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Characterization of Fibrous Residues from Agrowastes and the Production of Nanofibers

Wai-Yee Fung,[†] Kah-hay Yuen,[†] and Min-Tze Liong*,[‡]

[†]School of Pharmaceutical Sciences and [‡]School of Industrial Technology, Universiti Sains Malaysia, 11800 Penang, Malaysia

Oil palm trunk (OPT), oil palm frond (OPF), and okara are agrowastes generated abundantly by the palm oil and soy industries. There are vast potentials for these fibrous biomass rather than disposal at landfills or incineration. Fibrous materials (FM) and alkali-treated fibrous residues (FR) were produced from the selected wastes and subsequently characterized. Functional properties such as emulsifying properties, mineral-binding capacity, and free radical scavenging activity were also evaluated for possible development of functional products. Supernatants (FS) generated from the alkaline treatment contained soluble fractions of fibers and were also characterized and used for the production of nanofibers. Okara FM had the highest ($P < 0.05$) protein (31.5%) and fat (12.2%) contents, which were significantly reduced following alkali treatment. The treatment also increased total dietary fiber (TDF) in okara by 107.9%, in OPT by 67.2%, and in OPF by 25.1%. The increased fiber fractions in FR enhanced functional properties such as water-holding capacities and oil-holding capacities. Okara displayed the highest ($P < 0.05$) emulsifying properties compared to OPT and OPF. High IDF content of OPT and OPF contributed to high antioxidant activities (377.2 and 367.8% higher than that of okara, respectively; $P < 0.05$). The soluble fraction from alkali treatment of fibers was successfully electrospun into nanofibers, which can be further developed into nanoencapsulants for bioactive compound or drug delivery.

KEYWORDS: Agrowaste; oil palm; okara; nanofibers; electrospinning

INTRODUCTION

Increasing awareness of sustainable development and the deteriorating environmental status has fueled a race to reduce rapidly mounting processing and agriculture wastes. Biomass waste such as agricultural residues is creating great environmental concerns, with approximately 200 billion tonnes of lignocellulosic wastes being produced annually (*1*). Much effort has focused on finding alternatives and environmentally friendly handling and disposal of these wastes, including recycling to produce revenue-generating products. Although most biomass is suitable and is being converted to animal feed (*2*), there is still exploitation potential as agricultural residues are abundant, renewable, and relatively economical.

Among Asia's biggest commodities, oil palm (*Elaeis guianensis*) and soy (*Glycine max*) produce copious amounts of recyclable biomass that are generated from various processing stages. Only 10% of the oil palm plant is economically valuable, used for the production of palm oil and palm kernel oil, whereas the remaining 90% is constituted of oil palm trunks (OPT), oil palm fronds (OPF), empty fruit bunches (EFB), and palm pressed fibers (PPF) (*3*). Malaysia, the leading producer of palm oil, produced an estimated 56.9 million tonnes of oil palm biomass in 2000 (*4*), which is constituted of hemicelluloses, cellulose, and lignin. Consumption of soy and soy products has increased worldwide due to its well-documented health benefits. Soy consumption has been associated with alleviation of menopausal symptoms, increased bone mass in postmenopausal women, and prevention of hypoestrogenic effects due to the high content of isoflavones (*5*). The increase in soy foods sales is also partly attributed to the approval of a health claim linking soy with a reduction of the risk of coronary heart disease by the U.S. Food and Drug Administration (FDA). The booming soy industry has led to increased generation of okara, the solid byproduct of soy milk and tofu manufacturing. Okara is commonly disposed via dumping in landfills and burnt as waste. However, it is a beneficial waste, as okara is rich in protein and dietary fiber content.

Agrowastes are great sources of dietary fiber, which includes cellulose, hemicelluloses, lignin, pectin, gums, and other polysaccharides. The soluble and insoluble dietary fiber fractions (SDF and IDF) are known to confer a wide range of health benefits, including reduction in the risks of gastrointestinal diseases, cardiovascular diseases, and obesity. There is a need for supplementation of dietary fiber via fiber-rich foods as the normal daily intake of most populations is still below the recommended Dietary Reference Intake of 14 g of dietary fiber per 1000 kcal, or 25 g for adult women and 38 g for adult men (*6*). Hence, high-fiber products are gaining popularity as functional foods with a low glycemic index and hypocholesterolemic properties. However, high-fiber content in food is often associated with undesirable sensory properties due to the inherent properties of fibers being coarse and grainy. Healthy foods such as high-fiber

*Corresponding author (e-mail mintze.liong@usm.my; phone +604 653 2114; fax +604 657 3678).

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cereals are dry and have increasingly undesirable organoleptic properties as fiber content is increased. The food industry has developed processing methods and compound coatings that can effectively mask and reduce fibrous mouthfeel associated with dietary fiber. However, compound coatings are essentially made up of fats and carbohydrates, which increase the caloric value of the food upon intake. The diminutization of fibers to nanosize may reduce or even completely remove the undesirable organoleptic properties inherently and eliminate the need for additional processing steps or high-calorie additives, which may defeat the net purpose of high-fiber health functional foods.

This study aimed to produce and characterize fibrous residues (FR) from three major agricultural waste fiber materials (FM), namely, oil palm trunk (OPT), oil palm frond (OPF), and okara, for further functional food-based applications. Fibrous residues obtained were subsequently evaluated for their potentials to produce nanofibers for the development of organoleptically desired high-fiber foods. The successful application of agrowaste nanofibers using nanotechnology and chemical treatment will be a milestone in sustainable agricultural management. Agrowaste materials could be converted into valuable and functional materials, including food and drug carriers, thus extending the life cycle of the agriculture byproduct.

MATERIALS AND METHODS

Preparation of Fiber Material (FM). Okara (*G. max*) was obtained as a wet residue from a local factory. The wet residue was oven-dried (Memmert Oven UL40, Memmert, West Germany) until constant weight at 60 °C. OPT and OPF (Cedar Food Industries, Penang, Malaysia) were obtained as ground flakes. The fibers were processed with a super mill (Supermill TK-3168, Takasima, Penang, Malaysia) and sieved through a 150 μm mesh screen (Retsch GmbH, Haan, Germany). The fiber materials (FM) were vacuum-packed (AudionVac VMS 133, Audion Elektro BV, Weesp, The Netherlands) and stored at −20 °C until further use.

