International Journal of Agricultural Technology 2023 Vol .19(3):1165-1178
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Bacterial cellulose production and application on a fat replacer on fat-reduced Chinese sausage

Oonnom, P., Rungjindamai, N. and Ochaikul, D.*

Department of Biology, School of Science, King Mongkut's Institute of Technology Ladkrabang, Chalongkrung Rd. Ladkrabang, Bangkok, 10520, Thailand.

Oonnom, P., Rungjindamai, N. and Ochaikul, D. (2023). Bacterial cellulose production and application on a fat replacer on fat-reduced Chinese sausage. International Journal of Agricultural Technology 19(3):1165-1178.

Abstract Bacterial cellulose (BC) produced by *Acetobacter xylinum* TISTR 976 strain was used as a fat replacer in Chinese sausage and the properties of this product were investigated in the present study. The BC was prepared by fermentation with a coconut medium for 10 days. To remove bacterial cells, the purified BC was soaked in 1% (w/v) NaOH. The bacterial cellulose powder (BCP) was prepared by two methods including soaking in NaOH (BCP-M1) and grinding and soaking in NaOH (BCP-M2). The BC was neutralized, squeezed and kept in -20 ℃. The frozen BC were freeze dried at -50 ℃ for 8 h. The BC dried product was finally ground into fine powder (diameter 0.5 mm). The second method (BCP-M2) produced the better quality of BC by showing physical appearance, water holding capacity, oil holding capacity were not significant difference from commercial BCP. In addition, BCP-M2 presented non-cytotoxic effect to Vero cell by MTT cytotoxicity. BCP can be replaced for pork fat for Chinese sausage production. The fat contents of Chinese sausage were reduced significantly (1-2%) when BCP was incorporated into the Chinese sausage. Fiber contents were increased significantly in this product compared to those of control (containing no BCP and 24% fat). 1% BCP treatment and control treatment had the higher springiness scores than other samples. Textural hardness was significantly increased for BCP-added treatment. Moreover, the concentration of 1% BCP also increased acceptable sensory qualities. The product shelf life in the vacuum-package was studied at 4 ℃ and room temperature (30±2 ℃) for 28 days. Thiobarbituric acid value was increased at room temperature storage. When it was stored at 4 ℃, there is a few changes of concentration of thiobarbituric acid. The result indicated that BCP could be used to replace fat in the production of Chinese sausage.

Keyword: Bacterial cellulose, Chinese sausage, Fat replacer

Introduction

Nowadays, consumers are aware of the potential health risks associated with high-fat diets if consumed in excess has affect health and lead to serious disease such as obesity, cardiovascular disease and colon cancer (Jimenez-Colmenero *et al*., 2001). Therefore, health agency recommends reduced fat consumption as a way to reduce risk factors in the development of disease (Garcia *et al*., 2002). Thus leading to develop new

*Corresponding Author: Ochaikul, D.; **Email:** daungjai.oc@kmitl.ac.th

food recipes or transform traditional food products having a reduced fat content (Jimenez-Colmenero *et al*., 2001).

Chinese sausage is the typical meat product from Chinese that has gained widespread popularity among customers due to its good flavor and taste. Chinese sausage is made from pork and pork fat which is chopped or ground into small pieces, seasoned with sugar, salt, soy sauce and food additive. The marinated pork is filled in artificial sausage casing, dried in the air or hot air oven. On the other hand, Chinese sausage contains almost 30%-fat which it adverse health effect associated if overconsumption. To solve these issues the traditional chinese sausage fat content must be reduced by choosing for a fat replacer (Izidoro *et al*., 2007., Mun *et al*., 2009). However, fat influences the properties and quality such as flavor, texture and shelf life of food product. If the fat level of the Chinese sausage is reduced, the quality of the sausage may deviate from the original recipe. Therefore, it is necessary to find a fat substitute without altering the original quality.

Bacterial cellulose (BC) is produced by acetic acid bacteria (Esa *et al*., 2014). This aerobic gram-negative bacteria actively fermented at pH 3 to 7 and temperature between 25 to 30˚C (Castro *et al*., 2011) such as *Acetobacter* sp. and *Gluconacetobacter* sp. *Acetobacter xylinum* is the most common bacterium used to produce cellulose because of its high efficiency in cellulose production. Cellulose produced by bacteria has different properties and structures than cellulose produced by plants such as high polymer crystallinity, high purity and high water-holding capacity (Akoglu *et al*., 2018). Bacterial cellulose is commonly used as s thickening agent, stabilizer or fat replacer in food industry (Akoglu *et al*., 2015). In the research of Lin and Lin (2004) studied on the physicochemical, texture and quality characteristic of Chinese-style meatball (20% fat) containing varying level of bacterial cellulose found that bacterial cellulose could be used as functional ingredient in Chinese-style emulsified meat product and the research of Akoglu *et al.* (2015) studied on the effect of bacterial cellulose as a fat replacer in sucuk (Turkish dry fermentation sausage) found that bacterial cellulose could be used as fat replacer in sucuk. In addition, when adding bacterial cellulose caused a reduction in food retention in intestine of rat (Lin and Lin, 2014). Bacterial cellulose has been accepted by the Food and Drug Administration in 1992 “generally recognized as safe” (Khan *et al.*, 2007). Therefore, the aim of this study was investigated cellulose powder production from bacterium and the effect of addition bacterial cellulose power for fat substituted on some quality characteristics and sensory properties in Chinese sausage.

