

The high rate of population growth has caused an increase in food demand. This has resulted in an increasing use of plastic, which contributes to the economic benefits [20, 21]. Food industry still widely uses non-degradable plastic as a food packaging material. However, non-degradable plastic, such as polyethylene (PE), has an immensely slow degradation time under natural environmental conditions [22–24]. Thus, food industry indirectly affects the environment.

The utilisation of cheese whey to produce bioproducts, such as edible films, would be a potential course of action to protect the environment. Cheese whey fermentation can be applied to enhance the antimicrobial effect and the packaging ability of the edible film produced in order to extend the shelf life of packaged food. The aim of this paper was to review the potential of fermented cheese whey in the produce of edible films and active packaging systems.

**STUDY OBJECTS AND METHODS**

The paper was written with non-research methodology based on literature reviews from various sources.

**RESULTS AND DISCUSSION**

**Fermented cheese whey.** The protein content in milk is 3.5% which is, in turn, composed of 80% of casein (α-, β-, and κ-caseins) and 20% of whey proteins (β-lactoglobulin, α-lactalbumin, and others). Several proteins in milk have an antimicrobial effect as shown in Table 1 [31, 33–38]. Whey contains biological active substances, such as enzymes, trace elements, and immunoglobulins which contribute to the good health [25].

<table>
<thead>
<tr>
<th>Source</th>
<th>Protease</th>
<th>Peptide</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine α-casein</td>
<td>Chymosin</td>
<td>Caseidin</td>
<td>Gram-positive bacteria</td>
</tr>
<tr>
<td></td>
<td>Chymosin,</td>
<td>Isracidin asl-CN (f1–23)</td>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td></td>
<td>Chymotrypsin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bovine α-casein</td>
<td>Trypsin</td>
<td>Casoidin-f f (150–188)</td>
<td>Gram-positive and gram-negative bacteria, yeast</td>
</tr>
<tr>
<td>β-Casein</td>
<td>Trypsin and</td>
<td>β-Casein-derived peptides</td>
<td><em>Enterococcus faecium, Bacillus megaterium</em></td>
</tr>
<tr>
<td></td>
<td>chymotrypsin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1 Antimicrobial peptides in milk.
Bioactive peptides can be released in three ways: gastrointestinal digestion (in vivo), fermentation (in vitro), and hydrolysis (in vitro) [31]. Release of bioactive peptides in gastrointestinal tract is the result of enzymatic action. The enzymes are pepsin, trypsin, or chymotrypsin. Pepsin, which is produced from pepsinogen by hydrochloric acid (HCl), converted protein to peptides and amino acids [43]. Other enzymes, such as alcalase and thermolysin, can also stimulate gastrointestinal digestion to produce ACE inhibitory peptides, as well as anti-bacterial, anti-oxidative, immunomodulatory, and opioid peptides[44–49].

Other ways to produce bioactive peptides are microbial fermentation and hydrolysis. For microbial fermentation, such LAB as Lactococcus lactis and Lactobacillus helveticus are used. microbes will use distinct intracellular peptidases including endopeptidases, amino-peptidase, di-peptidase, and tri-peptidase [33]. Hydrolysis of protein molecules can be performed by proteinases, which leads to the release of bioactive peptides. Proteinases are obtained from the secretion of the bacterial and fungal sources [31].

Bioactive peptides produced from fermented whey can be purified to enhance their functional activities. Stepwise filtration can be carried out to extract bioactive peptides after fermentation. Afterwards, the extract is centrifuged at 7000 rpm for 10 min in a refrigerated centrifuge to obtain supernatant. The supernatant is filtered through 0.45 μm and then through 0.22 μm syringe filters. Ultrafiltrate of bioactive peptides of 10 kDa and 5 kDa in size can be obtained after passing through the 10 kDa and 5 kDa MWCO membranes [56].

**Edible film production.** Food products usually have a short shelf-life. In order to prolong it, most of manufacturers uses food packaging. A packaging system should protect the product from contamination during handling, storage, and sale until it reaches retailers and consumers [57]. Non-degradable packaging still has widely applied by food industry. the US Environmental Protection Agency (EPA) reports that 31% of municipal solid waste (MSW) is packaging waste [58]. Edible films as a packaging material can be an effective solution of reducing waste because of their degradable characteristics [57].

The ideal edible film has high water holding ability; controls gas exchanges; inhibits solute transport, organic vapour transfers, as well as oil and fat migration; improves mechanical properties of food to simplify handling and carriage; has neutral sensory characteristics, improving sensory properties of food.
products [57]. Edible films should consist of components produced mainly from edible biopolymers and food grade additives. The additives should meet safety requirements to food additives and to be at least GRAS (Generally Recognized as Safe) [59].

