Research Article

Application of Microencapsulated Bamboo Leaf Extract Powder to Control the Rancidity in Moo Yor (Vietnamese-style Sausage) During Refrigerated Storage

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Abstract
Recently, bamboo leaf extract (BLE) is considered as a new source of natural antioxidants. Microencapsulation technique is one of the potential methods to protect bioactive compounds. In this study, a microencapsulated bamboo leaf extract (MBLE) and its ability to prevent lipid oxidation in fat-containing product was investigated. Moo Yor, a Vietnamese-style sausage is one of the short shelf life meat products due to rancidity developed from lipid oxidation during refrigerated storage. A fat-rich Moo Yor, accounted for 14% fat content, was prepared and MBLE was added at 0, 1, 2, and 3% (w/w), comparing with the addition of conventional synthetic antioxidant butylated hydroxytoluene (BHT). All samples were collected every 2 days throughout 8 days of storage at 4°C to investigate the change of phenolic compounds and lipid oxidation. The gradually reduction of lipid oxidation throughout storage in Moo Yor with BHT was higher than those added with 2% MBLE and 1% MBLE except in Moo Yor with 3% MBLE. The remaining of total phenolic compound in Moo Yor samples was increased in the first 2 days and then decreased throughout storage. The decreasing of total phenolic compounds in control was higher than those of Moo Yor with 1% MBLE, 3% MBLE, BHT, and 2% MBLE, respectively. These results implied that 2% MBLE was the most effective antioxidant to prevent rancidity in fat-rich Moo Yor during 8-day storage at 4°C.

Keywords: Bamboo leaf extract, Natural extract, Microencapsulation, Antioxidant, Lipid oxidation

1 Introduction

Bamboos are group of giant, woody grass, which is abundantly distributed in both tropical and subtropical areas [1]–[3]. Although more than 1400 bamboo species distributed all over the world [4], but major species of bamboo are found in Asia Pacific and South America [2], [5]. Moreover, bamboo leaves have been used as traditional Chinese medicine for treating fever, detoxification, stomach heat, phlegm disorder, and hypertension for over 1000 years [2]–[4], [6].

Bamboo leaves have been considered as a natural source of antioxidant [7]. Recently, several studies of antioxidant activity in bamboo leaves carried out to identify the active compounds, including the development of extraction and analysis method. The three main bioactive components of bamboo leaf extract (BLE) are flavonoids, phenolic acids, and lactones [5], [6], [8]–[11]. The C-glycoside flavonoids were represented by orientin, isoorientin, vitexin, isovitexin [12]–[14], homovitexin [1], [12], and tricin [1], [6], while the major phenolic compounds were p-coumeric acid, chlorogenic acid, caffeic acid, and ferulic acid [1], [4], [15]. In 2005, Zhang [8] reported his clinical and animal studies and confirmed that BLE had an antioxidant activity. The antioxidant in BLE was able to block the chain reaction of lipid autoxidation, chelating metal ions of transient state,

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scavenging nitrite compounds, and blocking the synthetic reaction of nitrosamine [12], [15]. In China, BLE has been approved as a natural food additive for meat products, edible oils, cereals, bakery products, and fried foods [14], [16]. Even though, several studies have been carried out to investigate and identify the antioxidant activity from BLE, the mechanism of BLE to inhibit the lipid autoxidation has not been completely understood.

Normally, natural antioxidants such as flavonoids and phenolic compounds are known to sensitive to pH, metal ions, light, temperature, oxygen, and enzymatic activities [17]. The stability of antioxidant is an important aspect to consider for applying of antioxidants in foods. Therefore, microencapsulation is a technique providing longer shelf-life by protecting antioxidants with encapsulating agent and packing the sensitive ingredients within a coating material [18]. Maltodextrins is one of carbohydrates that have been extensively used as encapsulating agents. Maltodextrin is water soluble materials and it has low viscosity at high solids ratio. Moreover, it is available in different molecular weights, which provides different wall densities around the sensitive materials [19], [20].

