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Investigation on the Lipid- and Cholesterol-Lowering Abilities of Biocellulose

CHI-FAI CHAU,[†] PAT YANG,[§] CHAO-MING YU,[§] AND GOW-CHIN YEN*,[†]

Department of Food Science and Biotechnology, National Chung Hsing University, 250 Kuokuang Road, Taichung 40227, Taiwan, and Chia-Meei Food Industrial Corporation, 56 Dawei Road, Dali City, Taichung County 41259, Taiwan

The present study investigated and compared the physicochemical properties as well as the hypolipidemic and hypocholesterolemic effects between plant cellulose and biocellulose. Biocellulose had higher water-holding and cation-exchange capacities than plant cellulose (~2- and 6-fold, respectively). The results showed that the administration of plant cellulose and biocellulose to hamsters effectively ($P < 0.05$) decreased the concentrations of serum triglyceride (by 13.9–55.5%), serum total cholesterol (by 17.4–27.9%), serum low-density lipoprotein cholesterol (by 41.9–47.9%), liver total lipids (by 6.4–10.3%), and liver cholesterol (by 11.8–16.3%). Feeding plant cellulose and biocellulose also enhanced the excretion of total lipids (144–182%), cholesterol (136–203%), and bile acids (259–479%) in feces. The efficacy of biocellulose in lowering serum lipids and cholesterol in hamsters was significantly higher than that of plant cellulose. These results suggested that biocellulose could be a promising low-calorie bulking ingredient for the development of novel fiber-rich functional foods of different forms such as powder, gelatinous, or shred forms.

KEYWORDS: Biocellulose; plant cellulose; cholesterol; lipid; bile acids

INTRODUCTION

Research has demonstrated and health organizations recommend that sufficient consumption of plant cellulose and other common dietary fibers from cereals, fruits, and vegetables could promote beneficial physiological functions including blood lipid and cholesterol attenuation, laxation, and reduced risk of cardiovascular diseases (*1–3*). The hypocholesterolemic and hypolipidemic effects of food fibers are generally related to their composition, source, physicochemical properties, and abilities to enhance cholesterol and bile acid excretion (*4–7*). The importance of food fiber has encouraged a continuing search for new fiber sources of valuable functions and properties for functional food applications.

Unlike insoluble plant cellulose (the basic structural matrix of plant cell walls), biocellulose, also called bacterial cellulose or nata, is a gelatinous cellulosic pellicle produced by an acetic acid-resistant bacterium, *Acetobacter xylinum* (*8*). In Asia, biocellulose is a popular gelatinous and springy fiber-rich food ingredient used in baked goods, desserts, and snacks as well as added in drinks and beverages. It could be used as a thickener to maintain viscosity in food and to retard glucose and bile acid diffusion effectively (*9, 10*). It has also been used in a wide range of nonfood applications such as high-quality audio membranes, cosmetics, skin care products, medical materials, and nonwoven fabric or paper for old document repair (*10–12*). Although plant cellulose and biocellulose are chemically the same, biocellulose has some special physical and chemical properties such as high crystallinity and hydrophilicity, ultrafine network architecture, purity (free of lignin), and moldability during formation (*11, 13*). As there is a close relationship among the physicochemical properties, hypocholesterolemic action, and potential food applications of insoluble fibers (*5, 7*), it is worth studying the properties and physiological functions of biocellulose to exploit its potential food applications.

The present in vivo study was to investigate and compare the effects of biocellulose and plant cellulose on the absorption and excretion of lipids and cholesterol in hamsters fed diets supplemented with cholesterol (2.0 g kg^{-1} of diet). Some physicochemical properties of the biocellulose and plant cellulose were studied. Relationships between physicochemical properties and physiological functions of these fiber samples were also considered in this study.

MATERIALS AND METHODS

Biocellulose and Cellulose Samples. Fresh biocellulose pellicle synthesized by *A. xylinum* was supplied by the Chia-Meei Food Industrial Corp. (Taichung County, Taiwan). The moisture content of the fresh biocellulose pellicle sample was 981 ± 0.50 g kg^{-1} on a fresh weight basis. After lyophilization, the dried biocellulose sample was ground in an impact grinding mill (IKA MF10.2, Staufen, Germany) to pass through a 0.5 mm sieve. The biocellulose powder sample was kept in a desiccator until used. The plant cellulose (Alphacel 900453) was purchased from ICN Nutritional Biochemicals (Cleveland, OH).