Preparation of Fiber Residue (FR). FMs of OPT, OPF, and okara were defatted using petroleum ether (bp 40−60 °C) (QRec, Auckland, New Zealand) at a ratio of 1:10 (w/v). The defatted FM was suspended in water at a ratio of 1:10 (w/v), adjusted to pH 9 using 1 N NaOH (Hansa Fine Chemicals GmbH, Bremen, Germany), and autoclaved at 121 °C for 30 min. The suspension was left to cool to 25 °C followed by centrifugation at 2330*g* for 30 min at 25 °C to separate the soluble (FS) and insoluble fractions (FR). Fiber residue (FR) was washed three times sequentially with distilled water, ethanol (QRec), and acetone (QRec), followed by oven-drying (Memmert) until constant weight at 60 °C. The dried samples were processed with a supermill (Takasima) and sieved through a 150 μm wire mesh test sieve (Retsch). Fiber supernatant (FS) was stored at 4 °C, whereas FR was vacuum-packed (Audion Elektro BV) and stored at −20 °C until further use and analyses.

Production of Nanofibers from FS. The supernatant containing soluble fibers (FS) were electrospun (Electrospinz ES1, Electrospinz Ltd., Blenheim, New Zealand) to produce nanofibers (NF). Samples of FS were added with 5% (w/v) polyethylene oxide (PEO) (Aldrich Chemistry, Milwaukee, WI) solution and stirred until homogeneous. The mixture was spun at 25 °C by applying an electrical potential of 10−15 kV and collected on an aluminum foil serving as the ground collector. The morphology of the nanofibers produced was observed using a scanning electron microscope (SEM).

Morphology of FM, FR, and Nanofibers. The morphology of FM, FR, and nanofiber samples was assessed using SEM. Samples were affixed to sample stubs and subsequently gold-coated using a Polaron SC515 SEM Coating System (Bio-Rad sputter coater, Bio-Rad Ltd., Hemel Hempstead, U.K.). The samples were then viewed and photographed using a Leo Supra 50VP Ultrahigh Field Emission scanning electron microscope (FESEM) (Carl Zeiss, Oberkochen, Germany).

Chemical Analyses. Moisture, fat, ash, and protein contents of FM and FR were determined using AOAC methods (*7*). Moisture (method 925.40) was determined upon drying at 105 °C until constant weight. Fat was determined using the Soxhlet extraction procedure (method 920.39). Ash (method 923.03) was determined upon ashing in a furnace at 550 °C. Protein was calculated from the total nitrogen content (method 976.05), using a conversion factor of 6.25. Total carbohydrate was determined by difference (subtraction of the sums of protein, total fat, moisture, and ash). The liquid FS was also analyzed for proximate composition on a wet basis using the AOAC methods. Fat was determined using the Mojonnier method (*8*).

Total dietary fiber (TDF) and soluble dietary fiber (SDF) of FM and FR were determined using AOAC method 991.43. The insoluble dietary fiber (IDF) was calculated by subtracting the SDF portion from the TDF portion.

Mineral contents of FM and FR (Fe, Zn, Cu, Mn, Ca, K, Mg) were determined using atomic absorption spectrophotometry (AAnalyst 100 Spectrometer, Perkin-Elmer, USA). Microwave digests of weighed samples were analyzed using AAS and calculated on the basis of known concentrations of standard element solutions. Na, P, and Si were determined using inductively coupled plasma optical emission spectroscopic method (ICP-OES) (Optima 7300 DV ICP-OES with Scott Spray Chamber, Perkin-Elmer) (AOAC method 985.01).

Color Measurement. Color of FM and FR was measured across the full spectrum of visible light wavelengths (400−700 nm) using a spectrophotometer (Minolta CM-3500d, Minolta Co. Ltd., Osaka, Japan). Hunter color *L*, *a*, and *b* values were quantified using Spectra Magic software version 2.11 (Minolta Cyberchrom Inc., Osaka, Japan).

Water- and Oil-Holding Capacities. The water-holding capacity (WHC) of FM and FR was determined using the method of Chau et al. (*9*). Briefly, weighed samples were homogenized into distilled water and centrifuged. The supernatant volume was then measured. The oil-holding capacity (OHC) was determined by substituting the distilled water with palm olein oil (density = 0.897 g/mL). WHC and OHC were calculated using the following equation:

$$\mathrm{WHC/OHC} = \frac{(\text{weight of water/oil held (g)})}{(\text{weight of sample (g)})}$$

Emulsifying Activity (EA) and Emulsion Stability (ES). The EA of FM and FR was determined using the method of Yatsumatsu et al. (*10*). Briefly, water-suspended sample was emulsified in palm olein oil and centrifuged, following which the EA was calculated using the following equation:

$$\mathrm{EA}\ (\%) = \frac{(\text{height of emulsified layer (cm)})}{(\text{height of whole layer (cm)})} \times 100$$

The ES was determined using the method of Yatsumatsu et al. (*10*), by measuring the emulsified layer upon heat treatment at 80 °C for 30 min. The ES was calculated using the following equation:

$$\mathrm{ES}\ (\%) = \frac{(\text{height of emulsified layer after heating (cm)})}{(\text{height of emulsified layer before heating (cm)})} \times 100$$

Antioxidant Activity (AO). Antioxidant activities of antioxidant polar fractions of FM and FR were determined using the DPPH method as described by Hubert et al. (*11*). The antioxidant property was evaluated using the 1,1-diphenyl-2-picrylhydrazyl nitrogen-centered radical (DPPH$^{\bullet}$) (Sigma-Aldrich) as a scavenging target. The scavenging activity (SA) of extracts on DPPH radical was assessed by measuring the absorbance at 523 nm (UVmini-1240, Shimadzu, Tokyo, Japan). The inhibition percentage of the DPPH absorbance was calculated as SA using the equation

$$\mathrm{SA}\ (\%) = \frac{(A_a - A_b)}{A_a}$$

where A_a is the absorbance of the control mixture (methanolic DPPH without sample) and A_b is the absorbance of the solution containing the tested extract.

