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Research Article

The Action of Food Grade Polysaccharides from *Corchorus olitorius*, *Abelmoschus esculentus*, and *Pisum sativum* Wastes with Milk Proteins for Potential Application in Dairy Products

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ABSTRACT

It is the first time to prove the efficiency of lactic acid in polysaccharides extraction from wastes of *Corchorus olitorius* (molokhia), *Abelmoschus esculentus* (okra), and *Pisum sativum* (pea). The interaction between these new polysaccharides and milk proteins has been studied and compared with the behavior of pectin as control polysaccharides. Milk treated with the three types of food grad polysaccharides and pectin showed incensement of total proteins in Milk Supernatant Fraction (MSF) indicating its suitability to use in dairy products as stabilizer. Alkaline native Urea-PAGE presented that pectin, okra, and pea polysaccharides showed associative interaction with αS-casein while molokhia polysaccharide was more attractive to β-casein. Scanning Electron Microscopy (SEM) of coagulated skim milk samples showed an open matrix of casein treated with pectin and pea polysaccharides while a compact microstructure was associated with okra and molokhia polysaccharides samples. The interaction results indicated the potential use of pea polysaccharides in low-fat solid dairy products. While the okra and molokhia polysaccharides were more proportionally suitable to be used in beverages-based milk products. This finding could support the dairy industrial sector with new safe polysaccharide polymers, which could be used in different varieties of dairy products.

Keywords: Polysaccharides; Plant wastes; Polymer interaction; Milk protein; Food grade

INTRODUCTION

Corchorus olitorius, commonly known as (molokhia), *Abelmoschus esculentus* (okra), and *Pisum sativum* (pea), are kind of vegetables cultivated in equatorial and semitropical environments. They are known in the Mediterranean region and consumed as a mean vegetable food in the Middle East. The leaves and seeds are the eatable parts of molokhia and pea respectively, leaving a high percentage of wastes like stems of molokhia, pods of pea, and stem ends of okra fruits. These wastes accumulate in the local markets and fields of Egypt, which can affect negatively the environment. Reduction of food waste enhances food availability, turns on a sustainable application for natural resources, and positively augments the economy [1].

The potency of using food by-products and wastes for the production of nutrient ingredients and bioactive compounds generates new foods with health-promising effects such as antioxidants, antimicrobial, anti-aging, antitumor, immunoregulation, hypoglycemic and hypoglycemic [2-4]. Polysaccharides are a considerable part of these natural ingredients as texturizing agents, which potentially enhance the functional characteristics of foods. These polymers are intensively studied in the dairy sector [5-8].

Recently, manufacturers paid attention to plant based polysaccharides particularly plant polysaccharides since their extraction from the plant is costless and simple to industrialize [3]. Polysaccharides and other macromolecules such as proteins found in foods play a vital role to form the performance, stability, and consistency of the final products [4]. Natural polysaccharides are highly recommended by the consumer and food industry. It has different advantages over other synthetic polysaccharides such as biodegradability, physical and chemical

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modification ability, non-toxic, and friendly to the environment [9].

In the dairy industry, polysaccharides are widely used as texturizing, mouthful, and stabilizing agents, while the selection of polysaccharides is controlled by their interaction with milk proteins [9]. In the diluted solution, the two macromolecules are co-soluble and the system is stable. While some commercial polymers such as guar gum and xanthan gum tend to form phase separation in low-concentrated solutions [10]. Increasing the concentration of polysaccharides and proteins, they interact either by segregative (the macromolecules repeal each other) or associative action (the macromolecules attract one another) [11-13]. Casein micelles form a major key to improving the structure of dairy products, so polysaccharides-caseins interaction should be determined before designing the dairy formulation. The selection of polysaccharides associates with casein micelles and contributes to colloidal stability it is very important [9,12]. Consequently, avoid non-interacting polysaccharides with caseins and lead to phase separation [14].

This study aimed to develop a new edible method for polysaccharide extraction from different plant wastes and compare this method with other traditional extraction methods. Also, the interaction of the extracted polysaccharides and pectin with milk protein has been studied to evaluate the most suitable polysaccharide for fermented dairy products.