* Author to whom correspondence should be addressed (telephone +886-4-22879755; fax +886-4-22854378; e-mail gcyen@nchu.edu.tw).
[†] National Chung Hsing University.
[§] Chia-Meei Food Industrial Corp.

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Table 1. Formulations of the Experimental Diets

ingredient[a]	fiber-free diet	cellulose diet	biocellulose diet
casein	140	140	139
cellulose		51.7	
biocellulose			74.5
sucrose	100	100	100
corn starch	669	617	595
soybean oil	40	40	40
choline bitartrate	2.5	2.5	2.5
L-cystine	1.8	1.8	1.8
AIN-93 M vitamin mix	10	10	10
AIN-93 M mineral mix	35	35	35
cholesterol	2.0	2.0	2.0

[a] The ingredients are expressed as g kg^{-1} of diet (dry weight). Casein, cellulose, choline bitartate, L-cystine, AIN-93 M vitamin mix, AIN-93 M mineral mix, and cholesterol were obtained from ICN Nutritional Biochemicals (Cleveland, OH).

Chemical Analyses. According to AOAC methods (*14*), moisture (method 934.01) and total ash (method 942.05) were determined. Crude protein content was measured in duplicate and estimated by multiplying the nitrogen content measured with a CHN-OS rapid element analyzer (Heraeus F002, Hanau, Germany) by a factor of 6.25. The contents of insoluble and soluble dietary fibers (AOAC method 985.29) (*14*) were determined using a K-TDFR fiber assay kit (Megazyme, Wicklow, Ireland). Following AOAC method 994.13 (*14*) with slight modifications, the neutral sugar profiles of the insoluble dietary fiber (IDF) derived from the biocellulose were determined by hydrolyzing the IDF fraction in 12 M H_2SO_4 at 35 °C for 60 min and further boiling in 2 M H_2SO_4 for another 60 min. The residual indigestible substance in the hydrolysate was quantified as Klason lignin gravimetrically. The released neutral sugars in the hydrolysate were quantified as alditol acetates by gas chromatography (Hitachi G-5000, Tokyo, Japan) fitted with a flame ionization detector. Allose was used as an internal standard. GC operating conditions were as follows: column, Quadrex 007-225 (15 m × 0.53 mm i.d.); oven temperature, initially held at 100 °C for 3 min, then raised to 220 °C at a rate of 4 °C min^{-1}; injector and detector temperatures, 270 °C; gas flow rates, 2.1 mL min^{-1} (carrier gas, nitrogen) and 500 mL min^{-1} (air). The noncellulosic glucose contents in the IDF fraction and biocellulose powder were determined by hydrolyzing the powder sample with 2 M H_2SO_4 at 100 °C for 60 min. The cellulose content in the IDF fraction was calculated from the difference between its total glucose and noncellulosic glucose contents.

Determination of Physicochemical Properties. The water-holding capacity (WHC, mL g^{-1}), oil-holding capacity (OHC, g g^{-1}), bulk density (g mL^{-1}), and cation-exchange capacity (CEC, mequiv kg^{-1}) of the fiber powder samples were determined according to the methods described by Chau and Cheung (*5*) and Chau and Huang (*15*). The density of soybean oil was 0.88 g mL^{-1}.

Diets and Experimental Design. Following the formulation of the AIN93 M diet (*16*), three experimental diets including "fiber-free", "cellulose", and "biocellulose" diets were prepared and supplemented with cholesterol (2.0 g kg^{-1} of diet) to induce an alimentary hypercholesterolemia in hamsters (**Table 1**). Cellulose and biocellulose were the sole source of fiber in the cellulose- and biocellulose-containing diets, respectively. Twenty-four male Golden Syrian hamsters (6 weeks old) weighing 78.7 ± 8.1 g were obtained from the National Laboratory Animal Center (Taipei, Taiwan). The study protocol was approved by the Animal Care and Use Committee of National Chung Hsing University. After a 7-day acclimation period, the hamsters were divided into eight weight classes of three animals each. Then, the three diets were randomly allocated to one of the three animals in each weight classes. The animals were individually housed in stainless steel screen-bottomed cages in a room maintained at 24 ± 1 °C with a 12 h light/dark cycle. During the experimental period (30 days), food and drinking water were supplied ad libitum. The institutional guidelines for the care and use of laboratory animals were followed. Food intakes and body weights were recorded daily. Feces were collected, weighed, and analyzed for moisture content daily. A portion of the fecal sample left unused was stored at −20 °C for further analysis. At the end of the experimental period, animals were anesthetized by isoflurane (Halocarbon Laboratories, River Edge, NJ) after fasting for 12 h. Blood was collected from the orbital sinus, and serum was prepared for biochemical analysis. After laparotomy, liver and other visceral organs were removed, weighed, and stored at −70 °C for analysis.