Materials and methods

Preparation of starter culture

Acetobacter xylinum TISTR 976 was cultivated on standard Glucose-Yeast extract-Calcium carbonate Agar (GYC) at 30˚C for 3 days. Two loopfuls of the bacterium were transferred into 100 mL of a sterilized coconut water medium in a 250-mL Erlenmeyer flask. The coconut water medium consisted of coconut water, 5% (w/v) sucrose, 1% (w/v) ammonium sulfate and 1% (v/v) acetic acid. The starter culture was incubated statically at 30˚C for 3 days.

Bacterial cellulose (BC) production using coconut water medium

The bacterial cellulose production was performed in 500-mL fermented jars containing 100 mL of the sterilized coconut water medium. Each jar was inoculated with 10% (v/v) of the starter culture and incubated statically at 30 ℃ for 14 days. The thickness of cellulose membranes was measured using vernier caliper. The BC yield analysis was dried in hot air oven at 70 ℃ until constant weight was obtained. The bacterial cellulose yield was expressed as dry weight of BC per volume of culture medium (g/L).

Purification of BC sheet

The purified BC sheet was determined according to Kamal *et al*. (2020) with slight modifications. *Acetobacter xylinum* TISTR 976 was grown on coconut water medium at 30℃ for 10 days. After fermentation process, the produced BC membranes were washed with water and extruded water using squeezer and followed with purification process by soaking with different concentration of sodium hydroxide solution: 0% (control), 0.5%, 1.0%, 2.0% and 3.0% (w/v) at room temperature for 24 h to remove bacterial cells. The purified BC sheet were then boiled with water for 30 min and rinsed with water until the neutral pH and then kept in -20 ℃ for 24 h after that freeze dried at -50 ℃ in freeze dryer for 8 h. The purified BC sheet was analyzed using Scanning Electron Microscope, SEM (magnification of 5,000X). The SEM analysis sent for inspection with the Nanotechnology and Material Analytical Instrument Service Unit (NMIS) at King Mongkut's Institute of Technology Ladkrabang.

Preparation of bacterial cellulose powder (BCP)

The preparation of BCP was determined according to Lumbikananda *et al*. (2018) with slight modifications. The BCP was prepared by two methods including;

BCP-M1: BC sheet were rinsed with water to remove culture medium and soaked in 1% (w/v) sodium hydroxide at room temperature for 24 h to remove bacteria. After that, the BC sheet were then boiled with

water for 30 min and rinsed with water until the neutral pH and then kept in -20 ℃ and dried in freeze dryer (-50 ℃) for 8 h. The BCP-M1 was obtained using pin mill (sieve diameter 0.5 mm).

BCP-M2: BC sheet were rinsed with water and homogenized using electrical blender for 30s and soaked in 1% (w/v) sodium hydroxide for 24 h. BC pastes were boiled in water for 30 min and rinsed with water until the neutral pH and dried in freeze dryer for 8 h. The BCP-M2 was finely grind using pin mill (sieve diameter 0.5 mm).

Analysis of BCP

Water holding capacity (WHC) and Oil holding capacity (OHC): The preparation of WHC and OHC was determined to Ang (1991) with slight modifications. The samples 1 g of BCP was mixed with 20 mL of distilled deionized water for WHC analyzing and mixed with 15 mL of palm oil for OHC analyzing contained in 50 mL centrifuge tube. The slurry was allowed to stand for 10 min, the centrifuged at 3,000 rpm for 30 minute in a centrifuge (Hermil Labrtechnik GmbH, Germany). After centrifugation, the supernatant solution was drained and wet BCP precipitate was weighed. The water holding capacity (WHC) and oil holding capacity (OHC) was calculated as follow:

$$\text{WHC (g } H_2O \text{ absorbed/g BCP)} = \frac{\text{Final sample weight} - \text{Original sample weight}}{\text{original sample weight}}$$

$$\text{OHC (g Oil absorbed/g BCP)} = \frac{\text{Final sample weight} - \text{Original sample weight}}{\text{Original sample weight}}$$

Moisture: The moisture was determined according to AOAC (2000). The moisture can contained 3 g BCP was oven at 105 ℃ for 3 h. After that, the sample was placed in desiccator. The moisture was calculated as follows:

$$\% \text{ Moisture} = \frac{\text{Weight of sample before drying} - \text{Weight of sample after drying}}{\text{Weight of sample before drying}} \times 100$$

Colour measurement and water activity (a_w): It was made with a Hunter Lab colorimeter (USA) using the color space CIE lightness *L**, redness *a**and yellowness *b**system. Bacterial cellulose powder (BCP) 3 g was put on a plate and obtained three plates for each sample. Measurements were performed at three points on the powder.

