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Exploring the nanonisation physical strategies for nano-prebiotics production

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ABSTRACT

Prebiotics, non-digestible carbohydrate is consumed to improve the survival of probiotics in gut microbiota. The internalisation of prebiotics into probiotics potentially ensures survival and targeted delivery. To achieve the internalisation of prebiotic sources, nanonisation had been implemented to reduce the size of polysaccharides to nano size. In this study, two nanonisation techniques were applied which were water bath and probe sonication. Different sonication power was used at 50 and 100% amplitude for probe and water bath sonication at 3, 6, 9, 12 and 15 min. The maximum particle size reduction for Arabic gum can be determined by probe sonication treatment at 100% amplitude for 12 min with a 35% reduction from 253.98 nm to 165.43 nm, about 35% of its original size. Significant size reduction was observed from maltodextrin in probe sonicator for 6 min at 100% amplitude where the 81% reduction from 938.96 nm to 180.55 nm. The in vitro study on sonicated prebiotics also showed comparable viability of *Lactobacillus plantarum* compared to glucose of carbon-source. Therefore, nanonisation on prebiotics can be potentially applied to the internalisation process to ensure the successful delivery system of probiotics.

Keywords: *Prebiotics, Nanonisations, Waterbath sonications, Probe sonications, Probiotic encapsulation*

1. INTRODUCTION

Prebiotics have been defined as non-digestible food ingredients that beneficially affect the host by stimulating the growth and activity of one or a limited number of good bacteria in the colon [1]. The application of prebiotics is mentioned to have an advantage in altering the GI microflora which improves gut health (intestinal microbial balance). In addition, the immune system is altered thus improving the performance and enhancing the nutrient utilization of the poultry. Prebiotics also reduce the pathogen invasion that increases the resistance to colonization. The most common prebiotics are oligosaccharides, which are non-digestible carbohydrates. The mode of action of prebiotics is explained by supplying nutrients to the beneficial microbes (probiotics) or by tricking pathogenic bacteria into attaching to the oligosaccharide rather than the intestinal mucosa.

Efficient delivery of probiotics to the intestinal area is very critical for achieving the therapeutic effect of the probiotics, because of the harsh gastric conditions that cause low bioavailability of the oral delivery of probiotics [2]. Reports show that encapsulation can protect the probiotics from harsh conditions [3,4]. To enhance the efficiency of the probiotics, we aim to incorporate both probiotics and prebiotics by using a nanotechnology approach to ensure the bioaccessibility of the probiotic and its delivery to the targeted area [5]. The method of internalisation of prebiotics into probiotics is seen as a potential method to ensure the successful delivery of probiotics into the targeted area [4]. Therefore, it is proposed to encapsulate the probiotic into microcapsules (prebiotic nano-particles) to protect the cultures against harsh gastrointestinal conditions.

Nanonisation of prebiotics is a technique to reduce the size of prebiotics from macromolecules to nanomolecules. Nanonisation by sonication works as the sound waves from the sonication process agitates the particles in the solution. Additionally, it changes an electrical signal into a physical vibration that can disintegrate materials. As a result, these interruptions can mix solutions and accelerate the transformation of solids into liquids. Hypothetically the act of disintegration of material is aimed to break the particle of prebiotics and thus results in nano-prebiotic particles. Several types of nanonisations include probe sonication (direct) and water bath sonication (indirect). The difference between probe and water bath sonicators is explained by the generation of sound waves in the water bath where the samples are submerged or attached as probes directly to the sample to be sonicated. The application of nanonisation by sonication has been reported by several authors. Sompech et al. [6] reported that a 35% size reduction was analysed by $LaCoO_3$ sonication for up to 6 min. Better size reduction by 69% as a result of probe sonication as compared to bath sonication for only a 65% reduction in graphene oxide synthesis [7].

Physical treatment such as sonication can reduce the size of prebiotics to be internalised in probiotics during the encapsulation procedure. The primary objective of this research is to investigate the efficacy of sonication as a

nanonization technique for producing nano-prebiotics. Given the critical role prebiotics play in promoting gut health, there is a pressing need to enhance their bioavailability, solubility, and stability. Sonication, which utilizes high-frequency sound waves to create cavitation and shear forces, offers a potential solution by reducing prebiotic particles to the nanoscale. Therefore, this study aims to compare the water bath sonication and probe sonication techniques for prebiotics' size reduction. The study contributed to offering a foundation for the application of sonication to produce nano-prebiotic particles. Furthermore, this study also provided a basis for the optimisation of developing probiotic encapsulation.