Serum Cholesterol and Triglyceride. Total cholesterol (no. 402, Sigma Chemical Co., St. Louis, MO), high-density lipoprotein (HDL) cholesterol (no. 352, Sigma Chemical Co.), and triglyceride (Merckotest 14354, Merck, Darmstadt, Germany) in the serum samples were determined enzymatically using the commercial assay kits. Serum low-density lipoprotein (LDL) cholesterol was determined according to the method as mentioned by Chau and Huang (*4*).

Liver Cholesterol and Lipids. According to the method of Folch et al. (*17*), liver lipids and cholesterol were extracted from 1−2 g of liver with a chloroform/methanol mixture (2:1 v/v). The concentration of liver cholesterol in the lipids extract was determined colorimetrically at 490 nm (*18*). Total liver lipids were quantified gravimetrically by evaporating off the solvents in the lipids extract.

Fecal Cholesterol and Lipids. Fecal lipids in the lyophilized fecal samples were extracted with a chloroform/methanol (2:1 v/v) mixture using the method described by Folch et al. (*17*). Fecal cholesterol content in the fecal lipids extract was estimated according to the method of Searcy and Bergquist (*18*). Fecal total lipids were determined gravimetrically by evaporating off the organic solvent in the fecal lipids extract.

Fecal Bile Acids. During the last 3 days of the experiment, fecal samples were collected for analyses of fecal bile acids, which were extracted by boiling ethanol using the method of Beher et al. (*19*). According to the method of Turley and Dietschy (*20*), a reaction mixture containing 750 μL of Tris-HCl buffer (0.133 M Tris, 0.666 mM EDTA, pH 9.5), 500 μL of 1 M hydrazine hydrate (pH 9.5), 150 μL of 7 mM NAD^+ (N-7004, Sigma Chemical Co.), and 50 μL of bile acids extract or standard (T-0750, Sigma Chemical Co.) was preincubated at 30 °C. Into the mixture was added 50 μL of hydroxysteroid dehydrogenase solution (H-8879, Sigma Chemical Co.). After incubation at 30 °C for 60 min, the content of fecal bile acids was determined spectrophotometrically at 340 nm against a reagent blank in which the enzyme solution was replaced by Tris-HCl buffer.

Statistical Analysis. Results are expressed as mean ± standard deviation. Data were subjected to one-way analysis of variance and Duncan's multiple-range test using SAS software (Statistical Analysis System, Cary, NC). Values of $P < 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

In the present study, the moisture contents of the plant cellulose and biocellulose samples were 63.0 ± 1.05 and 41.1 ± 0.90 g kg^{-1}, respectively. Chemical analyses revealed that the IDF contents of plant cellulose and biocellulose samples were 967 ± 3.15 and 717 ± 4.31 g kg^{-1} of DW, respectively. A small amount of soluble dietary fiber (SDF) was present in the biocellulose powder (5.27 ± 0.60 g kg^{-1} of DW), and no detectable level of SDF was found in the plant cellulose. The IDF content of some common fruits and vegetables such as artichoke, asparagus, cereal bran, and orange might vary from 149 to 588 g kg^{-1} (*15, 21*). The results showed that the dried biocellulose powder could also be a promising source of insoluble food fiber. The plant cellulose sample contained only a small amount of ash (2.37 ± 0.43 g kg^{-1} of DW) and no detectable level of protein. The dried biocellulose powder was found to have protein (14.5 ± 0.39 g kg^{-1} of DW), ash (28.3 ± 1.04 g kg^{-1} of DW), glucose (184 ± 4.81 g kg^{-1} of DW), and some soluble nonpolysaccharide components (~56.1 g kg^{-1} of DW). In recent years, the total yield of biocellulose in Asia can be up to 10000 tons per year, and the dried biocellulose powder has been used as a fiber-rich food ingredient commercially available in Taiwan. It was inferred that the biocellulose powder available in large quantities could be exploited