Moo Yor or Yor is Vietnamese-style sausages. It is one of popular meat emulsion products in Thailand made by grinding and blending pork meat with ice cubes, fat, various curing agents such as salt, sugar, ground spices (pepper or garlic), or monosodium glutamate. Moo Yor has a paste-like texture in the raw state, but gradually changes into a more rigid structure during heating process. It has short shelf life approximately two weeks in refrigerator [21] because pork meat and fat can rapidly develop oxidative rancidity under chilled or frozen storage conditions [22]. Therefore, this research was aimed to study the effect of microencapsulated bamboo leaf extract (MBLE) on the qualities of Vietnamese-style sausages (Moo Yor) during refrigerated storage.

2 Material and Methods

2.1 Materials

Fresh leaves of Pai Ruak or Thai bamboo (*Thrysostarchy siamensis* Gamble) were obtained from Lampang province, Thailand. Gallic acid monohydrate and catechin were purchased from the Sigma–Aldrich (Switzerland). Maltodextrin 20DE was sponsored by Corn products (Thailand) Co., Ltd. Pork meat, lard, tapioca starch, baking powder, salt, and sugar were purchased from local market.

2.2 *Preparation of bamboo leaf extract*

Fresh bamboo leaves Pai Ruak were cleaned and dried using tray dryer at 45°C for 2 h, and then ground to coarse powder using mechanical grinder. Dried bamboo leaves powder 2% (w/v) was soaked with 75% aqueous ethanol and subjected to 48 h maceration with constant shaking (120 rpm) at room temperature. The mixture was then filtered and centrifuged at 5,000 rpm for 5 min.

2.3 *Microencapsulation of bamboo leaf extract*

Bamboo leaf extract (BLE) (5% v/v) was agitated at 600 rpm using overhead stirrer (VELP Scientifica, Italy). After that Maltodextrin 20DE (95% w/v) was gently added into 5% BLE, the mixture was continued stirring for 15 min and then desiccated under vacuum at a condenser temperature of –40°C for 24 h with freeze-dryer. Dried cakes were ground into powder and stored in an air-tight container at room temperature.

2.4 *Investigation the capacity of microencapsulated bamboo leaf extract (MBLE) against lipid oxidation in Moo Yor (Vietnamese-style sausage)*

2.4.1 Preparation of Moo Yor

Pork meat and lard were separately ground using grinding machine and mixed with baking powder, salt, sugar, and tapioca starch as the formula shown in Table 1. Marinated pork was frozen for 1 h before finely ground with ice using meat grinder. MBLE powder was added into marinated pork mixture at 0, 0.05, 0.15, and 0.2% (w/w), while BHT was added at 0.02% (w/w). All components were thoroughly mixed until homogenized. Raw marinated pork mixture was tightly packed into heat-resistant plastic box and steamed for 45 min. Moo Yor was subsequently cooled down before keeping in a refrigerator at 4°C.

Table 1: Basic formula of fat-rich Moo Yor (Vietnamese-style sausage)

Raw material	% (w/w)
Pork	76.87
Lard	19.22
Baking powder	1.20
Salt	1.15
Sugar	0.82
Tapioca starch	0.74

2.4.2 To study the changes of antioxidant activity in Moo Yor during storage

Moo Yor samples were collected on day 0, 2, 4, 6, and 8 of storage and were kept at –20°C to investigate the changes of phenolic compounds by Folin-Ciocalteu method [23].

2.4.3 To investigate the development of lipid oxidation during storage

Moo Yor samples were collected on day 0, 2, 4, 6, and 8 of storage and were kept at –20°C until the analysis of the development of lipid oxidation reaction by modified Thiobarbituric acid-reactive substances (TBARS) method [24]. TBARS assay was performed by standard method using malonaldehyde (MDA) in the range of 20 to 100 nm.