Mineral-Binding Activity. The mineral-binding activity of FM and FR was evaluated using AAS. AAS grade metal solutions of Fe, Zn, Cu, and Ca were diluted to 10 mg/L. Sample (250 mg) was added into 25 mL of metal solution (10 mg/mL), and the pH was adjusted to 5−6. The mixture was shaken in an orbital shaker for 1 h (150 rpm, 37.5 °C). The metal solution was recovered by centrifugation at 2330*g* for 15 min at 25 °C,

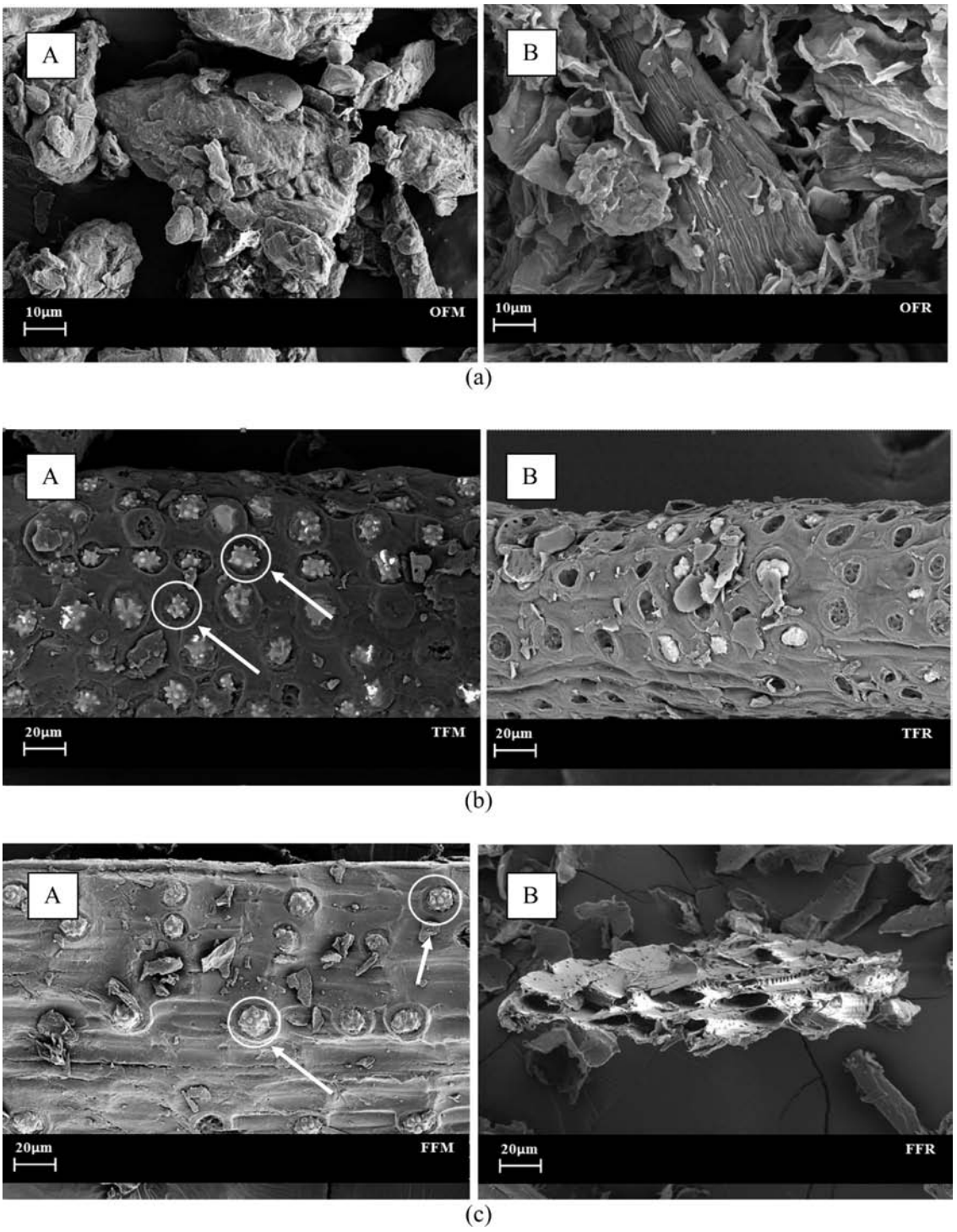

Figure 1. SEM micrographs of (A) fiber materials and (B) fiber residues of (**a**) okara (OFM and OFR), (**b**) oil palm trunk (TFM and TFR), and (**c**) oil palm frond (FFM and FFR). White circles and arrows indicate the presence of silica.

followed by filtration through Whatman no. 541 filter paper (20−25 μm). The remaining metal in the supernatant was quantified using AAS (Perkin-Elmer). The inherent minerals in fibers were taken into account by measuring the sample supernatant, with metal solution replaced with distilled water. A control mixture contained metal solution, without sample FM or FR. The net mineral-binding activity of FM and FR was calculated using the following equation:

$$\text{metal-binding activity } (\%) = \frac{(C_{\text{initial}} - C_{\text{final}}) \times 100}{C_{\text{initial}}}$$

Statistical Analysis. All determinations were performed in triplicates. Statistical analysis was performed using paired *t* test and one-way ANOVA. Post hoc determination was performed using Tukey's test (SPSS Inc., version 11.5; Chicago, IL) with α level at 0.05.

RESULTS AND DISCUSSION

Morphology of FM and FR. The SEM micrographs (**Figure 1**) showed obvious differences in surface morphology between the FM and FR samples. The FM of okara (**Figure 1a**) contained irregular clumps of fiber with smooth surfaces, whereas the FR sample appeared to be rough and flaky. Fibers in their native state have been reported to contain waxes and other encrusting substances such as hemicellulose, lignin, and pectin that form a

Table 1. Proximate Composition (Percent) of Fiber Materials (FM), Fiber Residues (FR), and Fiber Supernatant (FS) of Oil Palm Trunk (OPT), Oil Palm Frond (OPF), and Okara