MATERIALS AND METHODS

Materials

The seedless pea pod (*Pisum sativum*), the stem ends of okra fruits, (*Abelmoschus esculentus*), and the stems of Jew's-mallow (*Corchorus olitorius*) (Molokhia) were obtained from the different local markets at Alexandria city. The plant wastes were washed with tap water to remove the adhered surface dust particles and after with distilled water and dried at 100°C for 3 h using the oven (KOTTERMANN D 3165 Hanigsen/W-Germany). The dried wastes were ground into a fine powder using a heavy-duty mill, and stored in a tidy closed container at room temperature (25°C ± 5°C) until use.

Extraction of crude polysaccharides from plant wastes

Water extraction: Polysaccharides were extracted according to the method described previously [15], with some modifications. The ground waste of each vegetable was blended in a blender for 5 min with hot distilled water at 70°C using the solid-liquid ratio of 1:25. The blended mixture was incubated for 3 h in a digital thermostatic water bath (HH-S6, China) with continuous stirring at 150 rpm. The aqueous phase was separated by filtration using multiple layers of muslin cloth and centrifugation at 5000 × g for 20 min in table top centrifuge (Model:PLC-012 Gemmy Industrial Corp, Taiwan) and dried by using a tray drier (Edipan international, 28918 LEGANES, Madrid, Spain).

Alkaline extraction: Alkaline extraction was performed using the method described by He, et al. [16] with modifications. The ground wastes were mixed with different concentrations (0.2, 0.4, 0.6, 0.8, and 1 M) of sodium hydroxide, and a solid-alkaline ratio of 1:25, respectively. The succeeding experimental steps were the same as those in the water extraction section.

Acid extraction: Dried wastes were mixed with different concentrations (0.2, 0.4, 0.6, 0.8, and 1 M) of lactic acid, and a solid-acid ratio of 1:25, respectively. The succeeding experimental steps were the same as those in the water extraction section.

Microwave extraction: The polysaccharides extraction by microwave was performed using the method developed previously [15]. The plant wastes were mixed with distilled water and solid-liquid ratios of 1:25. The mixtures were placed in a microwave using power levels of 400 w and 600 w for 3 min. The succeeding experimental steps were the same as those in the water extraction section.

Determination of total carbohydrates concentration

The polysaccharide content in the combined extract was determined using phenol-sulfuric acid according to the method described previously [17]. Polysaccharide yield was reported as gram polysaccharides 100 g dried wastes. The extracts were freeze-dried using lyophilizer (FLD-BT-104, Labcon Scientific Limited, UK) and kept at -80°C till use.

Determination of Total Soluble Solids (TSS)

The total solids content was determined according to the official methods of analysis, [18].

Potential interactions between the extracted polysaccharides and milk protein

Preparation of polysaccharides and milk protein mixture: To investigate the interaction of crude extracted polysaccharides and milk proteins. Skim milk powder, and polysaccharides were added to the reconstituted skim milk at concentrates of (0%, 0.1%, 0.3%, and 0.5%) and incubated at 30°C for 1 h. After incubation, the treated milk samples were centrifuged for 20 min (5000 × g) to separate the Milk Supernatant Fraction (MSF) and the Milk Pellet Fraction (MPF). Samples (1 ml) of MSF were collected, and the MPF samples were re-dissolved in an equal volume (1 mL) of lysis solution containing 7 M urea for further analysis [19].

Determination of protein concentration: The protein content in MSF and MPF samples was determined using the Lowery method [20]. Bovine serum albumin (Sigma) was used as a standard protein.