2. METHODOLOGY

2.1. Preparation of nano-prebiotics

Maltodextrin (Sigma Aldrich, USA), DE: 10-12 and Arabic gum (Sigma Aldrich, USA) were mixed with deionized water at concentrations of 10% wt/v and 2% wt/v, respectively. The selection of prebiotic concentration was referring to previous reports on effective maltodextrin and Arabic gum concentrations for encapsulation matrix [8,9]. The prebiotics solutions were mixed at 255 rpm for 30 min before the sonication process. Water bath sonication (Cole Palmer, USA) and probe sonication (QSonica, Q500-220 + 4418 + 432B2, USA) were set at 50 and 100% amplitude to reduce the size of selected prebiotics. The sonication time was set to 3, 6, 9, 12 and 15 min. Different amplitudes of sonication and duration were referred to reports on producing polysaccharide nanoparticles [10].

2.2. Determination of nano-prebiotic particles

The average size and size distribution of nano-particle prebiotics were determined by dynamic light scattering (DLS) analysis using a commercially available instrument, namely, the Brookhaven Particle Size and Zeta Potential Analyzer (Brookhaven Instruments, Holtsville, USA).

2.3. Morphology of nano-prebiotic particles

The morphology of the treated nano-prebiotic particles was observed under a phase-contrast microscope (Olympus, CX33, Japan) at 40x magnification. The morphology of the sonicated prebiotics was observed for their structural shape and changes in comparison to untreated prebiotic sources.

2.3. *In vitro* prebiotic effect

The in vitro prebiotics effect was done according to Wang et al. [11] with some modifications. Nano-prebiotics particles were added in a carbon-free MRS medium as the only carbon source at a final concentration of 2.0% (wt/v). An equal amount of glucose was added as positive control, and another MRS medium with no carbon source was used as blank control. All mediums were autoclaved at 121°C for 15 min. The concentration in each medium was adjusted to 1 x 10^6 CFU/mL, and the *Lactobacillus plantarum* was cultivated at 37 ± 1°C for 24 h. *L. plantarum* was collected by centrifugation (5,000×g, 5 min, 4± 1°C). The *L. plantarum* strains were washed twice with sterile phosphate-buffered saline solution (pH 7.4 ± 0.1) to remove the left fermentation products adhered to the surface of *L. plantarum* strains.

2.4. Statistical analysis

All experiments were performed in triplicates to verify the repeatability of the results. The data were analysed using a One-way analysis of variance and Tukey's honest significance difference (HSD) test at $p<0.05\%$ using SAS software (Version 9.4, S.A.S. Institute Inc. North Carolina, USA). All data were presented as mean ± standard deviation.

3. RESULTS AND DISCUSSION

3.1. Effect of nanonisation techniques on prebiotics' particle size

In a way to produce prebiotic nano-particles for internalised encapsulation with probiotics, the condition of treatment was done to determine the optimised condition for reducing the prebiotic source. In this part of the study, the physical treatment of the water bath sonicator and probe sonicator were used at different power levels (%) and time (min) to compare their effect on reducing the size of the prebiotic.

Maltodextrin and Arabic gum have been tested to observe the difference in the particle size as a response to the physical treatment of 50 and 100% sonicator power in the range of 3 to 15 min of sonication. The results of the maltodextrin and Arabic gum variation on the treatment are shown in Figure 1 and Figure 2, respectively. Figure 1 and 2 show the impact of water bath (WS)- and probe sonication (PS) on the reduction of maltodextrin and Arabic gum particle size at different sonication times at an amplitude of 50% and 100% amplitude, respectively.