MTT cytotoxicity: The MTT cytotoxicity analysis sent for inspection with the Scientific Instrument Center at King Mongkut's Institute of Technology Ladkrabang. The cell line is African Green Monkey kidney

(Vero cell) at a concentration of $1x10^5$ cells/mL and the sample a concentration of 1000 µg/mL.

Formulation and process of Chinese sausage

Chinese sausages were prepared with the ingredients listed in Table 1. Different level of BCP (0.5%, 1.0%, 2.0% and 3.0% w/v) were added to the mixture including 23.50%, 23.00%, 22.00% and 21.00% of pork fat, respectively. Four different treatments were prepared. Control treatment contained no BCP but only 24.00% pork fat. Pork meat was mixed with the ingredients and stuffed in artificial sausage casing with a 2.1-cm diameter and 17-cm length. Then Chinese sausage samples were hung on stainless steel hangers and were place in tray dryer (Kluay Nam Thai Trading Group, Thailand). The dried temperatures were as follows: 85 ℃ for 3 h, 65 ℃ for 16 h and 60 ℃ for 3 h until drying process was ended. The samples were placed into the plastic bags and vacuum packaged.

Table 1. Formulation of Chinese sausage

Ingredients	**samples**				
	Control	**Cs1**	**Cs2**	**Cs3**	**Cs4**
Pork meat	55.00	55.00	55.00	55.00	55.00
Pork Fat	24.00	23.50	23.00	22.00	21.00
Bacterial cellulose powder	-	0.50	1.00	2.00	3.00
Sugar	20.00	20.00	20.00	20.00	20.00
Salt	0.80	0.80	0.80	0.80	0.80
Seasoning Sauce	0.16	0.16	0.16	0.16	0.16
Sodium nitrite	0.01	0.01	0.01	0.01	0.01
Spice powder	0.03	0.03	0.03	0.03	0.03
Total	100.00	100.00	100.00	100.00	100.00

Control = 24% fat with 0% BCP; Cs1 23.5% fat with 0.5% BCP; Cs2 23% fat with 1.0% BCP; Cs3 22% fat with 2.0% BCP and Cs4 21%fat with 3.0% BCP

Shelf life of Chinese sausage

All samples were packed in vacuum bag 2 piece/bag and kept at 4˚C and room temperature (30±2 ℃) for 28 days (Kumar *et al*., 2007). The samples were drawn at 7 days interval for assessment of texture properties and sensory attributes.

Analysis of Chinese sausage

Chemical analysis: Moisture content (oven air-drying), protein (Kjeldhal nitrogen) and ash (muffle furnace) content were determined according to the Association of Official Analytical Chemists (2000). Fat content was analyzed according to Soxhelet method. All determinations were performed in triplicate.

Color measurement: It was made with a Hunter Lab colorimeter (USA) using the color space CIE lightness *L**, redness *a**and yellowness *b**system. Chinese sausage samples were cut and obtained three slices for each sample. Measurements were performed at three points on the central part of the cut surface.

Textural analysis: The textural properties of the samples were determined according to Yu and Lin (2014) and slight modifications. The samples were cut having a 10-mm high then analyzed using a texture analyzer (Lloyd Instruments Ltd Fareham, England). A test speed 40 mm/s and 90% compression stain were used combining with a 1-KN load cell and P/50 adaptor (35-mm diameter cylinder) to determine parameters such as hardness, springiness, cohesiveness and chewiness.

Thiobarbituric acid (TBARS): The TBARS analysis sent for inspection with the Department of Nutrition, Faculty of Public Health at Mahidol University.

Sensorial analysis

Thirty panelists, student of the Department of Biology School of Science, King Mongkut's Institute of Technology Ladkrabang were performed about sensorial evaluation of this product. A test was carried out using hedonic scales, which the panelists evaluated different attributes: colour, odor, texture, flavor and overall acceptability (1= dislike extremely and 9= like extremely). The averages of scores were calculated and then the samples were sorted for preference of panelists.

Statistical analysis

An analysis of variance was used to evaluate the effects of different concentrations of BCP and pork fat on the overall attributes of Chinese sausage. The differences were tested by Duncan Multiple Rang Test (DMRT) at a confidence level of 5% ($P<0.05$) using the SPSS statistical package.

Results

BC production using coconut water medium

Acetobacter xylinum TISTR 976 was grown in coconut water medium for 14 days. BC yield and the thickness of cellulose membranes increased during 4 to 10 days and on day 10, BC yield had the highest (8.86 ±0.01 g/L). The BC yield and the thickness were slightly changed after day 10, as shown in Figure 1.

