

Antioxidant Property of Dry and Wet Mill Nanostructured *Zingiber Officinale* Rosc. (Ginger) Rhizome: A Comparative Study

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Abstract. The effects of the wet milling (WM) and dry milling (DM) on the physical and antioxidant activity of the nanostructured ginger rhizome powder (NG) were studied and compared with its micron (MG) and submicron particle (SM). Both dry and wet milling processes reduced the particle into nanoscale (in the range of 200 to 250 nm). DM showed a 3.6% increase in size compared to the WM nanostructured ginger rhizome. A higher degree of the agglomerated nanoparticle was found in the DM based on FESEM image. Greater value of TPC and TFC showed in DM sample (1178.33 mgGAE/g and 22.35 mgQE/g of dw respectively, followed by the WM with 9203.70 mgGAE/g; 20.16 mgQE/g of dw respectively, SM with 7595.27 mgGAE/g; 16.22 mgQE/g and the MG with 6938.33 mgGAE/g; 14.80 mgQE/g of dw correspondingly. No difference in the DPPH free radical inhibition between the DM and WM samples, but greater than the SM and MG. Moreover, the DM showed a strong ($p < 0.05$) ABTS free radical scavenging activity and FRAP compared to the other samples tested. The antioxidant activity was in the following sequence: DM>WM>SM>MG. Dry mill process had a potential to produce the nanostructured ginger rhizome with better antioxidant activities.

Keywords: Antioxidant, dry mill, wet mill, *Zingiber officinale* Rosc.

1. Introduction

Ginger (*Zingiber officinale* Rosc) is one of the well known spices and has been used since the past 19th century. It is classified as an important material due to its broad range of applications such as in medicine, pharmaceutical as well as in the food industries. On the other hand, ginger also possesses the obvious antioxidant properties both in vivo and in vitro systems due to the presence of its major phenolic substances collectively known as gingerols and its derivative [1]. However, it has been reported that the active compounds found in herbs and spices are ineffectively absorbed due to the poor solubility in water or oil, the larger particle size and complex chemical structure [2]. Abundant studies have been carried out to enhance the activity and improve the value in use. Nanotechnology is reported to improve the solubility and increase the dissolution rate of poorly water-soluble active pharmaceutical ingredients [3]. Nanotechnology is described as the “science of materials and systems with structures and components which demonstrate novel physical, chemical and biological properties or phenomena that exist in the nano size scale (1-100 nm)”. Nanoparticles are defined as colloidal systems with particles varying in size from 10 nm to 1000 nm [4]. Nanotechnology also shows great potential in nutraceuticals and functional foods manufacture for health improvement. The reduction of particle size of the various materials to nanosizes leads to some changes in their structure and surface area and brings some new outstanding characteristics that bulk materials do not previously possess as well as give new applications in both the academia and industry [5]. The particle size reduction causes larger external surface areas available for interaction which promotes efficient nutrient

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distribution, improves product shelf life, and increases absorption. Generally, greater intracellular uptake and bioactivity are shown by nanoparticles compared to microparticles due to their small size and relative mobility [6]. In addition, the active ingredients are also fully dissolved. When the plant medicine undergoes nanonization, the cell membrane and cell walls are crushed into pieces, causing the active constituents to directly be in contact with the outer solvent. Furthermore, the contact area of the nanoparticle with body fluid also increases promoting faster absorption and higher bioavailability [7]. Despite the emergence of nanotechnology and the benefits that it provides, there is little information on the effect of the milling condition on the antioxidant activity of the nanostructured ginger rhizome and thus needs to be clarified. Therefore, this study investigates the effects of the dry and wet milling on the antioxidant activity of the nanostructured ginger rhizome powder and the effectiveness of the activity with the conventional prepared sample.