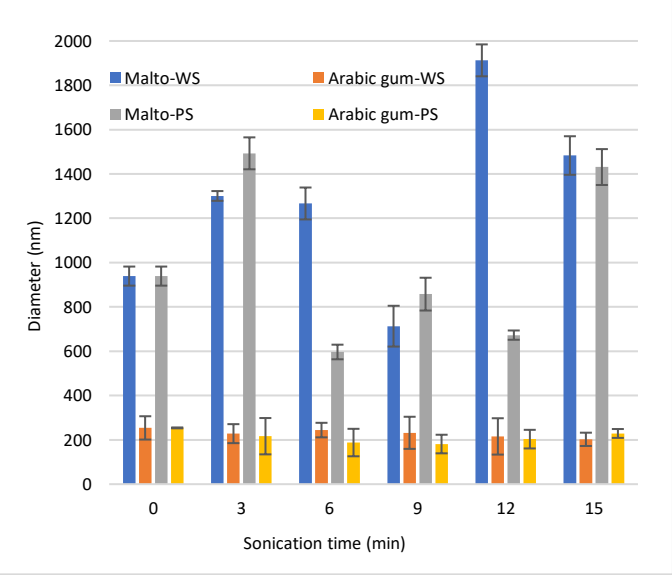

Figure 1. Impact of probe and water bath sonication on reduction of prebiotics' particle size at different times for 50% amplitude

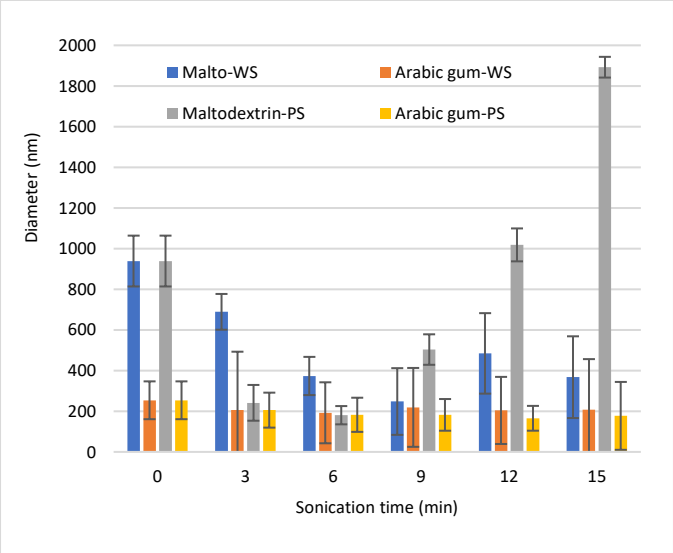

Figure 2. Impact of probe and water bath sonication on reduction of prebiotics' particle size at different times for 100 % amplitude

It was found that specific sonication parameters, particularly amplitude and duration, play a critical role in determining the size and uniformity of the nano-prebiotics. For Arabic gum, the original untreated particle was determined as 253.9833 nm in size. Generally, water bath and probe sonication at amplitude 50 and 100% showed no significant reduction in size after 3, 6-, 9-, 12- and 15-min. Maximum reduction of particle size can be determined by probe sonicator treatment at 100% amplitude and 12 min with 35% reduction to 165.4333 nm from its original size (untreated). Further increase in sonication time (15 min) resulted in a larger size of Arabic gum particles. A possible explanation for this result might be due to the increase in heat generated as time of sonication increased. The expansion of the objects takes up more space and explains the swelling of particle size as the temperature increases with sonication time [12].

On the other hand, the original maltodextrin has a larger particle size which was 938.9633 nm. By application of the physical treatment, the size of the particles was reduced with the highest reduction of 81% to 180.550 nm when treated with a probe sonicator at 100% amplitude for 6 min. The same trend was observed in the effect of sonication time on the particle size as a result of sonication, prolonged sonication time resulted in increment of maltodextrin particle size. It was also observed that prolonged sonication time increased the temperature of the particle solution. This might be due to when heat is added to a substance, faster vibration of the molecules and atoms. As atoms vibrate faster, the space between atoms increases. The motion and spacing of the particles determine the state of matter of the substance. The result of increased molecular motion is that the object expands and takes up more space which explains the swelling of the particle size with an increase in solution temperature as the sonication time increases. The result is also in line with a report by Ahmad et al. [10] on particle size expanding as a result of temperature increment. This result is in line with a report by Mellado et al. [7] that the probe sonicator resulted in a higher reduction compared to the water bath sonicator in the treatment of graphene oxide structure. Therefore, it can be concluded that probe sonication with 100 % amplitude for 6 min works well in producing small-size particles of prebiotics for encapsulation purposes with probiotics.