Alkaline native Urea-PAGE (Gel electrophoresis of milk proteins): The alkaline native Urea-PAGE was performed according to Andrews AT. MSF and MPF samples with or without plant polysaccharides were analyzed by, gel electrophoresis with gel separate of T=12.5%, C=4%, and 4.5 M urea (separation gels buffer 4.6% tris and adjusted by HCl to 8.9). Samples were separated using a running buffer composed

of 15 g tris+73 g glycine in 5 L water and running conditions were 25 V/cm for 75-90 min., till the Bromophenol blue tracing dye was close to the bottom of the slab. Staining was caring out for 1 h in 0.25% (w/v) commission blue G 250 in 50% methanol containing 12.5% TCA and destining in 7% acetic acid. The sample buffer was 10% staking gel buffer containing 8 M urea and 2% of 2-mercaptoethanol, and 0.01% Bromophenol blue. 0.01 g of casein or co-precipitate was dissolved in 1ml sample buffer and 5 µl of all the treatments were applied to the gel. The data were analyzed by total lab software (V1.11).

Microstructure analysis: A Scanning Electron Microscope (SEM) was used for microstructural observation of different milk samples treated with the tested polysaccharides. Sample preparation was carried out according to the method previously described. Briefly, specimens (1-5 mm^3) were fixed for 1 h in 3% glutaraldehyde and dehydrated in a graded ethanol series (30%, 50%, 70%, 80%, and 90%, 1 h in each). Samples were dried with acetone and amyl acetate and then coated with gold in a Fine Coat JEOL (JFC-1100 E, Ion Sputtering Device, Tokyo, Japan) Sputter coater. Samples were examined using JSM-5300 Jeol scanning electron microscope, Tokyo, Japan.

Statistical analysis

All the experiences were prepared twice in an independent manner and measurements were done in triplicate and analyses of variance were conducted by the procedure of General Linear MODEL (GLM) using CoStat (CoStat Software, Pacific Grove, CA, USA) program under Windows software version 6.311. The Least Significant Difference (LSD) test was employed to determine a significant difference at $P<0.05$.

RESULTS AND DISCUSSION

Polysaccharides extraction

The crude polysaccharides extraction from plant wastes was performed on seedless pea pods *Pisum sativum*, stem ends of okra fruits, *Abelmoschus esculentus*, and stems of *Corchorus olitorius* (Molokhia). Four methods of polysaccharides extraction, alkaline, acid, water, and microwave were applied in this study.

Lactic acid extraction: It is the first time to apply lactic acid in polysaccharides extraction from plant tissue. Lactic acid is produced by Lactic Acid Bacteria (LAB), which are widely used in food fermentation and is recommended as a safe and food-grade organic acid. A diluted concentration of 0.2 M lactic acid was the ideal concentration for polysaccharides extraction from pea and okra wastes. This method of polysaccharides extraction is considered a safe and economic method for modern industry. Lactic-extracted polysaccharides could be applied in the fermented food sector especially dairy-fermented products, in which the principal fermenter agents are LAB.

Polysaccharides yield of dry mass from pea shells was significantly increased gradually by increasing the concentration of lactic acid up to 1 M. High polysaccharides yield of 16.8 ± 1.2 and 18.60 ± 2.00 of dry mass was reported with a diluted solution of lactic acid (0.2 M) for pea and okra wastes respectively (Table 1). While polysaccharides yield by lactic extraction from molokhia steam was significantly very low compared with the other two tested wastes. Polysaccharides extraction by organic acids such as acetic acid from Jerusalem artichoke produced a high yield of 76%-81% of fructooligosaccharides over ethanol or acetonitrile extraction. Acid extraction of polysaccharides from *Laminaria japonica*, edible brown seaweed, was carried out with 0.1 M HCl and yielded 18.6% (w/w).

Table 1: Polysaccharides yields from plant wastes using acid extraction, 1:25 ratio of wastes dry matter to the extraction phase. Data are the means ± standard deviations.