proximate	sample[a]	okara[b]	OPT[b]	OPF[b]
moisture	FM	6.01 ± 0.02 B,b	6.99 ± 0.14 A,a	6.29 ± 0.09 A,b
	FR	9.75 ± 0.001 A,a	3.96 ± 0.14 B,c	6.27 ± 0.04 B,b
	FS	95.25 ± 0.001 c	96.56 ± 0.007 b	98.09 ± 0.001 a
protein	FM	31.46 ± 1.07 A,a	2.55 ± 0.40 A,b	3.45 ± 0.76 A,b
	FR	13.44 ± 0.53 B,a	2.44 ± 0.05 B,b	3.29 ± 0.32 B,b
	FS	0.24 ± 0.003 a	0.07 ± 0.005 b	0.05 ± 0.001 c
fat	FM	12.19 ± 0.04 A,a	1.71 ± 0.04 A,c	2.77 ± 0.11 A,b
	FR	0.34 ± 0.07 B,a	0.44 ± 0.0003 B,a	0.40 ± 0.23 B,a
	FS	0.01 ± 0.001 a	0.01 ± 0.0005 b	0.004 ± 0.001 b
ash	FM	4.61 ± 0.06 A,a	4.23 ± 0.01 A,b	3.31 ± 0.09 A,c
	FR	3.64 ± 0.03 B,a	2.30 ± 0.13 B,b	1.86 ± 0.01 B,c
	FS	0.02 ± 0.0004 a	0.01 ± 0.0001 b	0.007 ± 0.0001 c
carbohydrate	FM	47.09 ± 0.97 B,b	84.54 ± 0.47 B,a	84.46 ± 0.49 B,a
	FR	72.84 ± 0.63 A,b	90.70 ± 0.03 A,a	95.25 ± 2.27 A,a
	FS	4.47 ± 0.003 a	3.35 ± 0.003 b	1.84 ± 0.0003 c

[a] FM and FR were calculated on dry basis; FS was calculated on wet basis. [b] Means in the same row followed by different lower case letters are significantly different ($P < 0.05$). Means in the same column followed by different upper case letters are significantly different ($P < 0.05$).

thick and smooth outer layer. However, the chemical and physical treatments removed most of the surface waxes and noncellulosic materials, leading to rough and flaky fiber structures. The micrographs of FM samples of OPT and OPF revealed distinct silica structures embedded on the smooth surface of intact fiber bundles (**Figure 1b**,**c**). The SEM micrographs of FR samples showed that grinding and alkali treatments removed most of the surface structures and the cementing materials around fiber bundles, producing FR samples with exposed fiber microstructures (**Figure 1b**,**c**). The dissolution of primary cell walls upon alkaline treatment also partially removed hemicelluloses, pectin, and lignin. The silica structures abundantly embedded on the smooth surface of FM samples of OPT and OPF were also notably reduced in FR samples. The silica was removed along with the outer surface structures of the fibers by the grinding and alkali treatment. The removal of silica is desirable as it has been noted that silica poses a nutritional constraint in fiber foods due to decreased digestibility and toxicity concerns (*12*).

Chemical Compositions. *Proximate Analyses*. The proximate compositions of okara, OPT, and OPF are shown in **Table 1**. Okara FM contained a significantly higher ($P < 0.05$) crude protein content (31.5%) compared to the FM of OPT and OPF. Okara has been noted to have high protein content, a majority of which is solubilized in alkaline condition (*13*). As expected, protein was significantly ($P < 0.05$) reduced in all alkaline-treated FR samples, although the removal in OPT and OPF was markedly lower compared to okara due to their inherently lower FM protein content. Protein content in okara was reduced by 57.3%, which negatively affected the emulsifying activity of okara fiber as discussed later. The fat content of okara FM was also the highest ($P < 0.05$) compared with OPT and OPF. Soybean is rich in protein and oil, most of which remains in the okara after the extraction of soy milk (*2*), accounting for the high composition of fat and protein in okara FM. All samples exhibited a significantly reduced ($P < 0.05$) fat content in FR compared to FM due to the defatting procedure prior to alkaline treatment. Okara had the highest fat removal (97.2%) ($P < 0.05$) compared to OPF and OPT. The disruption of fiber cell walls has been shown to enhance oil and protein extraction from soybeans, whereas conditions that favor protein extraction also favored oil extraction (*14*). Thus, physical grinding and alkaline and autoclave conditions that disrupted fiber structures contributed to the high protein and oil removal from the fibers (FM). The reduction in fat and protein compositions in fiber samples following alkaline treatment contributed to the change in their functional properties such as WHC and OHC, as discussed below. The removal of protein and fat during alkaline treatment also led to increased purity of the fiber fraction, and this was exhibited in the increased total carbohydrate content. Carbohydrate content significantly ($P < 0.05$) increased by 54.7% in okara FR and by 7.29 and 12.78% in OPT and OPF FR, respectively. The carbohydrate comprised mainly insoluble fibers, which were quantified in dietary fiber analyses.

The fiber supernatants (FS) contained high moisture levels (95−98%), attributed to the addition of water during fiber treatment (**Table 1**). The remaining proximate components of protein, ash, and fat, which were quantified on wet basis, were low in all FS samples. The highest ($P < 0.05$) protein content was found in okara (0.24%) followed by OPT and OPF with 0.07 and 0.05% protein, respectively. The higher protein content in okara FM, which solubilized into the fiber supernatant during alkaline treatment, contributed to the significant ($P < 0.05$) difference between protein contents of the oil palm and okara fiber supernatants. Fat content was low (0.004−0.01%) in all FS samples due to prior defatting process. Low levels of ash (0.007−0.02%) and carbohydrate (1.8−4.5%) were also observed in all FS samples.

Fiber Composition. Alkaline treatment is the most common fiber treatment to extract protein, fat, and soluble fibers such as pectin to yield an insoluble fiber-rich fraction. Total dietary fiber (TDF) consists of soluble fiber (pectic substances and gums) and insoluble fiber fractions (cellulose, hemicelluloses, and lignin). The chemical treatment significantly increased ($P < 0.05$) the TDF content of okara by 107.9%, of OPT by 67.2%, and of OPF by 25.1%, due to removal of other components such as protein, fat, and soluble polysaccharides (**Table 2**). The TDF content of okara, OPT, and OPF FR are comparable to the TDF content of corn bran (87.8%) and higher than the TDF content of wheat bran (44.5%) and oat bran (23.8%) (*15*). The soluble dietary fiber (SDF) content of all fibers significantly ($P < 0.05$) decreased upon alkaline treatment, which solubilized and removed the soluble fiber fractions. Okara FM had the highest ($P < 0.05$) SDF value (13.5%), which was reduced to 3.8% upon alkaline treatment. The alkali treatment significantly ($P < 0.05$) increased the insoluble dietary fiber (IDF) values in all fiber samples, especially in okara FR (205.3%) followed by OPT and OPF FR, which increased by 64.4 and 27.4%, respectively. Increased content of IDF positively affected functional properties such as WHC and OHC of FR as discussed below. IDF, which possesses water absorption property, has been associated with increased satiety and improved digestive functions and has been clinically shown to prevent gastrointestinal disorders such as constipation and colon cancer (*16*). High IDF content in okara, OPT, and OPF FR suggests potential applications in dietetic products and dietary fiber supplements.