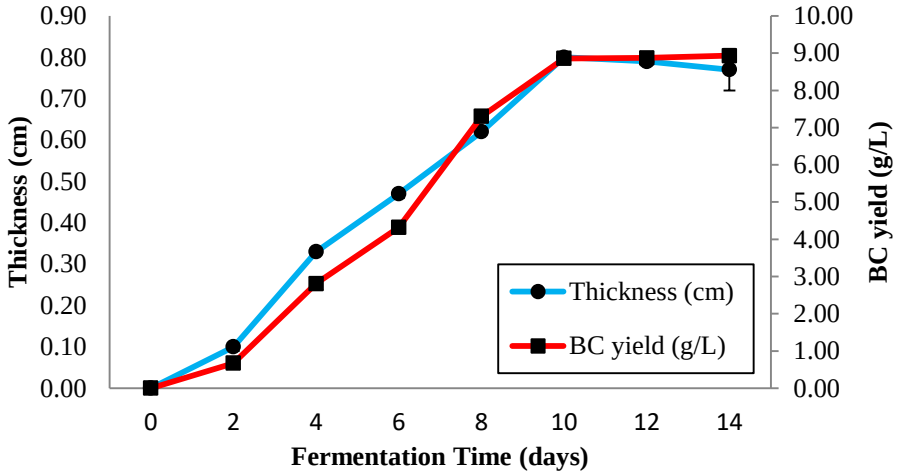

Figure 1. BC yield and thickness of BC membranes from *Acetobacter xylinum* TISTR 976 in coconut water medium

Purification of BC membranes

BC membranes were obtained from the cultivation of *Acetobacter xylinum* TISTR 976 on coconut water medium at 30 ℃ for 10 days. After incubation, BC membranes were rinsed with water and pressed with the press machine into sheets. BC membranes were subsequently treated with various concentration of NaOH solution (0.5%, 1.0%, 2.0% and 3.0% w/v) for 24 h to eliminate bacterial cell. The result shown that purified with 0.5% and 1.0% w/v had color of BC sheet similar to control (0% w/v, NaOH) whereas using 2.0% and 3.0% w/v, BC sheets became more yellow (Figure 2).

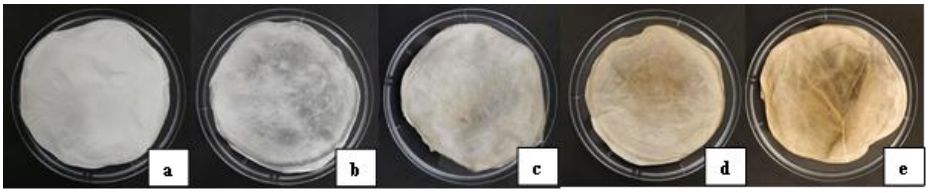

Figure 2. Purified BC sheets with various NaOH concentration (a) 0%, (b) 0.5%, (c) 1.0%, (d) 2.0% and (e) 3.0% w/v, NaOH

The purified BC sheet was analyzed using SEM, The surface morphologies were shown in Figure 3. The apparent bacterial cells were observed on the surface of 0% and 0.5% NaOH, while 1.0% NaOH can eliminate cell on surface of BC sheet.

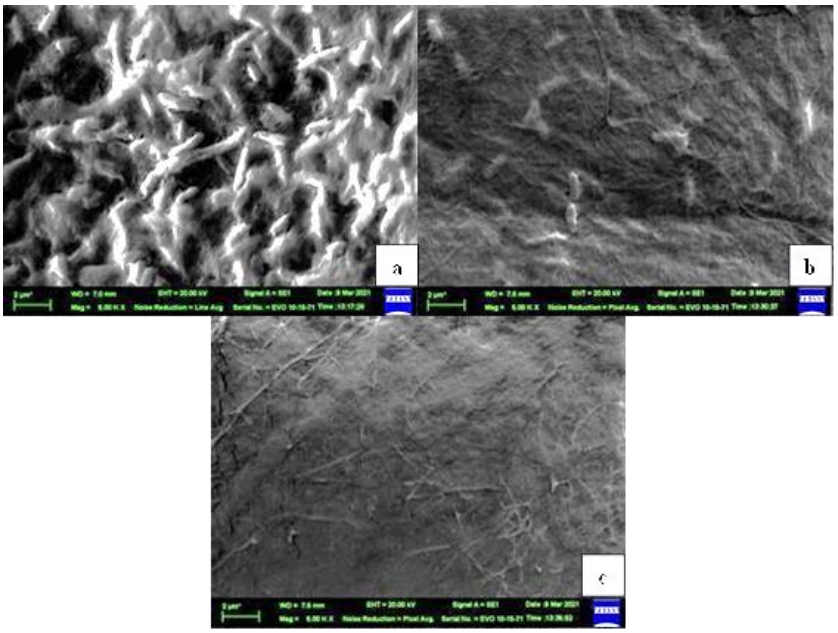

Figure 3. SEM images of surface of BC sheet (a) 0% NaOH (b) 0.5% NaOH and (c) 1.0% NaOH at a magnification of 5,000X

Production of bacterial cellulose powder (BCP)

The appearance of BCP was shown in Figure 4. The properties of BCP samples were shown in Table 2. BCP obtained from 2 methods (BCP-M1 and BCP-M2) had a significant effect (P<0.05) on some properties. The water holding capacity (WHC) and oil holding capacity (OHC) of BCP-M2 were similar to commercial BCP and higher than BCP-M1 except %cytotoxicity was lower. Then BCP-M2 was used in Chinese sausage.