2. Experimental Procedures

Fresh ginger (*Zingiber officinale* Rosc.) rhizome of the 'Bentong' variety was obtained from Bentong, in Pahang, Malaysia. Voucher specimens (SK 2049/12) of these plants were deposited at the Herbarium Institute of Bioscience, at University Putra Malaysia. The standards used in this study were supplied by the Sigma Integrated Company. The ginger rhizome was thoroughly washed, and dried in the cabinet dryer at 60 °C for 5 days. The dried ginger rhizome was ground using a food processor (Panasonic 176, China) for 5 min, into a micron particle (MG), whereas the submicron (SM) ginger was prepared using the hammer mill at 2890 rpm and sieved using a 250 µm sieve. The wet mill nanostructured ginger rhizome (WM) was prepared by introducing 0.5 gram of dried ginger powder to the planetary ball mill device (Retsch PM 200) with 2 mm zirconia beads. The ratio of the ginger rhizome powder to water was kept at 1:60, milled for 4 hours at 550 rpm which was the optimum ball-milled time for the ginger rhizome powder to show a strong antioxidant effect according to previous findings [8]. No stabilizer agent was used in this study. The dry milled ginger rhizome was prepared in a similar manner to the procedure applied to produce WM but no water was added. All collected samples were kept in an airtight container upon analysis. The aqueous extract of the MG, SM and DM ginger rhizomes were prepared by adding 0.5 g of ginger powder to 30 ml of distilled water. Whereas, for the WM sample, the previously prepared wet mill nanostructured ginger was used. All solutions were then sonicated for 30 minutes (UP400S – Hielscher, Germany), prior to a water bath heating (Memmert, Germany) at 90 °C for 30 minutes. The solution was then centrifuged at 4000 rpm for 15 minutes and filtered. The filtrate was collected and stored at 4 °C prior to analysis. The particle size distributions were measured by means of a laser sizer (Malvern Mastersizer S, UK) at 25 °C and the reading was taken at 173 °C scattering angle. Prior to measurement, the sample was dispersed into distilled water and homogenized for 30 minutes. The surface morphology was observed using the Field Emission Scanning Electron Microscope (FESEM) (Carl Zeiss SMT- SUPRA 40VP). The antioxidant activity was estimated using the Total Phenolic Content (TPC) and the Total Flavonoid Content (TFC). TPC was determined using the Folin–Ciocalteu reagent with analytical grade gallic acid as a standard. The absorbance was taken at 750 nm by using a UV/VIS spectrophotometer (UV–VIS Helios Zeta, Thermo Scientific, USA) and expressed as mg of gallic acid equivalents per g of dry weight (mgGAE/g). Meanwhile, TFC was determined by an aluminium chloride colorimetric method [9]. The absorbance was measured against distilled water as the blank at 510 nm and expressed as quercetin equivalents per gram of dry weight (mgQE/g dw). The antioxidant activities were measured using DPPH [10], FRAPS [11] and ABTS [12] assays. The absorbance was at 517 nm with ethanol as a blank for DPPH measurement, 593 nm against distilled water as a blank to measure FRAP and 734 nm to detect the ABTS activity. All experiments were carried out in triplicate and presented as mean and standard deviation. One-way analysis of variance (ANOVA) was used to analyze the data using the SPSS statistical software (version 21 SPSS Inc., USA). The means were compared with the Duncan's multiple comparison test (DMCT) and $p < 0.05$ was considered as statistical significance.

3. Results and Discussion

3.1. Particle Size and Surface Morphology

In general, the broad particle size distribution of the ginger rhizome powder with an average diameter of 20 μm was shifted into a bimodal sharp peak with an average diameter of 221.87 nm in WM and 233.37 nm DM after 4 hours of milling time respectively. The nanoparticle produced consisted of a mixture of smaller and larger particles. About 78.2% of WM and 88.7% of DM nanoparticles were in the range of 100 to 1000 nm with 5% of the WM sample containing particles bigger than 1000 nm showing that nanonization through the wet milling process produced a mixture of smaller and bigger particles. However, some degree of particle agglomeration was shown in the DM sample based on the FESEM image (Fig 1). Meanwhile, the non-aggregated solid spherical particle was shown by the MG and SM particles. The FESEM image showed that presence of flake like inhomogeneous granules with the diameter in the range of 200 to 700 nm in DM and more uniform nanoparticles with the range of 300 to 500 nm in the WM sample. Milling in wet condition produced more uniform particle size distribution by encapsulating the dry particle and preventing the re-agglomeration process. In contrast, the particle size of MG and SM were relatively inhomogeneous and varied from several micrometers to 50 micrometers. The results revealed that some physical changes occurred as a result of the wet and dry milling processes. The energy generated during the milling process forced the granule into breaking point. When the breakage point was reached, the granules broke into pieces leading to particle size reduction. Once the reaction happened, the granule became loose and developing more porous and small fragments [13].

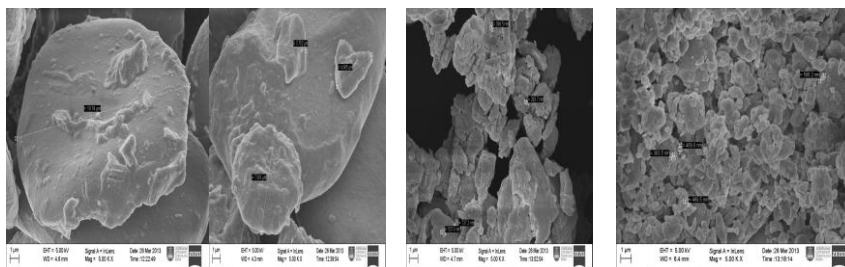


Fig. 1: FESEM images of ginger rhizome powder: a) micron ginger (MG); b) submicron ginger (SM); c) wet mill nanostructured ginger (WM); d) dry mill nanostructured ginger (DM).