Mineral Composition. Calcium and phosphorus made up the bulk of mineral content in all FM and FR samples (**Table 3**). Okara FM contained high amounts of calcium, potassium, magnesium, and phosphorus, with trace minerals zinc, copper, and manganese present in lower amounts. Soybean mineral content has been noted to be mainly constituted of calcium, potassium, and magnesium (*13*), accounting for similarly high contents in okara, where the minerals remained after extraction of soy milk. OPT and OPF contained high amounts of all the minerals analyzed, except for copper and zinc, which were present in relatively lower amounts. In general, alkali treatment led to

Table 2. Fiber Composition (Percent) Fiber Materials (FM) and Fiber Residues (FR) of Oil Palm Trunk (OPT), Oil Palm Frond (OPF), and Okara

fiber composition	sample	okara[a]	OPT[a]	OPF[a]
TDF	FM	38.61 ± 0.00 B,c	52.83 ± 0.014 B,b	73.56 ± 1.57 B[b], a
	FR	80.28 ± 0.014 A,c	88.33 ± 0.17 A,b	92.02 ± 1.414 A[b], a
SDF	FM	13.54 ± 0.014 A,a	2.51 ± 0.014 A,b	3.14 ± 0.021 A,b
	FR	3.75 ± 0.00 B,a	0.64 ± 0.00 B,c	2.34 ± 0.00 B,c
IDF	FM	25.07 ± 0.028 B,c	52.19 ± 0.156 B,b	70.42 ± 1.428 B,a
	FR	76.53 ± 1.442 A,b	85.82 ± 1.57 A,a	89.68 ± 1.414 A,a

[a] Means in the same row followed by different lower case letters are significantly different ($P < 0.05$). Means in the same column followed by different upper case letters are significantly different ($P < 0.05$). [b] Significantly different ($P < 0.1$).

Table 3. Mineral Content (Micrograms per Gram of Dry Agrowaste) of Fiber Materials (FM) and Fiber Residues (FR) of Oil Palm Trunk (OPT), Oil Palm Frond (OPF), and Okara

mineral	sample	okara[a]	OPT[a]	OPF[a]
Fe	FM	69.89 ± 0.15 A,c	184.05 ± 0.75 B,a	146.19 ± 0.37 B,b
	FR	58.93 ± 0.25 B,c	238.58 ± 4.87 A,a	150.19 ± 0.45 A,b
Zn	FM	29.00 ± 0.15 A,a	32.09 ± 0.3 A,b	8.88 ± 0.20 A,c
	FR	26.78 ± 0.07 B,b	29.30 ± 0.07 A,a	7.69 ± 0.05 A,c
Cu	FM	11.55 ± 0.05 A,a	3.61 ± 0.02 B,c	5.78 ± 0.07 A,b
	FR	5.97 ± 0.05 B,a	5.19 ± 0.07 A,c	5.89 ± 0.02 A,b
Mn	FM	17.57 ± 0.05 A,c	39.8 ± 0.25 A,b	112.16 ± 0.15 A,a
	FR	14.43 ± 0.17 B,c	32.50 ± 0.07 B,b	93.17 ± 0.20 B,a
Ca	FM	2291.46 ± 8.43 B,b	2123.36 ± 8.19 A,c	3939.21 ± 22.75 A,a
	FR	5126.56 ± 13.42 A,a	2401.7 ± 58.14 A,c	3472.39 ± 16.45 B,b
K	FM	299.69 ± 3.50 A,a	220.95 ± 5.77 A,b	133.03 ± 0.92 A,c
	FR	98.78 ± 0.53 B,a	15.41 ± 0.09 B,c	15.41 ± 0.00 B,b
Mg	FM	102.31 ± 0.64 A,a	97.88 ± 0.64 A,b	28.25 ± 0.28 A,c
	FR	65.37 ± 0.00 B,a	44.69 ± 0.32 B,b	14.64 ± 0.08 B,c
Na	FM	7.40 ± 0.00 B,c	342.2 ± 0.14 B[b], a	330.6 ± 1.41 B,b
	FR	1910.6 ± 0.71 A,a	354.8 ± 1.41 A[b], b	357.5 ± 0.07 A,b
Si	FM	44.85 ± 0.07 A,c	134.3 ± 0.00 A,b	418.5 ± 14.14 A,a
	FR	13.3 ± 0.00 B,c	60.7 ± 0.14 B,b	102.2 ± 1.41 B,a
P	FM	3457.1 ± 1.41 A,a	388.2 ± 0.14 A,c	632.8 ± 13.44 A,b
	FR	2014.4 ± 14.41 B,a	135.5 ± 0.00 B,b	164.5 ± 0.07 B,b

[a] Means in the same row followed by different lower case letters are significantly different ($P < 0.05$). Means in the same column followed by different upper case letters are significantly different ($P < 0.05$). [b] Significantly different ($P < 0.1$).

significant reductions ($P < 0.05$) in mineral contents, which was most prominent for alkaline minerals potassium and magnesium as well as phosphorus and silica. Potassium was significantly ($P < 0.05$) reduced by 93% in OPT, by 88.4% in OPF, and by 67% in okara FR samples. Magnesium was also significantly ($P < 0.05$) reduced in the FR samples, as observed in OPT (54.3% reduction), OPF (48.2% reduction), and okara (36.1% reduction). Reduction in phosphorus level was most prominent in OPF (74%), followed by OPT (65.1%) and okara (41.7%). FM of OPF, which contained the highest ($P < 0.05$) silica content (418.5 μg/g), showed a 75.6% reduction upon alkaline treatment. The silica in okara and OPT was significantly ($P < 0.05$) reduced by 70.3 and 54.8%, respectively. Alkaline treatment is often applied to remove silica from fibers to reduce toxicity (*12*).