Figure 4. Appearance of BC powder (a) commercial BCP, (b) BCP-M1 and (c) BCP-M2

Table 2. Properties of bacterial cellulose powder

	Commercial BCP	BCP-M1	BCP-M2
Water Holding Capacity ; WHC	9.63±0.09[a]	7.91±0.20[b]	9.15±0.10[a]
Oil Holding Capacity; OHC	7.30±0.20[a]	6.24±0.15[b]	7.39±0.05[a]
Moisture	4.02±0.12[a]	2.96±0.13[b]	2.66±0.06[b]
Water activity; a_w	0.59±0.10[a]	0.53±0.00[b]	0.52±0.00[b]
*L**	80.05±0.18[b]	78.57±0.18[c]	81.98±0.54[a]
*a**	1.46±0.05[b]	3.51±0.03[a]	1.67±0.14[b]
*b**	12.55±0.10[b]	14.77±0.08[a]	10.61±0.15[c]
% Cytotoxicity	22.58±0.01[b]	26.35±0.01[a]	20.66±0.02[b]

BCP-M1= soaking in NaOH, BCP-M2= grinding and soaking in NaOH
[a-c] Values with the different superscript letter in the same row show significant differences (P<0.05)

Effect of bacterial cellulose powder for fat replacer of Chinese sausage

Chemical properties of the Chinese sausage samples were shown in Table 3. Reduction of fat content and addition of BCP-M2 in different concentrations had a significant effect (P<0.05) on fat and fiber of samples. The treatments with reduced pork fat had higher in fiber content than that of the treatment of control. The fat content of the control was 23.48±0.01%, while Cs1, Cs2, Cs3 and Cs4 treatments had final fat content of 22.85±0.06%, 21.91±0.06%, 20.40±0.05% and 19.24±0.09%, respectively.

Table 3. Chemical properties of Chinese sausage samples

	Control	Cs1	Cs2	Cs3	Cs4
Moisture (%)	75.34±0.56[a]	74.63±0.04[a]	74.41±0.14[ab]	73.25±0.48[bc]	72.27±0.39[c]
Protein (%)	13.26±0.12[a]	12.68±0.23[bc]	12.73±0.14[ab]	12.59±0.10[bc]	12.13±0.21[c]
Fat (%)	23.48±0.11[a]	22.85±0.06[b]	21.91±0.06[c]	20.40±0.05[d]	19.24±0.09[e]
Ash (%)	2.48±0.01[b]	2.54±0.04[b]	2.56±0.02[b]	2.64±0.01[ab]	2.84±0.15[ab]
Fiber (%)	0.19±0.00[d]	1.19±0.06[cd]	2.10±0.40[c]	3.66±0.81[b]	8.98±0.31[a]

Control = 24% fat with 0% BCP; Cs1 23.5% fat with 0.5% BCP; Cs2 23% fat with 1.0% BCP; Cs3 22% fat with 2.0% BCP and Cs4 21%fat with 3.0% BCP
[a-e] Values with the different superscript letter in the same row show significant differences (P<0.05)

Color analysis

In the present study, the Chinese sausage samples showed no different in color between control sample and Chinese sausage in which BCP-M2 replaced of the pork fat. The Hunter *L**, *a**, *b** value in Chinese sausage samples were shown in Table 4. The appearance of Chinese sausage samples were shown in Figure 5.

Table 4. Color properties of Chinese sausage samples

	Control	Cs1	Cs2	Cs3	Cs4
*L**	28.12±0.03[a]	29.41±0.02[a]	31.91±0.03[a]	32.14±0.01[a]	32.29±0.02[a]
*a**	9.05±0.03[a]	9.06±0.01[a]	9.09±0.00[a]	9.07±0.00[a]	9.07±0.00[a]
*b**	1.76±0.05[a]	1.82±0.03[a]	1.73±0.12[a]	1.85±0.09[a]	1.75±0.03[a]

Control = 24% fat with 0% BCP; Cs1 23.5% fat with 0.5% BCP; Cs2 23% fat with 1.0% BCP; Cs3 22% fat with 2.0% BCP and Cs4 21%fat with 3.0% BCP

[a] Values with the different superscript letter in the same row show significant differences (P<0.05)

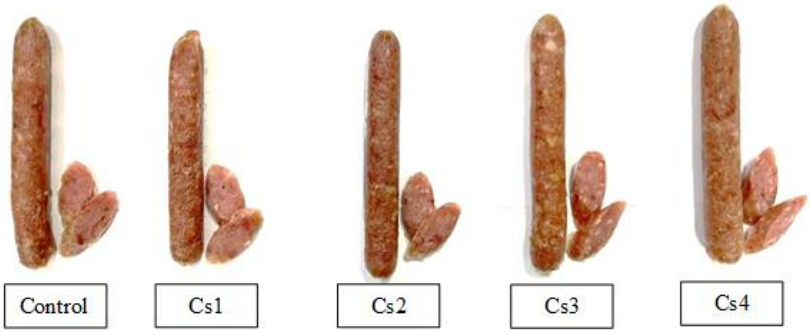

Figure 5. Appearance of Chinese sausage samples

Texture profile analysis

Texture profile (hardness, springiness, cohesiveness, chewiness) of Chinese sausage samples were compared with each other and result were shown in Table 5. Treatment with addition of BCP- M2 had significantly higher hardness, chewiness and cohesiveness values than the treatment with control. Springiness values of Cs1 and Cs2 treatments are not significant different from control treatment except Cs3 and Cs4 samples.