Plant wastes	Lactic acid concentration (M)	Polysaccharide yield (g/10 g dried wastes)[*1]	Total soluble solids (%)[*2]	Polysaccharides/DM (%)[*3]
Pea	0.2	1.61 ± 0.04^{b}	2.31 ± 0.43^{i}	16.80 ± 1.20^{c}
Okra		1.76 ± 0.14^{a}	2.65 ± 0.00^{h}	18.60 ± 2.00^{a}
Molokhia		0.58 ± 0.02^{f}	1.00 ± 0.40^{j}	5.90 ± 0.89^{g}
Pea	0.4	1.22 ± 0.14^{d}	2.62 ± 0.03^{h}	12.80 ± 1.56^{e}
Okra		1.57 ± 0.02^{b,c}	2.96 ± 0.05^{f,g}	16.60 ± 0.70^{c}
Molokhia		0.48 ± 0.12^{f}	2.31 ± 0.00^{i}	4.90 ± 0.46^{h}
Pea	0.6	1.43 ± 0.14^{c}	3.60 ± 0.06^{b,c}	15.05 ± 2.01^{d}
Okra		1.69 ± 0.14^{a,b}	3.81 ± 0.21^{b}	17.90 ± 2.23^{b}

Molokhia		0.36 ± 0.01^{g}	2.62 ± 0.02^{h}	3.70 ± 0.23^{i}
Pea	0.8	1.65 ± 0.04^{a,b}	3.25 ± 0.04^{e}	17.30 ± 2.00^{b,c}
Okra		1.55 ± 0.11^{c}	2.79 ± 0.28^{g}	16.40 ± 1.67^{c}
Molokhia		0.94 ± 0.04^{e}	3.15 ± 0.24^{e,f}	9.70 ± 0.98^{f}
Pea	1	1.70 ± 0.13^{a}	3.45 ± 0.22^{c,d}	17.70 ± 1.39^{b}
Okra		1.68 ± 0.00^{a,b}	4.09 ± 0.21^{a}	17.80 ± 1.29^{b}
Molokhia		0.90 ± 0.04^{e}	3.28 ± 0.03^{d,e}	9.30 ± 1.68^{f}

Note: *1 DM: Dried Matter contents in dried wastes of okra, pea and mellow were 94, 95 and 96%, respectively.

*2 Determined in the aqueous phase of extracted materials.

*3 Polysaccharides per dry matter of waste.

Numbers followed by the same subscript letters in each column are not significant different (P ≤ 0.05).

Alkaline extraction: The yield of extracted polysaccharides increased significantly as the alkaline concentration increased up to 0.8 M while decreasing at a concentration of 1.0 M (Table 2). The polysaccharides yields of dry mass from pea and okra wastes by tested alkaline concentrations ranged from 7.5% to 17.1% and 14.0% to 25.1% respectively, while low polysaccharides yields of 6.6% to 12.0%, obtained from molokhia steams. The extracted levels of polysaccharide with 0.8 M alkaline from the three vegetable wastes were higher than that extracted from microalgae *Chlorella* sp. [16], *Spirulina platensis*, and *Ziziphus jujuba* cv.

Table 2: Polysaccharides yields from plant wastes using alkaline extraction, 1:25 ratio of wastes dry matter to the extraction phase.

Plant wastes	NaOH concentration (M)	Polysaccharides yield (g/10 g dried wastes*1)	Total solid (%)*2	Polysaccharides/DM (%)*3
Pea	0.2	1.38 ± 0.02^{e,f}	3.35 ± 0.09^{h}	14.42 ± 1.20^{e,f}
Okra		1.70 ± 0.11^{c}	3.05 ± 0.05^{h}	18.00 ± 2.00^{c}
Molokhia		0.64 ± 0.05^{j}	2.42 ± 0.22^{i}	6.60 ± 0.50^{i}
Pea	0.4	1.26 ± 0.03^{f,g}	6.22 ± 0.27^{f}	13.10 ± 2.00^{g}
Okra		1.54 ± 0.02^{d,e}	6.56 ± 0.02^{e}	16.30 ± 1.20^{d,e}
Molokhia		0.85 ± 0.01^{h,i}	5.19 ± 0.25^{g}	8.80 ± 0.00^{h}
Pea	0.6	1.47 ± 0.20^{e}	6.30 ± 0.28^{e,f}	15.30 ± 2.50^{e}
Okra		2.09 ± 0.13^{b}	7.31 ± 0.26^{d}	22.10 ± 2.70^{b}
Molokhia		0.95 ± 0.01^{h}	6.41 ± 0.00^{e}	9.80 ± 1.60^{h}
Pea	0.8	1.64 ± 0.17^{c,d}	7.33 ± 0.20^{d}	17.10 ± 2.38^{c,d}
Okra		2.36 ± 0.15^{a}	7.78 ± 0.26^{c}	25.10 ± 3.40^{a}
Molokhia		1.16 ± 0.07^{g}	7.80 ± 0.05^{c}	12.00 ± 0,09^{g}
Pea		0.73 ± 0.00^{i,j}	8.62 ± 0.08^{a}	7.50 ± 1.14^{i}