Table 4. Reflectance (Hunter L^*, a^*, b^*) Values of Fiber Materials (FM) and Fiber Residues (FR) of Oil Palm Trunk (OPT), Oil Palm Frond (OPF), and Okara

reflectance value	sample	okara[a]	OPT[a]	OPF[a]
L^*	FM	82.41 ± 0.02 B,a	78.30 ± 0.01 A,b	78.35 ± 0.05 A,c
	FR	83.55 ± 0.02 A,a	71.34 ± 0.01 B,b	67.74 ± 0.01 B,c
a^*	FM	1.76 ± 0.03 A,c	3.11 ± 0.02 A,a	2.27 ± 0.02 B,b
	FR	1.57 ± 0.01 B,c	3.05 ± 0.01 B,b	4.53 ± 0.02 A,a
b^*	FM	20.96 ± 0.07 A,a	20.04 ± 0.03 A,b	19.87 ± 0.14 A,b
	FR	13.16 ± 0.08 B,c	14.48 ± 0.06 B,b	15.21 ± 0.01 B,a

[a] Means in the same row followed by different lower case letters are significantly different ($P < 0.05$). Means in the same column followed by different upper case letters are significantly different ($P < 0.05$).

Dialysis patients with high plasma silica levels of up to 385 and 235 μg/dL exhibited skin eruptions and folliculitis (*17*). The alkali treatment of fibers also resulted in an increased amount of sodium attributed to the use of sodium hydroxide. The sodium content of okara, OPT, and OPF increased significantly $P < 0.05$), with okara recording the highest increase of 1903.2 ppm (**Table 3**). Such an increase can be attributed to the higher SDF content of okara compared to OPT and OPF (**Table 2**). Soluble fibers such as pectin contain free carboxyl groups and free uronic acid residues that contribute to a high cation-binding capacity. This may have led to the increased binding of sodium by okara FR, thereby retaining sodium in the fiber matrix during alkaline treatment. Oil palm fibers were found to contain higher ($P < 0.05$) levels of iron compared with okara, whereas an opposing trend was observed for phosphorus whereby okara contained a significantly higher ($P < 0.05$) level of phosphorus compared with oil palm fibers. This could be attributed to soil mineral content and chemical attributes of the minerals with high binding affinity. High-phosphorus soil has been noted to have low levels of available iron due to binding of free iron, whereas phosphorus deficiency resulted in increased iron availability in plants, suggesting the antagonistic interaction between these minerals in plant nutrition (*18*). Hence, it is postulated that soybean, the source of okara, was cultivated in high-phosphorus soil, which resulted in high phosphorus content, but low iron availability. A low level of zinc (7.7−32.1 ppm) in the presence of high levels of calcium (2123.4−5126.6 ppm) and phosphorus (135.5−3457.1 ppm) detected in all fiber samples could be due to reduced zinc uptake by plants in soils with high calcium and phosphorus levels (*19*).

Color. The color of the native and treated okara, OPT, and OPF samples is shown in **Table 4**. L^* indicates the degree of lightness or darkness, wheras a^* indicates redness or greenness and b^* indicates yellowness or blueness. The samples had a high degree of lightness, and this was most prevalent in both okara FM and FR samples ($P < 0.05$) compared to OPT and OPF samples. FR of OPF exhibited the highest ($P < 0.05$) redness, whereas FR of okara exhibited the lowest ($P < 0.05$) redness. Lightness of samples significantly ($P < 0.05$) increased in okara FR but decreased in OPT and OPF FR upon alkaline treatment. a^* values denoting redness decreased in okara and OPT FR, whereas greenness decreased in OPF FR after alkaline treatment. All FR samples showed significantly ($P < 0.05$) lower b^* values, which denote yellowness as compared to FM samples, attributed to the defatting process during alkaline treatment that removed oil-soluble pigments.

Functional Properties. *WHC and OHC*. WHC is a desirable attribute of dietary fiber, which can contribute to stool bulking and have various applications in viscosity and texture modification in the food industry. Our results showed that the WHC of all

Table 5. Functional Properties of Fiber Materials (FM) and Fiber Residues (FR) of Oil Palm Trunk (OPT), Oil Palm Frond (OPF), and Okara

physicochemical property	sample	okara[a]	OPT[a]	OPF[a]
water-holding capacity (g of water/g of sample)	FM	2.36 ± 0.04 B,c	2.72 ± 0.01 B,b	3.28 ± 0.02 A,a
	FR	6.99 ± 0.01 A,a	4.61 ± 0.14 A,b	4.85 ± 0.22 B,b
oil-holding capacity (g of oil/g of sample)	FM	1.46 ± 0.11 A,c	2.97 ± 0.02 A,a	2.70 ± 0.13 A,b
	FR	3.11 ± 0.08 B,c	4.98 ± 0.04 B,a	4.37 ± 0.03 B,b
emulsifying activity (%)	FM	66.3 ± 0.6 A,a	6.6 ± 0.1 A,b	4.0 ± 0.1 A,c
	FR	14.0 ± 0.2 B,a	5.7 ± 0.1 A,b	1.5 ± 0.1 B,c
emulsion stability (%)	FM	99.3 ± 0.0 A,a	98.6 ± 0.2 A,a	31.2 ± 0.7 B,b
	FR	96.0 ± 2.6 A,a	75.5 ± 1.9 B,c	86.2 ± 0.7 A,b
antioxidant activity (scavenging activity %)	FM	19.50 ± 1.25 A,b	93.06 ± 0.10 A,a	91.23 ± 1.65 A,a
	FR	14.78 ± 1.90 A,c	61.99 ± 1.24 B,a	53.80 ± 2.30 B,b

[a] Means in the same row followed by different lower case letters are significantly different ($P < 0.05$). Means in the same column followed by different upper case letters are significantly different ($P < 0.05$).

samples increased ($P < 0.05$) upon alkaline treatment (**Table 5**), which corresponds to increased TDF in all samples (**Table 2**). The highest ($P < 0.05$) increase was observed in okara FR (196.2%), followed by OPT and OPF, which significantly increased ($P < 0.05$) by 69.5 and 47.9%, respectively. This trend is in tandem with a trend of TDF increment, which was highest ($P < 0.05$) in okara (107.9%), followed by OPT (67.2%) and OPF (25.1%) (**Table 2**). Porous fiber matrices form hydrogen bonds with water molecules, which contribute to the high WHC. Alkaline treatment under autoclave condition and subsequent grinding led to particle size reduction and, hence, increased the surface area for water holding and entrapment within the porous fiber matrix. The decreased ($P < 0.05$) fat content (**Table 1**) also led to increasing WHC (**Table 5**) of FR samples compared to FM. The fat content of fibers has been noted to be inversely related to hydration properties of fibers, as residual oil trapped in the fiber matrix restricts the entry of water molecules, leading to decreased hydration (*20*). The increased WHC of FR samples is desired in food applications requiring hydration and viscosity, such as meat products, and conservation of freshness, such as baked goods (*16*).