Table 2. Functional Properties[a] of Biocellulose Powder in Relation to Cellulose

fiber sample	bulk density (g mL^{-1})	water-holding capacity (mL g^{-1})	oil-holding capacity (g g^{-1})	cation-exchange capacity (mequiv kg^{-1})
cellulose	0.38 ± 0.03w	3.87 ± 0.14w	2.58 ± 0.11w	11.1 ± 3.6w
biocellulose	0.34 ± 0.03w	7.41 ± 0.15x	2.67 ± 0.13w	67.5 ± 7.4x

[a] Data are expressed as mean ± standard derivation ($n = 4$). Values within columns with different letters are significantly different (Duncan, $P < 0.05$).

as a potential ingredient for the development of novel fiber-rich food products.

Furthermore, monosaccharide analyses revealed that cellulosic glucose was the only monomeric sugar released from the IDF of biocellulose, showing that the IDF was pure cellulose. Plant and bacterial cellulose have the same chemical formula and structure, but the degree of polymerization differs from about 13000–14000 for plant to 2000–6000 for bacterial cellulose (*10*). Bacterial cellulose has a crystalline structure, and its microfibrils agglomerate to form a cellulose ribbon (*22*).

Table 2 presents the bulk density, WHC, OHC, and CEC of plant cellulose and biocellulose. The bulk density (0.34–0.38 g mL^{-1}) and OHC (2.58–2.67 g g^{-1}) of the plant cellulose and biocellulose were comparable to each other. The WHC of the biocellulose (7.41 mL g^{-1}) was about 2-fold higher ($P < 0.05$) than that of plant cellulose. The WHC and OHC of insoluble fibers from some common fruits, vegetables, and cereals (e.g., artichoke, asparagus, apple, orange, wheat, and oat) might vary from 2.80 to 13.2 mL g^{-1} and from 0.9 to 5.09 g g^{-1}, respectively (*15, 21*). As shown in **Table 2**, the CEC of the biocellulose (67.5 mequiv kg^{-1}) was approximately 6-fold higher ($P < 0.05$) than that of plant cellulose. The high water retention ability of biocellulose is an important property for its medical applications such as wound dressing or artificial blood vessels for microsurgery (*23*). The desirable WHC of biocellulose therefore suggested its potential uses in food applications requiring moisture retention.

In the present study, the three experimental diets were isonitrogenous. Whereas the IDF contents of plant cellulose and biocellulose samples were 967 and 671 g kg^{-1}, respectively, the amounts of plant cellulose and biocellulose added into the cellulose and biocellulose diets were adjusted to 57.1 and 74.5 g kg^{-1} of diet, respectively, to provide insoluble fiber at a level of 50 g kg^{-1} of diet (**Table 1**). Throughout 30 days of observations, all animals remained healthy and active. The food intake (6.19–6.55 g day^{-1}) and body weight gain (0.54–0.68 g day^{-1}) of hamsters among the three diet groups were comparable to each other after the feeding experiment. Moreover, there were no significant variations in the weights of different visceral organs including small intestine (13.3–15.3 g kg^{-1} of body weight), cecal wall (6.26–6.58 g kg^{-1} of body weight), colon plus rectum (10.7–11.3 g kg^{-1} of body weight), and kidney (7.07–7.30 g kg^{-1} of body weight) in hamsters among the three diet groups.

Table 3 presents the effects of plant cellulose and biocellulose on the concentrations of serum triglyceride and serum cholesterol. Compared with the fiber-free and cellulose diets (168−195 mg dL^{-1}), the serum triglyceride concentrations in hamsters fed the biocellulose diet were significantly ($P < 0.05$) reduced by 55.5 and 46.6%, respectively. Dietary fiber might hinder lipid absorption directly and thus reduce serum triglyceride concentration and the risk of cardiovascular diseases (*24*). As shown in **Table 3**, the supplementation of plant cellulose and biocellulose led to significantly ($P < 0.05$) lower concentrations of serum total cholesterol (−17.4 and −27.9%, respectively). Biocellulose was more effective ($P < 0.05$) than cellulose in lowering the serum total cholesterol (by −12.8%). Dietary fibers having high CEC could entrap, destabilize, and disintegrate a lipid emulsion, leading to a decrease in diffusion and absorption of cholesterol and lipids (*25*). It was therefore inferred that the better hypolipidemic and hypocholesterolemic effects of biocellulose in relation to plant cellulose were partly attributed to its markedly higher CEC (**Table 2**).