Okra	1	1.32 ± 0.00[f]	8.29 ± 0.01[a,b]	14.00 ± 2.14[f,g]
Molokhia		0.90 ± 0.03[h]	8.19 ± 0. 12[b]	9.30 ± 0.89[h]

Note: *1 DM: Dried Matter contents in dried wastes of okra, pea and mellow were 94, 95 and 96%, respectively.

*2 Determined in the aqueous phase of extracted materials.

*3 Polysaccharides per dry matter of waste.

Superscript letters following numbers for the same strain denote significant differences (P ≤ 0.05). Data are the means ± standard deviations.

Microwave extraction: Polysaccharides yield from plant wastes using microwave intensity of 400 and 600 watts, for 3 min (Table 3). This study using microwave in polysaccharides extraction showed low yields compared with alkaline and acid extraction methods for all tested plant wastes. The polysaccharides yield of 14.5 ± 1.7 from pea waste by 400 watts power was significantly higher than the same extraction using 600 watts. While polysaccharides extraction from okra and molokhia wastes did not affect by microwave power. Also, the lowest polysaccharides yield was reported for molokhia wastes waste compared with the same yield of the other two plant wastes.

Table 3: Polysaccharides yields from plant wastes using microwave assisted extraction, and 1:25 ratio of wastes dry matter to the aqueous phase. Data are the means ± standard deviations.

Plant wastes	Microwave intensity (watt)	Polysaccharide yield (g/10 g of dried wastes*1)	Total soluble solids (%)*2	Polysaccharides/DM (%)*3
Pea	400	1.39 ± 0.07[a]	1.45 ± 0.19[a]	14.5 ± 1.7[a]
Okra		1.27 ± 0.08[b]	1.13 ± 0.04[b]	13.5 ± 1.2[b]
Molokhia		0.26 ± 0.02[d]	0.74 ± 0.17[d]	2.7 ± 0.3[d]
Pea	600	1.01 ± 0.03[c]	0.94 ± 0.03[c]	10.5 ± 0.9[c]
Okra		1.27 ± 0.05[b]	0.29 ± 0.00[e]	13.4 ± 2.0[b]
Molokhia		0.28 ± 0.09[d]	1.16 ± 0.09[b]	2.8 ± 0.4[d]

Note: *1 DM: Dried Matter contents in dried wastes of okra, pea and mallow were 94%, 95% and 96%, respectively.

*2 Determined in the aqueous phase of extracted materials.

*3 Polysaccharides per dry matter of waste.

Superscript letters following numbers for the same strain denote significant differences (P ≤ 0.05).

The obtained results indicated that the microwave power did not have a noticeable effect on polysaccharides extraction yield with all tested wastes. This finding was not in agreement with the results reported by Liang Y, et al.; GU C, et al., who suggested that the high power of microwave extraction results in a high yield of polysaccharides from plant tissue. They theoretically explained this finding because the high power of microwave helps the selective migration of the polysaccharides from the materials to the surrounding at a more rapid rate.