The OHC of fiber affects cooking properties when applied in food products. The OHC of FR samples increased ($P < 0.05$) compared to the FM samples (**Table 5**), where an increase of 113% was noted for okara, 67.7% for OPF, and 61.9% for OPT. The structural and chemical modifications of fiber upon alkaline and physical treatments could have affected the OHC in a similar manner to WHC. Fat removal during defatting, as evidenced in low fat content (**Table 1**), and the rupture of the fiber matrix provided increased surface area for oil holding. OHC is also associated with IDF, which can absorb oil (*21*). The higher levels of IDF in OPT and OPF (**Table 2**) conferred higher ($P < 0.05$) OHC to the oil palm fibers compared with okara. The relatively low OHCs of FR and FM are desirable for the development of fried food products, as low OHC provides a nongreasy sensation (*20*).

EA and ES. EA refers to the ability of a molecule to facilitate dispersion of two immiscible liquids (*20*), whereas ES refers to the maintenance of the emulsion system. Okara possessed the highest ($P < 0.05$) emulsifying properties (**Table 5**), attributable to the high protein content (**Table 1**) acting as strong emulsifying agents. In general, results indicated that the EA and ES decreased upon alkaline treatment. The EA of okara FR was 78.9% lower ($P < 0.05$) than the EA of okara FM, mostly attributed to a high loss of proteins (**Table 1**). Similarly, the low EA observed in OPT and OPF samples corresponded with a lack of proteins (**Table 1**) in these fibers. Generally, all agrowaste samples displayed high ES. Our results indicated that okara FM and FR possessed higher ($P < 0.05$) ES (99.3 and 96%, respectively) than both OPF FM and FR (31.2 and 86.2%, respectively) and also OPT FM and FR (98.6 and 75.5%, respectively). Thus, okara may be more suitable for application in emulsion-based food systems requiring a long shelf life. Low EA may not necessarily indicate poor ES. OPT samples and OPF FR samples displayed high ES despite low EA and could potentially be used in emulsion-based food matrices requiring prolonged stability. Studies have associated good emulsification properties of dietary fiber with improved blood cholesterol levels, mostly attributed to the binding of biliary acids, hence limiting its absorption in the small intestine, and leading to increased expenditure of cholesterol for biliary acid synthesis (*22*).

Table 6. Mineral-Binding Capacities (Percent) of Fiber Materials (FM) and Fiber Residues (FR) of Oil Palm Trunk (OPT), Oil Palm Frond (OPF), and Okara

mineral binding activity	sample	okara[a]	OPT[a]	OPF[a]
Fe	FM	83.12 ± 0.19 A,a	23.51 ± 0.08 A,c	60.72 ± 0.70 A,b
	FR	71.23 ± 1.30 A,a	62.82 ± 6.93 A,a	63.80 ± 0.14 A,a
Zn	FM	66.38 ± 0.42 B,a	52.45 ± 0.07 B,b	67.03 ± 1.26 A,a
	FR	90.69 ± 0.27 A,a	88.42 ± 0.14 A,b	84.84 ± 0.85 A,c
Cu	FM	67.60 ± 0.32 B,a	53.80 ± 2.17 B,b	48.94 ± 1.58 B,b
	FR	85.83 ± 0.25 A,c	92.33 ± 0.02 A,b	93.78 ± 0.00 A,a
Ca	FM	38.52 ± 1.68 A,a	5.24 ± 2.12 A,b	4.15 ± 0.08 A,b
	FR	56.51 ± 2.53 A,a	22.78 ± 2.10 A,b	5.48 ± 0.39 A,c

[a] Means in the same row followed by different lower case letters are significantly different ($P < 0.05$). Means in the same column followed by different upper case letters are significantly different ($P < 0.05$).

Antioxidant Activity. The antioxidant activity of samples was measured as percentage scavenging activity in the presence of the DPPH free radical. The antioxidant activities of OPT and OPF FM were higher ($P < 0.05$) than that of okara FM (377.2 and 367.8% higher, respectively) (**Table 6**), mostly attributed to their higher IDF contents (**Table 2**). OPT fiber material contains 30% lignocelluloses (*3*), whereas OPF fiber material consists of 15.2% lignin (*4*), an effective free radical scavenger (*23*). OPT has been reported to possess a high oxygen radical absorbance capacity (ORAC) (*24*). The antioxidant activity of oil palm fibers was also exhibited in our present study (**Table 6**), and such a high scavenging activity for DPPH radical suggests potential of OPT