Table 5. Textural properties of Chinese sausage samples

	Control	Cs1	Cs2	Cs3	Cs4
Hardness (N)	188.02±1.34[c]	187.09±2.99[c]	205.71±2.46[c]	277.97±11.75[b]	486.19±9.50[a]
Springiness	0.67±0.00[b]	0.67±0.00[b]	0.67±0.00[b]	0.64±0.00[a]	0.64±0.00[a]
Cohesiveness	0.13±0.00[c]	0.14±0.00[c]	0.14±0.00[c]	0.16±0.00[b]	0.17±0.01[a]
Chewiness (Nm)	0.14±0.01[c]	0.14±0.01[c]	0.14±0.00[c]	0.34±0.05[b]	0.45±0.07[a]

Control = 24% fat with 0% BCP; Cs1 23.5% fat with 0.5% BCP; Cs2 23% fat with 1.0% BCP; Cs3 22% fat with 2.0% BCP and Cs4 21%fat with 3.0% BCP

[a-c] Values with the different superscript letter in the same row show significant differences (P<0.05)

Sensorial analysis

The effect of BCP-M2 on the sensory properties of Chinese sausage samples were shown in Table 6. There were no difference in colour, odor, texture, flavor and overall acceptability value between the control, Cs1 and Cs2 treatment. The Cs3 and Cs4 treatment had lower texture and overall acceptability value.

Table 6. Sensory properties of Chinese sausage samples

	Control	Cs1	Cs2	Cs3	Cs4
Colour	7.47±0.15[a]	7.43±0.19[a]	7.43±0.12[a]	7.50±0.09[a]	7.47±0.12[a]
odor	7.10±0.17[a]	6.93±0.20[a]	6.90±0.18[a]	7.10±0.19[a]	6.93±0.14[a]
Texture	6.83±0.20[a]	6.80±0.27[a]	6.73±0.26[ab]	5.83±0.18[b]	4.70±0.28[c]
Flavor	6.43±0.34[a]	6.50±0.31[a]	6.63±0.34[a]	6.73±0.33[a]	6.27±0.31[a]
Overall acceptability	6.83±0.24[a]	6.87±0.21[a]	6.80±0.34[a]	563±0.25[b]	5.43±0.26[b]

Control = 24% fat with 0% BCP; Cs1 23.5% fat with 0.5% BCP; Cs2 23% fat with 1.0% BCP; Cs3 22% fat with 2.0% BCP and Cs4 21%fat with 3.0% BCP

[a-c] Values with the different superscript letter in the same row show significant differences (P<0.05)

The Cs1 and Cs2 treatment were the higher fiber content and lower fat. In addition, they were acceptable by consumers similar to the control treatment. Meanwhile, Cs2 treatment can be reduce fat and higher in fiber than Cs1. This research was investigated BCP used as a fat replacer in Chinese sausage so the shelf-life of the Cs2 treatment has been further studied.

Shelf life of Chinese sausage

The Cs2 treatment was packed in vacuum bag, kept at room temperature (30±2 ℃) and 4 ℃ for 28 days. The changes in texture properties during storage were shown in Table 7. The texture characteristics were not much altered during storage.

Table 7. Texture properties of Chinese sausage during storage at room temperature (30 ±2 ℃) and 4 ℃ for 28 days

Day	Temperature (℃)	Hardness (N)	Springiness	cohesiveness	Chewiness (Nm)
0	Room temperature (30±2 ℃)	313.24±0.01[a]	0.49±0.07[a]	0.21±0.05[a]	0.29±0.06[a]
7		318.47±0.03[a]	0.47±0.01[a]	0.19±0.02[a]	0.38±0.03[a]
14		339.45±0.02[a]	0.41±0.03[a]	0.13±0.01[a]	0.24±0.07[a]
21		336.15±0.04[a]	0.37±0.07[a]	0.16±0.04[a]	0.28±0.13[a]
28		341.90±0.01[a]	0.39±0.02[a]	0.15±0.02[a]	0.31±0.04[a]
0	4	313.24±0.01[a]	0.49±0.07[a]	0.21±0.05[a]	0.29±0.06[a]
7		347.77±0.03[a]	0.33±0.05[b]	0.12±0.02[ab]	0.27±0.04[bc]
14		342.39±0.04[a]	0.33±0.01[ab]	0.15±0.02[ab]	0.29±0.04[ab]
21		342.34±0.05[a]	0.33±0.01[ab]	0.15±0.02[ab]	0.31±0.01[c]
28		349.52±0.02[a]	0.28±0.06[b]	0.09±0.03[b]	0.30±0.04[bc]

[a-c] The column different letter of each temperature show significant differences (P<0.05)

The TBARS of Chinese sausage was shown as Figure 6. The Cs2 treatment kept at 4 ℃ for 28 days, TBARS was lower (23.64±0.55 μg MDA/g) than that of room temperature (28.40±0.39 μg MDA/g)

For sensory test, the Cs2 treatment was no differences in the colour, odor, flavor and texture during storage time. The Chinese sausage was well accepted after 28 days of storage at room temperature and 4 ℃. The result was shown in Figure 7.