Water extraction: Hot water extraction of polysaccharides is a traditional method and is recognized as a safe and food grade method without using any chemicals, which can keep the natural and physical characteristics of polysaccharides molecules. In this study, the yield of polysaccharides with hot water extraction was lower than those obtains from alkaline and acid extraction for all tested plant wastes. Pea, okra, and molokhia waste polysaccharides yield of dry mass by water extraction were 7.70 ± 0.78, 12.60 ± 0.88, and 2.90 ± 0.19%, respectively, (Table 4). A higher polysaccharides yield of 16.21 ± 1.12% by water extraction from pea shell compared with present results was previously determined. Polysaccharides extraction from okra pods (the edible part) using hot water yielded crude polysaccharides of 1.1 % of fresh okra, which is so lower than the presented results from okra waste. Extracted polysaccharides from the leaves of Jew's-mallow (the edible part of the plant), in hot water, the polysaccharides concentration was 4.2% on a wet basis.

Table 4: Polysaccharides yields from plant wastes using water, and 1:25 ratio of wastes dry matter to the aqueous phase. Data are the means ± standard deviations.

Plant wastes samples	Polysaccharide yield (g)	Total solid (%)	Polysaccharides/dry matters (%)
Pea	0.74 ± 0.02[b]	0.43 ± 0.16[c]	7.7 ± 0.78[b]
Okra	1.19 ± 0.04[a]	1.24 ± 0.04[a]	12.6 ± 0.88[a]
Molokhia	0.28 ± 0.00[c]	0.76 ± 0.28[b]	2.9 ± 0.19[c]

Note: Superscript letters following numbers for the same strain denote significant differences (P ≤ 0.05).

Polysaccharides and milk protein interaction

Influence of extracted polysaccharides on milk protein: Dried polysaccharides obtained from tested plant wastes by lactic acid extraction and pectin as control polymer was added to skim milk samples with different concentrations of (0, 0.1, 0.3, and 0.5%) and incubated at 30°C for 1 h, and the concentration of total protein in Milk Supernatant Fraction (MSF) were determined (Table 5). Pectin addition to milk samples increased the total protein concentrations in MSF up to 29 ± 1.17 mg/mL by adding 0.3% of pectin.

While the significant decrease in protein concentration was associated with adding 0.5% of pectin, which indicated the precipitation of milk protein by increasing the pectin concentration. Milk samples treated with Molokhia polysaccharides showed high protein levels in MSF with all polysaccharides concentrations. The highest increase in total protein (26.6 ± 0.12 mg/mL) was associated with 0.5% of Molokhia polysaccharides.

Table 5: Changes in the total protein content in Milk Supernatant Fraction (MSF) treated with different concentrations of extracted plant polysaccharides.

Plant wastes samples	Protein Concentration mg/L		
	Polysaccharides concentration (%)		
	0.1	0.3	0.5
Pea	21.01 ± 0.50[d]	25.01 ± 0.01[b]	25.01 ± 0.08[a,b]
Okra	26.57 ± 0.21[a,b]	25.36 ± 0.17[b]	24.77 ± 0.58[b]
Molokhia	23.42 ± 0.08[c]	23.74 ± 1.45[c]	26.57 ± 0.12[a]
PC	27.33 ± 0.96[a]	28.95 ± 1.16[a]	16.24 ± 0.08[c]

Note: Control 22.33 ± 1.20

Data are the means ± standard deviations.

Superscript letters following numbers for the same strain denote significant differences (P ≤ 0.05).

Total protein levels of MSF samples treated with okra waste polysaccharides were significantly elevated over the control with all polysaccharides concentrations. Also, a gradually decreasing of total protein was indicated by increasing the okra polysaccharides concentration was observed. Overall, the addition of okra polysaccharides contributed to stabilizing protein content in the MSF. MSF of milk samples treated with pea polysaccharides showed decreasing in total protein with only 0.1% of polysaccharides concentration. Equal levels of proteins of 25.01 ± 0.08 mg/mL and higher than the control were related to adding 0.3% and 0.5% of pea polysaccharides.

In this study treating milk with the four types of polysaccharides increased the total protein levels in MSF concerning the different concentrations of polysaccharides. The results were in disagreement with the results obtained with chitosan, where the total protein levels decreased in MSF by adding chitosan up to 0.8% of chitosan and forming milk coagulation at normal milk pH [19]. Generally, our results indicated that the tested polysaccharides provided a stabilization stat of milk protein and these results support their application in the dairy industry.