3.2. Morphology of nano-prebiotics particle

Optical microscopy was used to observe the morphological characteristics of the treated prebiotic without any other process applied. No dehydration or drying process took place as the image of the sonicated prebiotics was aimed to be observed and studied. This explains the selection of optical microscopy to observe the morphological characteristics and thus visualize the effect of each sonication on the prebiotic surface. Figure 3 and 4 show optical images of the maltodextrin and Arabic gum that were probe sonicated with 100% amplitude at different times. In all sonication conditions, the dispersion of the particles can be seen clearly.

According to the results, the sonicated maltodextrin and Arabic gum have smooth or porous surfaces and are oval or circular. Samples showed tiny, smooth-surfaced polygonal granules, although some also had dents or hollows at one end. This is in line with a report by Noman et al. [13] that the production of nano-particle starch resulted in regular and round-shaped patterns. Further imaging (i.e. electron microscopy) should be applied to analyse the details of morphological changes as effect to sonication for the production of nano-prebiotic materials as well as the average distribution size of the nano-prebiotics produced.

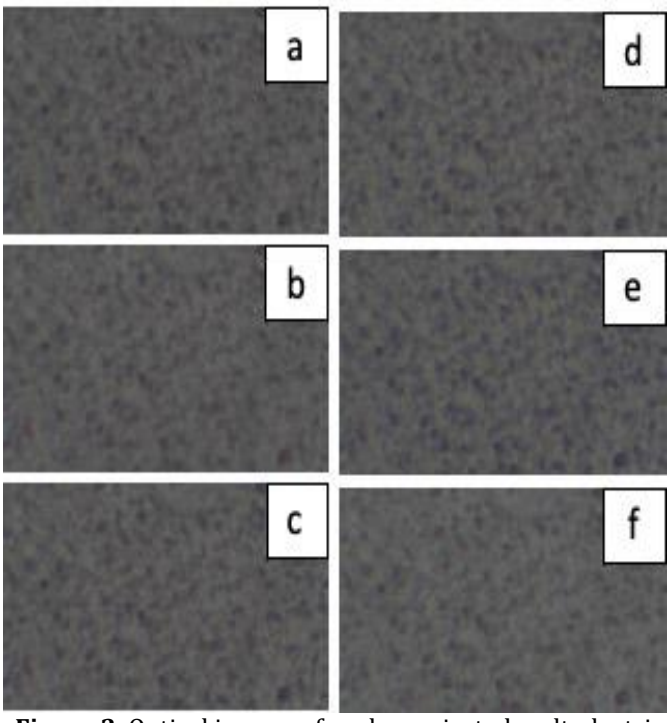

Figure 3. Optical images of probe sonicated maltodextrin at 100 % amplitude at different times for (a)0 min; (b) 3

min; (c) 6 min; (d) 9 min; (e) 12 min and (f) 15 min at 40x magnification

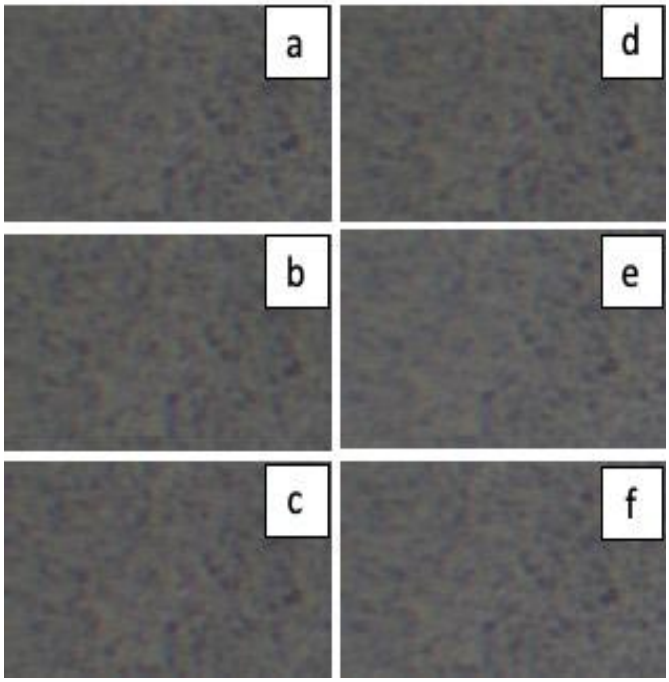

Figure 4. Optical images of probe sonicated Arabic gum at 100 % amplitude at different times for (a)0 min; (b) 3 min; (c) 6 min; (d) 9 min; (e) 12 min and (f) 15 min at 40x magnification.