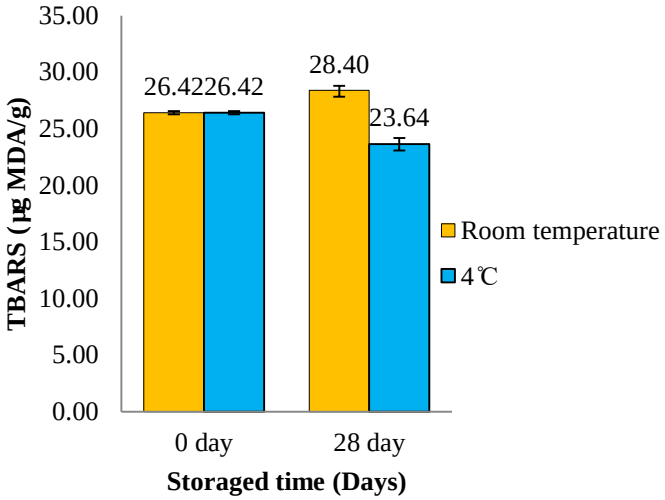

Figure 6. TBARS during storage at room temperature (30±2 ℃) and 4 ℃ for 28 days

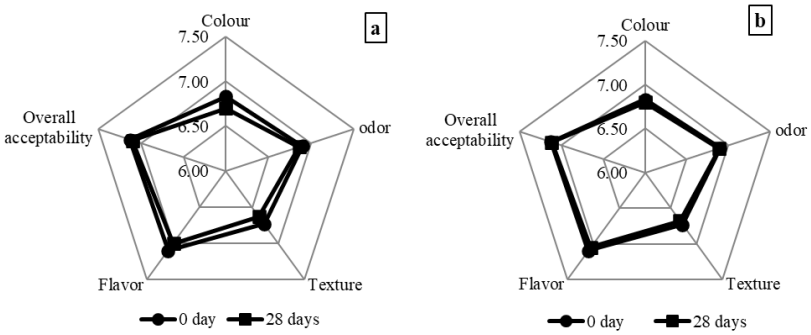

Figure 7. Sensory properties of Chinese sausage samples during storage a: room temperature, b: 4 ℃

Discussion

In keeping with the current health trend toward lower fat and increased fiber intakes in food, this research provided the bacterial cellulose (BC) production and application on a fat replacer on fat-reduced Chinese sausage. The BC production using coconut water medium of *Acetobacter xylinum* TISTR 976 were high on 10 days of fermentation process, which BC yield was 8.86±0.01 g/L. This result is consistent with the report of Sutthiphatkut *et al*. (2019) and Nugroho and Aji (2015). 1.0% w/v of NaOH can be removed bacterial cell from BC membranes and color of BC membranes no changed. Santos *et al*. (2014) reported that the thermal treatment could not remove the bacterial cell from BC membrance. In contrast, no bacterial cells were observed after the alkaline treatment. In addition, Kamal *et al*. (2020) reported that 1.0% NaOH solution had

provided better results in the effective removal of impurities with minimum change on BC membrane, similar to the present study. The bacterial cellulose powder (BCP-M2) production from grinding BC membranes and soaking in 1.0% w/v, NaOH had water holding capacity and oil holding capacity (OHC) similar to commercial BCP and lower %cytotoxicity (20.66±0.02) than BCP-M1. Lumbikananda *et al*. (2018) reported that the wet grinding BC powder method was white colour and soft texture, %cell viability was higher (98.07%) which were greater than 70%. The same observation was noted in the present study.

For this reason, the BCP-M2 was replaced fat in Chinese sausage. The reduction of fat content and addition of different concentrations of the BCP-M2 was effect on properties of Chinese sausage. The results indicated that the treatments with reduced pork fat had higher in fiber content than that of the control treatment. At the same time, textural had increase when added of BCP-M2 due to BCP is insoluble fiber, non-cholesterol, low-fat and low-calories (Shah and Brown, 2005). There was no significant difference in colour between control treatment and samples treatment, which was similar to Muguerza *et al*. (2001). Increasing BCP-M2 resulted in significantly difference in texture and overall acceptability of the sensory attributes tested except of colour, odor and flavor. The Cs1 and Cs2 treatment were higher fiber content and lower fat including the acceptable by consumers. Akoglu *et al*. (2015) reported that the addition of BC affected hardness value of sucuk samples. Whereas no significant differences were observed in odor, color, flavor and overall acceptability between samples. In the present study, the Cs2 treatment (23% fat with 1.0% BCP) was more amount of the BCP than that of Cs1 treatment. The properties of Cs1 and Cs2 and was no differences. So, the shelf-life of the Cs2 was investigated at 4 ℃ and room temperature (30±2 ℃) for 28 days. Thiobarbituric acid value was less at 4 ℃ due to TBARS has been used to determine the degree of lipid oxidation if the storage temperature is low, it will reduce lipid oxidation of the product (Wenjiao *et al*., 2014). There is no significant difference in most of textural properties and sensory attributes test. They are acceptable to consumers throughout the shelf life. The results indicated that BCP could be used as fat replacer in the production of reduced fat Chinese sausage.