Effects of different polysaccharides on casein as determined by alkaline native urea-PAGE: Casein is the principal milk protein and forms about 80% of total milk proteins. It composed of αS-casein, β-casein and κ-casein. Milk samples were treated with (0.0, 0.1, 0.3, and 0.5%) of pectin and different wastes polysaccharides from lactic extraction, and MSF was analyzed by alkaline native Urea-PAGE (Table 1). Then the resulting figure was analyzed by total lab software (V1.11) program to estimate the concentration of different casein components in the MSF compared with the control.

Protein and polysaccharides are macromolecules that interact together in the solution and play a very important role in the stabilization of food structure by controlling the mouthful, texture, and microstructure. The interaction of two macromolecules in the solution is varying depending on the

concentration of the polymers. This interaction may be segregate (the two macromolecules repel each other and are shown as incompatible) or associative (the two macromolecules attract one another) [11]. In the highly diluted solutions, the two polymers are co-soluble, and the system stays very stable. Increasing polymer concentration leads to phase separation in the case of segregate interaction. While associative interaction forms a co-soluble system up to certain concentrations of two polymers, increasing polymers concentration forms large attractive molecules and leads to polymers precipitation or coacervation [13].

In the present study, alkaline native Urea-PAGE, analyses showed that αS-casein can associate with pectin and increased αS-casein concentrations in Milk Supernatant Fraction (MSF). The highest increase in αS-casein was associated with 0.3 % of pectin, which indicated that this concentration of polymer provides high associative interaction and stabilization of αS-casein in the solution. Reduction of β-casein concentration by about 14% was associated with MSF treated with all tested pectin concentrations. While the concentration of κ-casein remains constant or slightly increases by about 5 % with adding 0.1% and 0.3% of pectin. These results indicated the high absorption of pectin on casein micelles, especially at αS-casein. High absorption of pectin on entire casein micelles and casein micelles cleaving-off κ-casein was proven previously and our results complete this finding by proving that the αS-casein is a more attractive casein fraction to pectin.

Polysaccharide extracted from pea wastes behaves the same behavior as pectin in the solution. All tested pea polysaccharides concentrations increased the concentration of αS-casein in the supernatant (Table 1). These results indicated high associative interaction between the αS-casein and pea polysaccharides. Also, stabilization and slight reduction from 2% to 15% were observed with β-casein and κ-casein respectively, in MSF samples treated with different pea polysaccharides concentrations. These results indicated the high affinity between αS-casein and the polysaccharide extracted from the pea notably a concentration of 0.3% polysaccharide.

In contrast, the polysaccharide extracted from molokhia wastes interacts with casein components completely different from pectin and pea polysaccharides. It caused a reduction between 10% to 30% and 13% of αS-casein and κ-casein respectively, in MSF. Its effect with β-casein is depending on the polysaccharide concentration. It showed a slight decrease of about 5% of β-casein in MSF treated with 0.3% and 0.5% of polysaccharides, while a slight increase of β-casein was reported in MSF treated with 0.1% of polysaccharides (Table 1).

The concentration of 0.1% of okra polysaccharides appeared to be the most effective concentration to stabilize casein micelles in MSF treated samples. This concentration increased the concentration of αS-casein and κ-casein by 194% and 16.5% respectively, while the same concentration reduced the amount of β-casein by 5% lower than the control. While 0.3% and 0.5% of okra polysaccharides decreased the amount of αS-casein and κ-casein by various levels, the lowest decrease was associated with the 0.5% of okra polysaccharide. This phenomenon may be attributed to the high degree of associative interaction between okra polysaccharide and κ-casein, which formed a large macromolecule in the case of increasing polymers concentration and consequently coacervation and precipitation.