There are two ways to create an edible film: wet process and dry process. Wet process applies dispersion in a solvent such as water, alcohol, or mixture of water and alcohol, or other solvents. The film-forming solution is then casted and dried to obtain films. The dry process does not require any solvent. It can be produced by compression, molding, or extrusion [60, 61]. The film-making and coating processes include melting and solidification of solid fats, waxes, and resins; conservation of hydrocolloid; complex conservation of two hydrocolloids; and thermal gelation or coagulation by heating [57]. Therefore, the dry process usually needs more equipment, which results in higher cost compared to the wet process.

In edible film production, the incorporation of a certain additive is possible to form an advanced system called active packaging [62]. The additive compound enhances shelf-life and stability of the product, as well as improves its microbiological safety and sensory attributes [63]. The following additives can be used in edible films: flavouring agents, spices, antimicrobial substances, antioxidants, pigments, light absorbers, salts, etc. Antioxidants and antimicrobial additives are commonly used in order to prevent spoilage and thus enhance safety. Antimicrobial agents, being used in active packaging, can overcome the hurdles of uncontrolled migration and interaction of an active compound of various natural antioxidants used directly in food [64, 65].

**Comparison of characteristics from various film bases.** Edible film or even active packaging usually use polysaccharide, protein, lipid, or composite base to make a film forming solution. Thus, fermented whey can be one of multifunctional ingredients and act as a film-forming base and an antimicrobial agent.

Researchers have focused on the use of composite based films to explore the complement advantages of each component [64, 66]. A composite based film can be both one-layer and multiple-layer. The matrix of hydrophilic and hydrophobic lipid, which is called bi-layer composite system, has better functional characteristics than pure hydrocolloid films. However, one of disadvantages of bi-layers composite systems is longer preparation process. It requires two casting and two drying stages, which has made these laminated films less popular in food industry [67]. In order to enhance holding properties of active packaging, scientists have studied its mechanical properties (Table 3). These are transparency, oxygen permeability, carbon dioxide permeability, water vapor permeability, emulsion stability, and glass transition temperature.

Generally, lipid films have the less structural integrity compared to protein or polysaccharide films [68]. The use of lipids in edible films has resulted in heterogenous film structure that has an impact on discontinuities in the polymer and production of a strong emulsion matrix [69]. A composite film based on polysaccharide has the greatest mechanical properties, which allows its using in gastronomy. Along with protein added, polysaccharide film is an optimal active packaging.

Besides the film based component, the composition of the edible film is also an important factor. Plasticizer is one of substantial components to create a flexible film by reducing interaction between intermolecular starch [70]. Examples of plasticizer are polyol groups such as glycerol, xylitol, sorbitol, mannitol, and sucrose [18, 70]. Xanthan gum and carrageenan are also promising plasticizers which provide the product with strength and durability with great sensory properties. They demonstrate high stabilizing ability and resistance to water, [71]. The plasticizer is able to reduce intermolecular bonds between amylose, amylpectin, and amylose-amylopectin in the starch matrix and replace them with hydrogen bonds between plasticizer and starch. This reduces brittle and enhances flexibility [68].

**Table 3 Edible film characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Effective components for base making</th>
<th>Ineffective components for base making</th>
<th>Additional info</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical properties</td>
<td>Flexibility and texture of film</td>
<td>Composite, pure hydrocolloid matrix</td>
<td>Lipid (good in coating, bad in film)</td>
<td>[72]</td>
</tr>
<tr>
<td>Transparency</td>
<td>Lightness and colourless of film</td>
<td>Emulsified films with lipid</td>
<td>–</td>
<td>[73]</td>
</tr>
<tr>
<td>Oxygen permeability</td>
<td>O&lt;sub&gt;2&lt;/sub&gt; transfer through film</td>
<td>Hydrocolloid, protein</td>
<td>Hydrophobic</td>
<td>[74]</td>
</tr>
<tr>
<td>Carbon dioxide permeability</td>
<td>CO&lt;sub&gt;2&lt;/sub&gt; transfer through film</td>
<td>Cellulose films, protein</td>
<td>Lipid (stearic acid and palmitic)</td>
<td>[75]</td>
</tr>
<tr>
<td>Water vapour permeability</td>
<td>Moisture transfer through film</td>
<td>Hydrophobic (lipid)</td>
<td>Polysaccharide, Protein</td>
<td>[76]</td>
</tr>
<tr>
<td>(WVP)</td>
<td>Lower WS needed to protect food from moisture loss</td>
<td>Lipid</td>
<td>–</td>
<td>[77]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Better at smaller and more homo-genous lipid distributed</td>
<td>[78]</td>
</tr>
</tbody>
</table>

The flexibility of the film depends on the concentration of the plasticizer in the film-forming solution. High or low concentrations would result in anti-plasticization. For example, glycerol in the amount of over 30% used in the starch-based film is the case. It will result in a decrease in such characteristics as elongation at break. It was established that strong interaction between plasticizer and other molecules blocked the macromolecular mobility [70, 81]. On the other hand, if the plasticizer concentration is too low, the film formed will be brittle and hard to handle. Generally, the optimal concentration of plasticizer is 20–45% [82].