Sample was weighed 10 g and homogenized with 30 mL of 7.5% Trichloroacetic acid (TCA) by using centrifugation (HERMLE® Z200, Wehingen, Germany) at 5,000 rpm for 15 min, followed by filtration (No.1 Whatman®, USA). Extract (1 mL) was mixed with 1.5 mL of 0.8% Thiobarbituric acid (TBA) in ethanol. Then, the tube was heated to 70°C and hold for 30 min in water bath. After cooling, the samples were measured for the absorbance at OD 531 nm (UNICO S1200, NJ, USA).

3 Results and Discussion

The effect of MBLE on the inhibition of lipid autoxidation was studied by adding MBLE powder into fat-rich Moo Yor at various contents (1, 2, and 3% w/w) comparing with control (no antioxidant) and synthetic antioxidant as BHT (0.02%). From the preliminary experiment, the amount of MBLE added to Moo Yor should not be higher than 3% (w/w)

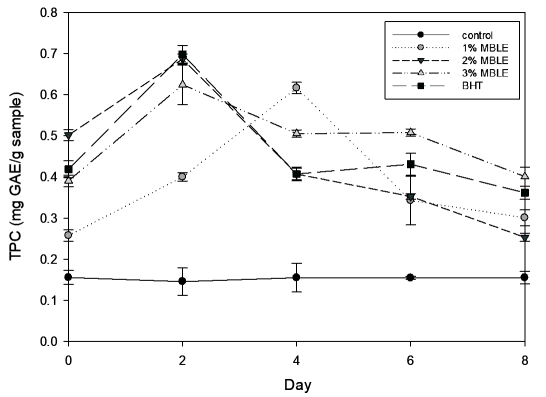

Figure 1: The changes of total phenolic compound in fat-rich Moo Yor with various antioxidants during 8-day refrigerated storage.

because the addition of high amount of MBLE powder affected the texture and sensory quality of Moo Yor. It caused the rougher texture of Moo Yor. Moreover, few panellists could detect the glitter during sensory evaluation and left their comments about sandy texture in the questionnaires. For clearly observation of the development of lipid autoxidation, fat-rich Moo Yor was formulated by substitution 25% of lean meat with lard in the basic formula.

Fat-rich Moo Yor collected on the day 0, 2, 4, 6, and 8 of storage at 4°C and then kept at –20°C for further investigation of the development of lipid oxidation and the remaining antioxidant activity. Even though, a previous study reported that shelf life of Moo Yor was approximately 2 weeks under refrigerated storage [21], [25], fat-rich Moo Yor had shorter shelf life than general formula according to its high fat content. From our preliminary experiment, fat-rich Moo Yor could be able to keep in refrigerator only for 1 week.

Frozen Moo Yor samples were thawed and blended with water. The supernatants were used to investigate the amount of antioxidant activity by Folin-Ciocalteu method using gallic acid as the standard. As shown in Figure 1, the total phenolic compounds in control was lowest amount and was not change throughout storage as expected. Interestingly, Moo Yor without MBLE showed low level of antioxidant activity even though it supposed to have no antioxidant activity. These results implied that there was some antioxidant appeared in control sample and this should be one of the ingredients used to produce Moo Yor.

As shown in Table 1, the ingredients in Moo Yor recipe included pork, lard, baking powder, salt, sugar, and tapioca flour which baking powder is a potential ingredient that has an antioxidant activity. Amorati and Valgimigli [26] reported that the presence of any reducing agents provided the positive result for Folin-Ciocalteu method. Therefore, it could be assigned as an antioxidant. Previous studies reported the reducing activity of baking powder, a mixture of baking soda and various acidic ingredients. Stahl studied the effect of baking soda and baking powder on the antioxidant activity in homemade cocoa products and they found that the increasing ratio of baking powder showed the higher amount of total phenolic compound (TPC) [27]. Thus, the trace amount of TPC in fat-rich Moo Yor with no antioxidant possibly resulted from the activity of baking powder in the formula.