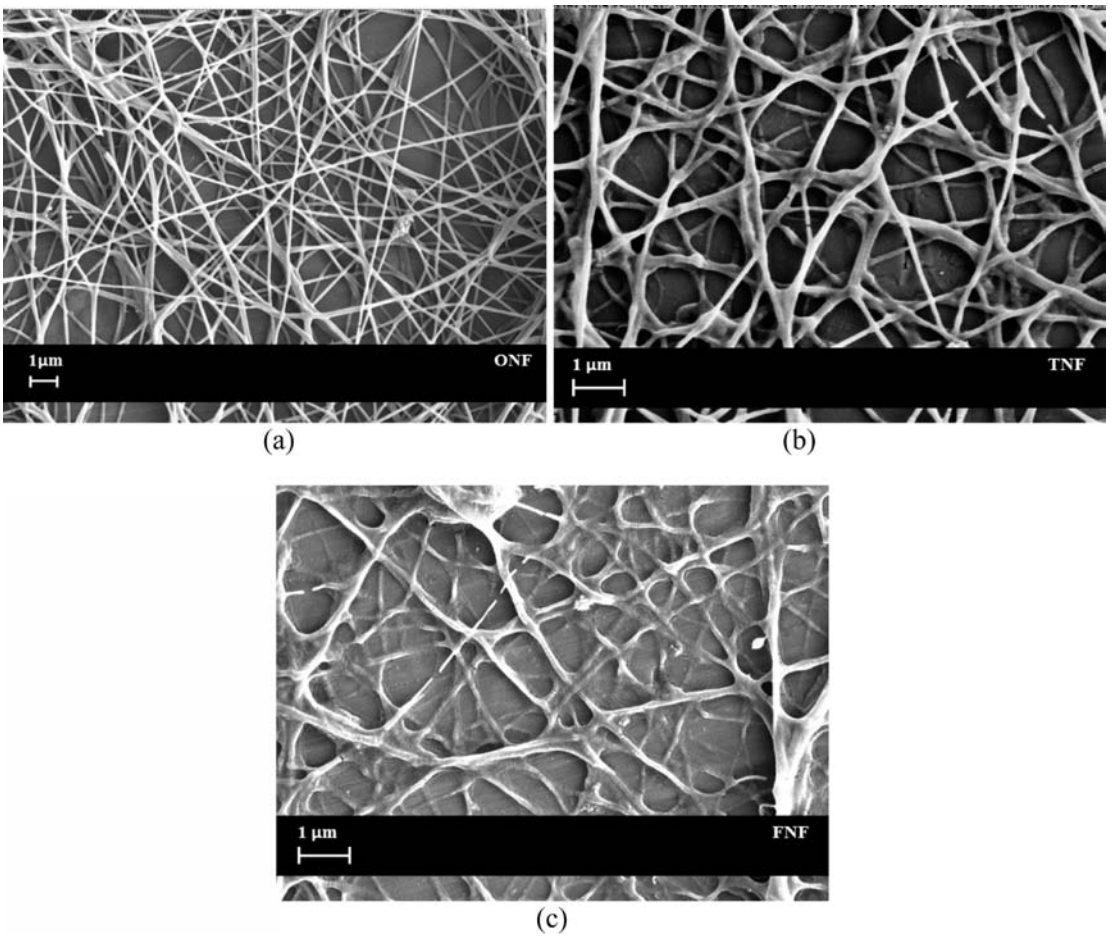

Figure 2. SEM micrographs of nanofibers produced from (**a**) okara (ONF), (**b**) oil palm trunk (TNF), and (**c**) oil palm frond (FNF).

as a functional food with antioxidative effects. Results also showed a reduced scavenging activity in FR samples compared to FM. Dizhbite et al. (*25*) previously reported that the radical scavenging activity of lignin, an insoluble fiber, was positively correlated with increased concentration of heterogeneous lignins and inorganic cations. These compounds reportedly increased the formation of phenolates with stronger antioxidative potentials than their native phenolice counterparts. We postulate that the alkaline treatment had altered the chemical composition of lignins and partially removed inorganic cations, leading to a reduced scavenging activity. Free radical scavenging activity and antioxidant activity are important functional properties of dietary fiber. Laboratory in vitro evidence and clinical data have associated the antioxidative effects of dietary fiber with protection against regenerative diseases and free radical induced tissue damages (*22*).

Mineral-Binding Activity. Agrowaste fibers are used in various industries and water treatment systems to bind heavy metals as hemicelluloses, pectin, lignin, and cellulose have notable metal binding abilities. The metal-binding property of dietary fiber is favorable to remove heavy metals ingested through food, but it is also antinutritive if it interferes with the absorption of beneficial minerals by the gut. The mineral-binding activity of fibers generally increased in FR compared to FM (**Table 6**), attributable to the increased IDF fraction in FR samples (**Table 2**). IDF possesses a high affinity for minerals such as iron, calcium, zinc, and copper. Additionally, alkaline treatment has been reported to expose mineral-binding sites within the fiber polymers (*26*), thus accounting for the increased mineral binding in alkali-treated FR. FR of okara and OPT showed the highest increase of zinc and copper binding compared to FM samples. Zinc binding increased by 36.6 and 68.6% in okara and OPT FR, respectively, compared to FM, whereas copper binding increased by 27 and 71.6% for okara and OPT, respectively. The saturation of certain minerals inherent to the fiber may affect its mineral-binding ability (*22*). Okara showed the highest binding activity for iron, attributed to its lower iron content, compared to OPT and OPF (**Tables 3** and **6**). Similarly, the binding of calcium was lower compared to other minerals tested, possibly due to the high inherent calcium content in all of the fiber samples (**Tables 3** and **6**). The ability of these fibers to bind with minerals is advantageous for the development of functional foods such as high-iron and calcium-fortified products. Cellulose binds weakly to the minerals and easily releases them in the host upon disruption. Additionally, the fermentation of these fibers by indigenous gut microflora would release the bound minerals for increased colonic absorption.

Production of Nanofibers. The supernatant of the alkaline extraction of FM was used to produce nanofibers. Nanofibers are defined as submicrometer-sized fibers with diameters ranging from 100 to 500 nm, which may be useful for drug delivery and functional food applications (*27*). Upon alkaline hydrolysis, the supernatant contained dissolved soluble fibers and hemicelluloses. The SEM micrographs (**Figure 2**) of the electrospun nanofibers showed the morphology of continuous tubular fibers with diameters of < 500 nm. These tubular fibers can serve as scaffolds or vehicles to deliver bioactive compounds within the tubular structure or attached on its functionalized surface. Electrospun polycaprolactone (PCL) was used to successfully encapsulate medically pure drugs resveratrol (antioxidant) and gentamycin sulfate (antibiotic), which exhibited sustained release for

enhanced drug delivery (*28*). In tissue-engineering studies, alginate−polyethylene oxide (PEO) electrospun nanofibers showed promising potential as compatible scaffolding for good adherence of cartilage chondrocyte-like cells (*29*). Electrospun polyvinyl alcohol (PVA) was used to immobilize lipase, with enzyme loading of up to 50% and superior activity compared to crude enzyme following exposures to elevated temperatures and humidity (*30*). These studies continue to support the vast potential of electrospun nanofibers as vehicles for entrapment of bioactive compounds and scaffoldings in various biomedical and bioactive applications. Agrowaste-based nanofibers may serve as potential alternatives to these synthetic polymers adapted for similar applications in functional and value-added products.

In conclusion, the alkaline and physical treatments of FM from okara, OPT, and OPF successfully reduced unwanted residues such as protein, fat, and ash to produce FR with increased TDF and IDF contents. FR appeared rough and flaky upon removal of the smooth waxy outer layers compared to FM and revealed fiber microstructures. Increased fiber fraction in FR influenced the functional properties such as WHC and OHC, emulsifying capacity, and mineral-binding capacity. Both FM and FR demonstrated high activities in mineral-binding and antioxidative scavenging activities and could be further evaluated as new functional foods. The alkaline treatment also solubilized the soluble fibers and hemicelluloses, which were subsequently used to produce nanofibers. These nanofibers possessed a continuous tubular morphology and can be further developed as food adjuncts or nanoencapsulators.

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