The potential of fermented whey for active packaging composite. The composite of edible film can be made to complement each single material-based film characteristics. Protein is usually used as one of material-based because of its nutritional value [83]. The comparison of the composite edible film using protein-based is shown in Table 4. From all of the sources of protein, soya and whey have been mostly used.

As a food barrier capability, the addition of oil has resulted in a lower WVP, which is showed in a comparison of soya protein with oil and with pectin. Thereby, we can conclude that a composite protein film with oil has better barrier properties. However, the water solubility of whey protein with starch is higher than that of soya protein with pectin. In terms of mechanical properties, whey protein with starch provides a better result than soy protein with pectin. However, some other parameters cannot be compared because of the difference between the film production and the analysis method. Based on the description above, it is possible to conclude that the whey protein is more effective than soya protein to produce the edible film.

Functional characteristics of fermented whey make it promising raw material for active packaging. Bioactive peptides from fermented whey have had their ability to act as an antimicrobial agent; immunomodulatory peptides regulate cell-mediated and humoral immune functions; ACE inhibitory peptides lower blood pressure; and opioid peptides are effective against hypotension, lack of appetite, etc., as well as exhibit antioxidant properties, protecting cells from free radicals [31].

Several bioactive peptides derived from whey protein are also known for its capability to enhance the defence towards various pathogenic bacteria and yeast. Their antimicrobial activity can inhibit the growth of such microorganisms effectively. The incorporation of bioactive peptides into film is more effective way to lower the concentration of microorganisms than direct using them in food. Thus, it allow avoiding unwanted flavours and odor of food [88].

In terms of the characteristics, a good characteristic can be achieved if the protein contained in the cheese whey is mixed with starch that contained polysaccharides. With the addition of plasticizer, this composite base film will have good mechanical properties as well as the barrier ability to prolong food shelf-life.

<table>
<thead>
<tr>
<th>Table 4 Characteristics of composite film base</th>
<th>Source</th>
<th>Method of making</th>
<th>WVP, g/mm·h·kPa</th>
<th>Water solubility, %</th>
<th>Tensile strength, MPa</th>
<th>Elongation at break, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Native wheat flour</strong></td>
<td>Glycerol</td>
<td>Dissolving (separately), heating at shaking (25°C, RH 40%, 24 h)</td>
<td>1.10 ± 0.08</td>
<td>0.008 ± 0.005</td>
<td>0.95 ± 0.008</td>
<td>3.29 ± 0.09</td>
</tr>
<tr>
<td><strong>Soya protein isolate</strong></td>
<td>Vegetable glycerin, sorbitan monostearate as surfactant</td>
<td>Emulsification (mixing 1300 rpm, 5 min), casting, drying (RH 55%, 25°C, 30 min)</td>
<td>0.113 ± 0.008</td>
<td>2.70 ± 0.046</td>
<td>1.60 ± 0.005</td>
<td>0.70 ± 0.18</td>
</tr>
<tr>
<td><strong>Almond protein</strong></td>
<td>Glycerol</td>
<td>Dissolving (60°C, 30 min), cooling, mixing (1300 rpm, 5 min), casting, drying (25°C, RH 55%, 25°C, 30 min)</td>
<td>0.119 ± 0.002</td>
<td>5.52 ± 0.43</td>
<td>0.007 ± 0.005</td>
<td>1.10 ± 0.60</td>
</tr>
<tr>
<td><strong>Almond protein</strong></td>
<td>Vegetable glycerin, sorbitan monostearate as surfactant</td>
<td>Emulsification (mixing 1300 rpm, 5 min), casting, drying (RH 55%, 25°C, 30 min)</td>
<td>0.113 ± 0.008</td>
<td>2.70 ± 0.046</td>
<td>1.60 ± 0.005</td>
<td>0.70 ± 0.18</td>
</tr>
<tr>
<td><strong>Whey protein isolate</strong></td>
<td>Vegetable glycerin, sorbitan monostearate as surfactant</td>
<td>Emulsification (mixing 1300 rpm, 5 min), casting, drying (RH 55%, 25°C, 30 min)</td>
<td>0.113 ± 0.008</td>
<td>2.70 ± 0.046</td>
<td>1.60 ± 0.005</td>
<td>0.70 ± 0.18</td>
</tr>
</tbody>
</table>
Advanced process to create composite fermented cheese whey film. Nowadays, an advanced process to create an edible composite is based on film-forming solution. The film with cellulose, whey and sunflower oil based are stirred with Ultraturrax homogenizer at 11000 rpm for 3 min to decrease the droplet sizes of oil. As a result, the smaller droplet sizes can disperse uniformly in the hydrocolloid matrix. Therefore, the penetration of water into film will be harder, which will result in better water vapour transfer. The combination of the degassed method under vacuum (80 kPa) and a vacuum pump for 5 min will result in the film production with tensile strength of 8.59 MPa, elongation at break 35.94%, WVP 3.211 g/m² s Pa, and transparency of 3.637 % [79].