Table 3. Effects of Biocellulose on the Serum Triglyceride and Cholesterol Concentrations[a] in Hamsters

diet	triglyceride (mg dL^{-1})	total cholesterol (mg dL^{-1})	HDL cholesterol (mg dL^{-1})	LDL cholesterol (mg dL^{-1})	HDL/total cholesterol ratio
fiber-free	195 ± 28.5x	247 ± 19.1x	123 ± 16.8x	85.0 ± 8.3x	0.50 ± 0.03x
cellulose	168 ± 24.0x	204 ± 16.0y	121 ± 16.4x	49.4 ± 4.3y	0.60 ± 0.03y
biocellulose	86.7 ± 5.54y	178 ± 10.1z	116 ± 14.3x	44.7 ± 6.6y	0.65 ± 0.02z

[a] Data are expressed as mean ± standard derivation ($n = 8$). Values in the same column with different letters are significantly different (Duncan, $P < 0.05$).

Table 4. Effects of Biocellulose on Relative Liver Weight, Liver Total Lipids, and Liver Cholesterol[a] in Hamsters

diet	rel liver wt (g kg^{-1} of body wt)	liver total lipids (mg g^{-1} of liver)	liver cholesterol (mg g^{-1} of liver)
fiber-free	46.8 ± 2.5w	126 ± 6.3w	35.6 ± 2.8w
cellulose	44.5 ± 2.8w	118 ± 3.1wx	31.4 ± 1.6wx
biocellulose	42.0 ± 4.5w	113 ± 1.6x	29.8 ± 0.8x

[a] Data are expressed as mean ± standard derivation ($n = 8$). Values in the same column with different letters are significantly different (Duncan, $P < 0.05$).

Despite the fact that a relatively higher HDL cholesterol concentration was expected to decrease the risk of cardiovascular diseases, no significant changes in the HDL cholesterol concentrations (116–123 mg dL^{-1}) were observed in hamsters among the three diet groups (**Table 3**). However, the significantly ($P < 0.05$) higher HDL/total cholesterol ratios for the cellulose- and biocellulose-supplemented groups (0.60 and 0.65, respectively) versus the fiber-free group (0.60) suggested their antiatherogenic potentials. It was reported that the HDL/total cholesterol ratio was negatively correlated with the risk of coronary heart disease (*26*). These results also implied that biocellulose had a higher antiatherogenic potential than plant cellulose. Compared with the fiber-free diet, consumption of the cellulose and biocellulose effectively ($P < 0.05$) reduced the serum LDL cholesterol concentrations by 41.9 and 47.4%, respectively. In general, our results agreed with other authors' findings that the hypolipidemic and hypocholesterolemic abilities of dietary fibers were associated with their types, quantities, and physicochemical properties (*5, 27*).

Table 4 shows that there were no significant differences in the relative liver weight (42.0–46.8 g kg^{-1} of body weight) of hamsters among the three diet groups. The variations in the liver weight in rats were related to the amounts of cholesterol and lipids being absorbed from the diet (*28*). As insoluble fiber (i.e., plant cellulose and biocellulose) was the major variable in the ingredients of different experimental diets (**Table 1**), differences in the liver weights were expected to be little. In **Table 4**, chemical analyses on liver tissues revealed that the inclusion of biocellulose in the fiber-free diet was effective ($P < 0.05$) in reducing the concentrations of liver total lipids (−10.3%) and liver cholesterol (−16.3%) after 30 days of feeding.