Fat-rich Moo Yor containing BHT, 2% MBLE, and 3% MBLE showed the same trend that TPC increased in the first two days of storage, then decreased throughout storage at 4°C, while the treatment with 1% MBLE showed the highest TPC on 4 day. These results implied that the rate of BLE releasing to fat-rich Moo Yor was slower in sample containing 1% MBLE than the other sample. The increasing of TPC during the first two or four days was caused by lesser lipid oxidation at the initial stage. Thus, TPC was released from MBLE and accumulated in the sample before it was used to retard the lipid oxidation later.

Figure 2 showed the changes of lipid oxidation developed in fat-rich Moo Yor during 8-day refrigerated storage as the present of Malonaldehyde (MDA). MDA is a dicarbonyl compound which is well known as one of many aldehyde products of lipid peroxidation [28]. MDA has been widely used for many years as a convenient biomarker for lipid autoxidation especially fish and meat products [29] because of its reaction with thiobarbituric acid (TBA) to form an intensely pink-red colored [28]–[30]. Thus, the present of MDA implied the development of lipid autooxidation. The lower MDA developed in the sample with the addition of antioxidant implied that the antioxidant has the better antioxidant property.

As the results, the lipid oxidation in fat-rich Moo Yor reduced during the storage except for 3% MBLE that the lipid oxidation increased at the beginning, slightly constant, and decreased during the end of storage. These results implied that BHT, 1% MBLE, and 2% MBLE showed antioxidant activity to inhibit the development of lipid oxidation, resulting in a reduction of lipid oxidation during the storage, while the excess MBLE (3%) could be acted as prooxidant and activated the development of lipid oxidation.

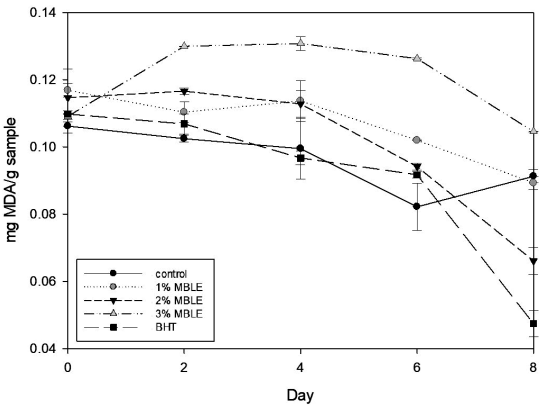

Figure 2: The changes of lipid oxidation product Malonaldehyde (MDA) in fat-rich Moo Yor with various antioxidants during 8-day refrigerated storage.

In 1997, Cao *et al*. reported the prooxidant activity of flavonoids. They noted that prooxidant activity of flavonoid depended upon the number of OH substitutions in the flavonoid structure. Many previous studied also reported the important flavonoid in BLE that were orientin, isoorientin, vitexin, isovitexin [12]–[14], homovitexin [1], [12] and tricin [1], [4]. Therefore, it could be assumed that these specific flavonoids found in MBLE acted as prooxidant if MBLE added at a high amount (more than 2% w/w) increasing the lipid oxidation in fat-rich Moo Yor during the early of 8-day storage. The best MBLE content to retard lipid oxidation in Moo Yor during 8-day storage was found to be 2% (w/w) MBLE.

4 Conclusions

BLE was microencapsulated by freeze drying in order to protect it during application in the fat-rich Moo Yor from degradation which can reduce its antioxidant activity. Addition of MBLE effectively reduced the development of lipid oxidation in fat-rich Moo Yor throughout 8 days of refrigerated storage. This result could be regarded as an important factor for the reduction of malonaldehyde in fat-containing foods by addition of MBLE. In this study, 2% (w/w) MBLE

was found to be the most effective to retard the lipid oxidation. Meanwhile, the mechanism of antioxidant activity of MBLE release mechanism and packaging conditions in foods will be further considered and evaluated.

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