3.2. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The phenolic compounds are compounds released by plants which are formed in response to the reactive oxygen species (ROS) due to stress and are known to possess antioxidant activities [14]. Gallic acid is a pungent phenolic compound that can be found in ginger. Table 1 indicates the TPC values of the nanostructured ginger rhizome prepared in different conditions against the micron and submicron counterparts. Generally, both the WM and DM nanostructured ginger rhizome contained a significantly higher ($p < 0.05$) TPC value compared to the MG and SM sample. The results obtained confirmed the effectiveness of nanotechnology. The TPC value of the dry weight varied between 6937.5 and 11783.33 mgGAE/g. Among the samples tested, the DM nanostructured ginger had the greater value which was 11783.33 mgGAE/g dw followed by WM which contained 9203.70, SM with 7298.61 while MG showed the lowest value (6937.50 mgGAE/g) correspondingly. The TPC value in the tested samples were in the order of DM > WM > SM > MG. The same trend was also observed in the TFC value where the nanostructured ginger rhizome regardless of the milling condition showed a significantly higher TFC value compared to the non-nano counterpart. The TFC value was in the range of 14.80 to 22.35 mgQE/g dw. No significant differences were observed in the MG and SM samples in terms of the TFC value. The results revealed that the size reduction into the nanoscale distinctly increased almost 69.83% of the TPC and 51.01% of the TFC values as compared to its non-nano particle. This was due to the fact that the broken cell wall during the milling process exposed the active ingredient and effective reaction with solvents; therefore, becoming more bioavailable [15]. This is aligned with [16] where they found that the TPC value increased 49% in the decreased size of the apple pomace powder compared to the coarse particle.

3.3. Antioxidant Activity

The comparative study of the antioxidant capacity of the ginger rhizome powder as a result of the wet and dry milling processes conducted by using ABTS, DPPH, and FRAP assays is shown in Table 1.

In this study, the ABTS radical scavenging activity was in the range of 364.85 to 503.79 mgTE/g dw. The DM sample possessed the highest ABTS discoloration antioxidant activity (503.79 mgTE/g dw) followed by the WM (452.45 mgTE/g dw), SM (430.85 mgTE/g dw) and MG (364.85 mgTE/g dw). The results obtained corresponded to the particle size of the tested material. The smaller particle size posed by the DM and WM nanostructured ginger rhizome led to a greater antioxidant activity. The bioavailability of poorly water soluble material is often fundamentally associated to the primary size particle of the material [20]. Thus, particle size reduction through the wet and dry milling processes exposed the active compound to the surrounding which led to a greater ABTS radical scavenging ability. Meanwhile, the same trend of antioxidant activity was also observed in the ferric reducing antioxidant power (FRAP) where the nanoparticle sample was markedly higher than the non-nano samples. In this study, the FRAP value was in the range of 994.14 to 1421.17 mMTE/g dw with DM showing the highest value which was 1421.17 mMTE/g at the concentration of 100 µg/ml ($p < 0.05$). Meanwhile, the reducing capacity of WM and SM were 1247.30 mMTE/g and 1065.77 mMTE/g dw respectively. Among all the samples, the MG sample contained the lowest ferric reducing antioxidant activity. The antioxidant activities of the plants that contained the polyphenol components depended on their capacity to donate the hydrogen atoms and inactivate the DPPH free radicals. In this study, the DPPH radical inhibition percentage for the tested samples is shown in Table 1. It was noted that the free radical inhibition percentage for all the tested samples was comparable to each other with the DM attaining significantly higher ($p < 0.05$) radical inhibition percentage (68.34%) compared to WM (66.76%) and the percentage was 15.42 % higher than the SM and MG sample. Chinese Traditional Herbs prepared using nanotechnology exhibited more DPPH radicals and were significantly better in ferric reducing antioxidant power compared to its traditionally ground material [17].

Table 1: Antioxidant Activities of Ginger Rhizome Powder as a Result of Wet and Dry Milling Processes

Sample	Antioxidant Estimation		Antioxidant activity		
	TPC (mgGAE/g)	TFC (mgQE/g)	ABTS (mgTE/g dw)	DPPH (% inhibition)	FRAP (mM TE/g dw)
MG	6938.33 ^c	14.80 ^c	364.85 ^c	57.7 ^b	944.14 ^d
SM	7299.27 ^c	16.21 ^c	430.85 ^b	57.8 ^b	1065.77 ^c
WM	9203.70 ^b	20.23 ^b	452.45 ^b	66.76 ^a	1247.30 ^b
DM	11783.33 ^a	25.02 ^a	503.79 ^a	68.34 ^a	1421.17 ^a

Values are expressed as mean \pm standard deviation (n=3) for micron (MC); submicron (SM); wet mill nanostructured ginger (WM); dry mill nanostructured ginger (DM). Values marked by the different superscript letters (from "a" to "d") within a column denote statistically significant differences ($p < 0.05$)

4. Conclusion

In this study it was found that milling conditions either wet or dry influenced the physical as well as antioxidant activity of the tested samples. Both the wet and dry milling processes successfully reduced the ginger rhizome particle to an average diameter of 221.87 nm and 233.37 nm respectively. A more homogenize nanoparticle was observed in the WM sample while some degree of agglomerated nanoparticle was performed on the DM sample. The size reduction into nanoscale led to a significantly ($p < 0.05$) higher TPC and TFC value with greater antioxidant activity compared to the SM and MG samples while the DM sample showed the highest value in all the experiments conducted. Thus, it can be concluded that the production of the nanoparticle in the dry condition enhanced the antioxidant activity of the plant material.

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