There are several methods available to increase the stability and the quality of characteristics of active packaging. The addition of enzyme is one of methods to enhance the film quality in the complex edible film. The presence of Transglutaminase (TGase) has caused enzymatic cross-linking in P/P soluble electrostatic aggregates. Thus, TGase can strongly produce composite bioplastics by escalating the mechanical and barrier characteristics. Supramolecular structure of P/P complex as enzyme substrate is crucially influencing pH of a film-forming solution. With the addition of TGase, film characteristics at pH (pH complexation around 3.25–5.5, when soluble P/P complexes occur) create better characteristics than higher pH. The pH can significantly increase tensile strength and elongation at break, and reduce Young’s modulus and WVP [89].

For the usage of fermented cheese whey in the active packaging, there is an advanced process that can be added in the fermentation process. Candida sp. is one of the yeast that has already found in Serro Minas, a cheese from Brazil [90]. Recently, a study for identifying the indigenous yeast that contained in homemade mozzarella whey has also found that Candida sp. contained in mozzarella whey and Greek fermented whey [91]. It means that Candida sp. is naturally contained in cheese whey and can live to ferment the whey [92]. Candida spp. is also known as the most effective yeast to produce xylitol with 63–70% w/w yields. According to several studies, C. tropicalis mutant maximises the xylitol production, reaching 100% yields [93]. It is known that xylitol is one of sugar alcohol that can be utilized in the film production as a plasticizer [18]. Fermented cheese whey can act as an antimicrobial agent and natural plasticizer.

Prospects of the use of edible films and active packaging with cheese whey. The edible film can be an effective solution to reduce plastic waste of food packaging. Addition of several antimicrobials can also be used to prolong the shelf life to reach a proper packaging system which is similar to the plastic packaging. Thus, fermented cheese whey as a base ingredient of composite film system is able to meet this requirement. Despite some disadvantages of protein, its combination with other ingredients make is possible to obtain an excellent film with required characteristics.

Besides various modification of film manufacture, the cost in creating edible film must be taken into account. The edible film should be cost-effective compared to plastic, paper, or any other packaging that can harm the environment. Thus, advancing the edible film production is important to make film characteristics as high as characteristics of plastic packaging.

The simplified process of cheese whey fermentation using indigenous yeast can also increase the antimicrobial properties of the fermented cheese whey. In the future, advancing film manufacture process from fermented cheese whey can be one of massive ways to create modern environmentally-friendly packaging.

CONCLUSION
Cheese whey, a by-product of cheese-making process, has several functional effects, including inedible film formation. Bioactive peptides contained in native cheese whey can be enhanced by fermentation to generate high antimicrobial activity. In addition, a composite edible film can be produced from fermented whey and starch to gain good mechanical characteristics as well as a good barrier to prolong food shelf-life. The utilisation of fermented cheese whey as an edible film material allows obtaining an active packaging system with high antimicrobial activity.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

ACKNOWLEDGEMENTS
Authors thank the Ministry of Research, Technology and Higher Education of The Republic of Indonesia that funded the research through ‘Penelitian Tesis Magister’, 2019. This article’s publication is supported by the United States Agency for International Development (USAID) through the Sustainable Higher Education Research Alliance (SHERA) Program for Universitas Indonesia’s Scientific Modeling, Application, Research, and Training for City-centered Innovation and Technology (SMART CITY) Project, Grant #AID-497-A-1600004, Sub Grant #IIE-00000078-UI-1.

REFERENCES


ORCID IDs

Gemilang L. Utama https://orcid.org/0000-0003-3382-2855