3.3. *In vitro* prebiotic effect

The type of carbon source influences a probiotic's growth performance [11,14]. Table 1 shows the viability of *L. plantarum* cells after 24 h incubation for different carbon sources in MRS media. The initial viability of the culture was set at 6.12 ± 0.247. The viability of sonicated prebiotics was compared to the viability of *L. plantarum* in no carbon source media (negative control) and glucose (positive control).

Table 1 Viability of cultures after 24 h incubation in different carbon source MRS broth

Time (h)	Log_{10}CFU				
	Initial	NC	Glu	SM	SAG
0	6.12 ±0.247[a]				
24		4.87 ±0.50[a]	9.87 ±0.032[b]	9.95 ±0.063[b]	9.68 ±0.452[b]

Values are means of three (3) replications ± standard deviation. NC: non-carbon source; Glu: glucose; SM: sonicated maltodextrin; SAG: sonicated Arabic gum. Values marked by the same letter are not significantly different (p>0.05).

Sole-carbon source of sonicated maltodextrin and Arabic gum resulted in comparable viability of *L. plantarum* in the range of 9.68 to 9.95 log_{10}CFU. The highest viability was observed in sonicated maltodextrin, slightly higher than the positive control which was glucose. Sonicated Arabic gum showed lower viability at 9.68 log_{10}CFU. The variation of the viability after 24 h incubation might be due to the availability of the carbon source to promote the growth of *L. plantarum* in the MRS medium.

L. plantarum is one of the lactic acid bacteria that may have a specific substrate fermentation mechanism that resulted in potentially utilizing the prebiotics selectively [15]. However, there is no significant difference between positive control and sonicated prebiotics. These results suggest that the treated (sonicated) prebiotics have the potential to replace carbon sources in the media, and hence potentially be used as prebiotic sources. For future works in synthesising prebiotics nano-particles, sonication potentially can be adopted as one of the nanonisation techniques as it is a rapid, stable and chemical-free method.

The insights gained from this research could inform other areas of nanotechnology, particularly in the food and pharmaceutical industries. This finding serves as basic knowledge with broad implications and extension of information on nano-prebiotic production that includes potential application in the functional food industry. The ability to produce highly bioavailable nano prebiotics might open new possibilities in the formulation of functional foods and dietary supplements. The nano-prebiotic produced by the sonication technique also can be potentially applied in the targeted delivery system with enhanced bioavailability to reach specific sites within the gastrointestinal tract.

4. CONCLUSIONS

The exploration of nanonization by sonication for nano-prebiotic production represents a significant step forward in the field of functional foods and dietary supplements. The research highlights the importance of optimizing sonication parameters to achieve the desired particle size and distribution, which in turn enhances the viability of probiotic culture. Nano-prebiotic particles (maltodextrin and Arabic gum) were successfully synthesised by the sonication method. The significant reduction in the size of maltodextrin illustrated that probe sonication offers better treatment physically to produce nano-prebiotic particles. It can be concluded that the nanonisation technique of probe sonication with 100% amplitude for 6 min works well as one of the nanonisation techniques to produce nano-sized prebiotic particles, particularly maltodextrin. The viability of *L. plantarum* in vitro study of sonicated maltodextrin was slightly higher than sonicated Arabic gum and comparable to macromolecules of glucose. A rapid and simple method of nanonisation ensures the stability of the prebiotic for internalisation in probiotics thus increasing the efficiency of the probiotic delivery system. The broader implications of these findings suggest potential applications in targeted delivery systems, functional foods, and beyond, offering new opportunities for innovation in health and nutrition. As the field continues to evolve, further research into the long-term stability and efficacy of sonicated nano-prebiotics will be crucial in fully realising their potential.

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