As shown in **Table 5**, the fecal moisture content of hamsters fed the biocellulose diet (320 g kg^{-1} of feces) was significantly

Table 5. Effects of Biocellulose on the Fecal Moisture Content, Fecal Dry Weight, Fecal Total Lipids, Fecal Cholesterol, and Fecal Bile Acids[a] in Hamsters

diet	fecal moisture content (g kg^{-1} of feces)	fecal dry wt (g day^{-1})	fecal total lipids (mg day^{-1})	fecal cholesterol (mg day^{-1})	fecal bile acids (μmol day^{-1})
fiber-free	233 ± 25.0x	0.45 ± 0.02x	50.5 ± 4.7x	17.7 ± 1.1x	11.6 ± 4.0x
cellulose	266 ± 28.6x	0.64 ± 0.03y	72.8 ± 6.8y	24.0 ± 3.9y	30.0 ± 3.6y
biocellulose	320 ± 17.5y	0.67 ± 0.02y	92.0 ± 8.0z	35.9 ± 1.4z	55.5 ± 8.7z

[a] Data are expressed as mean ± standard derivation ($n = 8$). Values in the same column with different letters are significantly different (Duncan, $P < 0.05$).

($P < 0.05$) higher than those fed the fiber-free and plant cellulose diets (137 and 120%, respectively). The higher fecal moisture content with the biocellulose diet was probably related to the superior ability of biocellulose in retaining moisture (**Table 2**). Our results also revealed that the addition of plant cellulose and biocellulose into fiber-free diet at a 5% level increased ($P < 0.05$) the fecal dry weight by 142 and 149%, respectively. No significant differences in the fecal dry weight were observed between the plant cellulose and biocellulose groups.

Furthermore, **Table 5** presents the effects of plant cellulose and biocellulose on the concentrations of lipids, cholesterol, and bile acids in the feces of hamsters. Compared with the fiber-free diet group, the feeding of plant cellulose and biocellulose resulted in significantly ($P < 0.05$) higher concentrations of fecal total lipids (144 and 182%, respectively), cholesterol (136 and 203%, respectively), and bile acids (259 and 479%, respectively). The results indicated that biocellulose was able to incur a higher output of total lipids, cholesterol, and bile acids in feces than plant cellulose. In general, the ability of dietary fibers to bind bile acids in the intestinal lumen could prevent the bile acids from reentering into circulation and eventually being lost through excretion (*29*). Moreover, the phenomena might also be attributable to other mechanisms that dietary fiber having high CEC might destabilize, entrap, and disintegrate the micelles, resulting in the reduced diffusion and absorption of lipids, cholesterol, and bile acids (*25*). It was therefore inferred that the elevated concentrations of fecal total cholesterol and bile acids in hamsters fed the biocellulose-supplemented diet might be partly due to the significantly higher CEC of biocellulose (67.5 mequiv kg^{-1}) compared to plant cellulose (11.1 mequiv kg^{-1}) (**Table 2**).

In **Tables 3**−**5**, the potential hypolipidemic and hypocholesterolemic abilities of plant cellulose and biocellulose could be further interpreted by the apparent negative relationships between serum triglyceride and fecal total lipids, between serum total cholesterol and fecal cholesterol, between serum total cholesterol and fecal bile acids, between liver cholesterol and fecal cholesterol, and between liver cholesterol and fecal bile acids. It was inferred that the reductions in the concentrations of serum triglyceride, serum cholesterol, and liver cholesterol were associated with the enhanced excretion of total lipids, cholesterol, and bile acids through feces. All of these observations could be explained by the mechanisms whereby dietary fibers lowered the concentrations of serum lipids and cholesterol by a combination of different physiological effects including reduced number of intact micelles, higher bile acid adsorption, increased cholesterol catabolism to bile acids, retarded cholesterol biosynthesis, and reduced absorption of lipids and cholesterol (*3, 6, 7, 30*).

Our results demonstrated that biocellulose was a promising source of insoluble fiber and had a significantly ($P < 0.05$) higher WHC and CEC than plant cellulose. Compared with the fiber-free group, the consumption of plant cellulose and biocellulose effectively ($P < 0.05$) decreased the concentrations of serum triglyceride, serum total cholesterol, serum LDL cholesterol, liver total lipids, and liver cholesterol to different extents by means of enhancing ($P < 0.05$) the excretion of total lipids, cholesterol, and bile acids via feces. The efficacy of biocellulose in lowering serum lipids and cholesterol was higher than that of plant cellulose. It was suggested that the biocellulose could be a promising low-calorie bulk ingredient for different food applications such as dietary fiber enrichment, baking, and dietetic snacks of different forms (e.g., powder, gelatinous, or shred forms). Biocellulose as a high-value product of biotechnology might offer industries opportunities to develop new formulations of fiber-rich functional foods.

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