Acknowledgements

This work was supported by King Mongkut's Institute of Technology Ladkrabang (Grant number KREF016309).

References

Ang, J. F. (1991). Water retention capacity and viscosity effect of powdered cellulose. Journal of Food Science, 56:1682-1684.

Akoglu, A., Cakır, I., Akoglu I. T., Karahan, A. G. and Cakmakci, M. L. (2015). Effect of bacterial cellulose as a fat replacer on some quality characteristics of fat reduced sucuk. GIDA, 40:133-139.

Akoglu, A., Caktr, I., Karahan, A. G. and Cakmakci, M. L. (2018). Effects of bacterial cellulose as a fat replacer on some properties of fat-reduced mayonnaise. Romanian Biotechnological Letters, 23:13674-13680.

AOAC. (2000). Official method of analysis of AOAC international: The Association of Official Analytical Chemists, 17th. Washington D.C.

Castro, C. E., Dong, J., Boyce, M. C., Lindquist, S. and Lang, M. J. (2011). Physical properties of polymorphic yeast prion amyloid fibers. Biophysical Journal, 101:439-448.

Esa, F., Tasirin, S. M. and Rahman, N. A. (2014). Overview of bacterial cellulose production and application. Agriculture and Agricultural Science Procedia, 2:113-119.

Garcia, M. L., Dominguez, R., Galvez M. D., Casas C. and Selgas, M. D. (2002). Utilization of cereal and fruit fibres in low fat dry fermented sausages. Meat Science, 60:227-236.

Izidoro, D., Sierakowski, M. R., Waszczynskyj, N., Haminiyk, C. W. I. and Scheer, A. D. P. (2007). Sensory evaluation and rheological behavior of commercial mayonnaise. International Journal of Food Engineering, 3:1-15.

Jimenez-Colmenero, F., Carballo, J. and Cofrades, S. (2001). Healthier meat and meat products: their role as functional foods. Meat Science, 5:5-13.

Kamal, A. S. M., Misnon, M. I. and Fadil, F. (2020). The effect of sodium hydroxide concentration on yield and properties of bacterial cellulose membranes. The 1st Annual Technology, Applied Science and Engineering Conference, 1-7.

Khan, T., Park, J. K. and Kwon, J. H. (2007). Functional biopolymers produced by biochemical technology considering applications in food engineering. Korean Journal of Chemical Engineering, 24:816-826.

Kumar, M., Sharma, B. D. and Kumar, R. R. (2007). Evaluation of sodium alginate as a fat replacer on processing and shelf-life of low-fat ground pork patties. The American Journal on Addictions, 20:588-597.

Lin, K. W. and Lin, H. Y. (2004). Quality characteristics of Chinese-style meatball containing bacterial cellulose (nata). Journal of science, 69:249-257.

Lumbikananda, N., Siridamrong, P., Vairojanawong, T., Poonthananiwatkul, C., Laungsopapun, G., Srichan, R. and Boonruang, A. (2018). Preparation of bacterial celluloae powder for medical materials: part 1. Srinakharinwirot Science Journal, 31:278-286.

Muguerza, E., Gimeno, O., Ansorena, D. and Bloukas, J. g. (2001). Effect of replacing pork backfat with pre-emulsified olive oil on lipid fraction and sensory quality of chorizo de Pamplona-a traditional Spanish fermented sausage. Meat Science, 59:251-258.

Mun, S., Kim, Y. L., Kang, C. G., Park, K. H., Shim, J. Y. and Kim Y. R. (2009). Development of reduced-fat mayonnaise using 4GTase-modified rice starch and xanthan gum. International Journal of Biological Macromolecules, 44:400-407.

Nugroho, D. A. and Aji, P. (2015). Characteristics of nata de coco produced by fermentation of immobilized *Acetobacter xylinum*. Agriculture and Agricultural Science Procedia, 3:278-282.

Shah, J. and Brown, R. M. Jr. (2005). Towards electronic paper displays made from microbial cellulose. Applied Microbiology and Biotechnology, 66:352-355.

Santos, S. M., Carbajo, J. ., Quintana, E., Ibarra, D., Gomez, N., Ladero, M., Eugenio, M. E. and Villar, J. C. (2014). Characterization of purified bacterial cellulose focused on its use on paper restoration. Carbohydrate Polymers, 116:173-181.

Sutthiphatkut, T., Suwanposri, A. and Ochaikut, D. (2019). Optimization of bacterial cellulose production from wastewater of noodle processing by *Komagataeibacter* sp. PAPI and bio-cellulose paper production. Walailak Journal, 17:1241-1251.

Wenjiao, F., Yongkui, Z., Yunchuan, C., Junxiu, S. and Yuwen, Y. (2014). TBARS predictive models of pork sausages stored at different temperatures. Meat Science, 96:1-4.

Yu, S.Y. and Lin, K.W. (2014). Influence of bacterial cellulose (nata) on the physicochemical and sensory properties of frankfurter. Journal of Food Science, 79:1117-1122.

(Received: 12 August 2022, accepted: 10 April 2023)