These results concluded that the pea polysaccharides and pectin reported high stabilization of casein in the solution of MSF up to 0.5% concentration, and showed high affinity to αS-casein rather than other casein fractions. Okra polysaccharides showed high affinity to αS-casein with a concentration of 0.1% only. Molokhia polysaccharide has different interaction from all the tested polysaccharides. It showed low affinity and stabilization to all casein components at all tested concentrations. These results may indicate the unsuitability to use Molokhia polysaccharide in dairy products. Finely, indeed more studies about the composition, structure, and interaction with milk proteins for all newly extracted polysaccharides.

Microstructure of the milk samples treated with extracted polysaccharides: Designing dairy fermented products needs to regard protein-polysaccharide interactions, which play a very important role in the formation of texture and microstructure of dairy products [12]. Selection of polysaccharides type during dairy products design is a very important key to ensure the interaction between milk proteins and polysaccharides in the end dairy product, since the integration between these biopolymers may control segregative reaction or phase separation during the storage period of the product. Scanning Electron Microscopy (SEM) of coagulated skim milk samples treated with 0.1% of pectin and plant wastes polysaccharides is presented in Table 2. Milk gelation is usually formed by acidification or renneting, the presence of polysaccharides in the gelation matrix affects the formation of casein aggregates.

Milk samples treated with pectin (Table 3) and pea polysaccharides (Table 4), had an identical microstructural appearance that could be described as an open matrix of casein clusters in which fat globules (voids) and whey pockets (minute pores) were evenly distributed. Similarly, Wang, et al., described the microstructure of fermented milk treated with 0.25% and 0.5% pectin as an extended open protein matrix in which large fat globules and whey pockets were embedded and protein clusters appeared denser compared with pectin-free samples. Previously reported that pectin is strongly adsorbed onto the casein matrix as the pH of fermented products is lowered. The formation of a dense casein matrix in samples stabilized with pea polysaccharides was identical to that formed by pectin, which may indicate that polysaccharides extracted from pea interact with casein molecules in the same manner as pectin did.

While milk samples treated with polysaccharides extracted from okra and molokhia wastes (Table 5), developed the most compact microstructure in which casein aggregates lost their identity and fused strongly to form a continuous matrix with the presence of few voids and minute pores. This may probably be attributed to the ability of these polysaccharides to crosslink casein aggregates and tighten the protein matrix resulting in strengthening the overall structure of milk gelation. Similarly, Xu, et al., reported that the addition of 0.08% okra polysaccharides in fermented milk formulation reduced the porous structure of the gel and induced the formation of larger protein clusters leading to a more compact protein network.

CONCLUSION

This study introduced a new safe polysaccharide extraction method from plant tissue using lactic acid. A diluted solution of lactic acid was very effective in extracting the polysaccharides from *Corchorus olitorius* (molokhia), *Abelmoschus esculentus* (okra), and *Pisum sativum* (pea) wastes. The extracted polysaccharides showed good stabilization of milk proteins, which indicates its suitability to use in the dairy industry. Alkaline native Urea-PAGE presented that pectin, okra, and pea polysaccharides showed associative interaction with αS-casein while molokhia polysaccharide was more attractive to β-casein. Scanning Electron Microscopy (SEM) of coagulated skim milk samples showed an open matrix of casein treated with pectin and pea polysaccharides, which indicated the suitability of two polymers to use in low-fat dairy products. While compact microstructure was presented in milk samples treated with okra and molokhia polysaccharides.

AUTHOR CONTRIBUTION

Goher N: Formal analysis (equal); methodology (equal); and conducting a research and investigation process. Kheadr E: Conceptualization (equal); formal analysis (equal); investigation (lead); methodology (lead); project administration (lead); validation (lead); and writing-review and editing (equal). Hassan A: Formal analysis (equal); methodology (equal); and conducting a research and investigation process. Elganam M: Conceptualization (equal) Alnemr T: Data analysis Dabour N: Conceptualization (equal); writing-original draft (lead); data analysis (lead); and writing-review and editing (equal).

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Not applicable.

DATA AVAILABILITY STATEMENT

All data are available within the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could influence the work presented in this paper.

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