Effect of multi-function disintegrator grinding on the particle size of *Garcinia indica* and *Garcinia cambogia* rind powder

Abstract

Ultra-fine formulations edible dried fruit rind material provides a vast range of opportunities for the development of new products and applications in the food systems. The *Garcinia indica* and *Garcinia cambogia*, both species have a range of uses from culinary to healthcare applications. The dried rind of both species was attempted to study size and proportion of ultra-fine powder formed using Multi-function disintegrator as a function of different durations of grinding. The particle size and relative proportions were assessed using Zetasizer dynamic light scattering apparatus. A set of three major peaks in each sample was analyzed for particle size and intensity. In *G. indica* the result in peak1 for size and intensity, the minimum size of particle recorded was 175.8 nm from the sample ground 60 minutes. In *G. cambogia* minimum particle size recorded was 461.01nm from the sample ground for 60 minutes. The different particle sizes ranging from 48.13 nm to 3705.63 nm was recorded in powder resulting from different durations of grinding at full speed of the disintegrator. The results suggested that samples ground for longer duration in a simple particle disintegrator could accumulate ultra-fine particles in both *G. indica* and *G. cambogia* rind samples.

Keywords: *Garcinia indica*, *Garcinia cambogia*, Powder, Particle size

Introduction

Garcinia indica (Choisy) and *Garcinia cambogia*, commonly known as *kokum/ murugalu / punarpuli* and *uppage,* respectively, are evergreen tree species cultivated on small scale in coastal regions and hilly areas of the *Western Ghats* of India. The relative distribution of these tree species covering the *Western Ghats* of Karnataka, Kerala, Maharashtra, Goa with varying intensity (Hegde, 2019).. The *kokum* fruit is spherical in shape, purplish orange to pinkish-red in colour, fleshy and has an acidic flavour (Hegde, 2019). Similarly, the fruits of *uppage* are green, ovoid berry, yellow or red when ripe, with 6-8 grooves, seed 6-8, smooth, large - about 5 cm long and 2 cm wide surrounded by a succulent aril. *Kokum* and *uppage* have become popular in the recent past due to their fruits, which are rich in (-)- Hydroxicitric acid (HCA) content. The rind of the fruits of *uppage* contains about 24-30 per cent HCA which is highest among the species of the genus *Garcinia (*Ashish *et al.,* 2008). The dried rind of both species is used as a substitute for tamarind. The fruits have many medicinal properties, including antiseptic hypolipidemic, hydragogue, diuretic, anti-bacterial, anti-oxidant, and anti-obesity activity (Han *et al.,* 2004, Mathew *et al.,* 2011 and Shivakumar *et al.,* 2013). The fruits of both species are extensively used in pharmaceutical and nutraceutical industries (Shameer *et al.,* 2016). *Garcinia gummi-gutta* and *G. indica* species have been reported to be stabilized in the forest ecosystem of the *Western Ghats* and have been the source of sustainable livelihood for many farmers in that region (Nagaraja *et al.,* 2011).

Garcinia species are known to have a rich diversity in their phytochemical traits (Anu *et al.*, 2016). Among various organic compounds present in different parts of *kokum* and *uppage,* (-)-Hydroxycitric acid (HCA) is the major acid, especially in fruit rinds. The HCA has been reported to have anti-obesity activity by inhibiting lipid synthesis in the body (Lewis and Neelakantan, 1965). The species *Garcinia cambogia* and *Garcinia indica* gained popularity in the pharmaceutical sector due to their high HCA content (Asish *et al.,* 2008). *Garcinia* powder has attracted more food Science research interest due to its unique chemical composition and high efficiency as a medicinal plant including its anti-obesity, anti-cancer anti-diabetic properties (Zhu 2016). The (-)- Hydroxycitric acid is a chiral compound that is derived from citric acid (Zhu 2016). This compound is having an additional –OH group when compared to citric acid and there are two asymmetrical carbons, making the compound possible to form four isomers (*Gogoi et al.*, 2014). HCA is an unstable compound due to the presence of two hydroxyl and three carboxyl groups. Therefore, it easily forms lactones with cations like Ca^{2+}, K^{+} *etc.* (Antony *et al.,* 1998). These fruit properties have rendered both species important in many human health, culinary and industrial applications.

Recently, nanotechnology is providing a vast range of opportunities for the development of new products and applications in the food system. Functional foods, nutraceuticals, bioactive, pharma foods, *etc*. are some of the areas where ultra-fine formulations developed through nanotechnology are impacting. In human food processing, ultra-fine formulations have been used as ingredients, additives, nutritional supplements, and functional foods as food ingredients and additives. Many of these ultra-fine formulations have been known to impart protective barriers, flavour, taste, controlled release and better dispensability for water-insoluble food ingredients (Mahmoud 2015). Prospecting nano / micro-sized *Garcinia indica* and *G. cambogia* rind are expected to aid in better absorption in the human gut (Chaudhry *et al*. 2008).

Superfine grinding technology can produce a powder with superior properties to conventional particles and it's thus, being used increasingly with variety of food materials to improve the quality of powder (Zhang *et al*., 2010). Micronization is the process of reducing the average diameter of solid materials, particle size (Duodu *et al*,.2002 and Skrabanja *et al.*, 2004). Superfine grinding methods include airflow grinding liquid flow grinding, low-temperature grinding, ball milling, ultrasonic disintegrator grinding etc Zhao *et al*, 2009. The ultrafine grinding technology has been applied in biotechnology and achieve various foodstuffs (Wu *et al*., 2012 and Niu *et al*., 2014). They found that the ultra-fine grinding improved the solubility, oil holding capacity and brightness of powder the contents of total saponins, minerals, phenols, and flavonoids were highest and the antioxidant activity was best, in the smallest particle size of the powder (Wu *et al*., 2020). In this paper, we reported the effect of grinding rind samples of both species for the different duration with commonly available Multi-functional disintegrator for particle size and morphology.

Material and method

The rind of *Garcinia indica* was prepared from the fruits harvested from the trees in the *ex situ* field gene bank of the College of Horticulture, Bengaluru, while the rind of *Garcinia cambogia* was obtained from M/S. Kadambha Farmer Producers Organization (FPO), Sirsi, Karnataka. The rind was briefly sun-dried. The material was dried using a hot air oven (KEMI, India) at 50 ^{0}C for four hours. The moisture content of the rind was estimated to be 2.5 per cent. The rind material was ground using 2000g Multi-function disintegrator at full speed of the motor (Made in China) for different durations *viz*., 5, 10, 15, 20, 25, 30, 45 and 60 minutes. The treatments involving more than 30 minutes of grinding required intermittent cooling of content for about 5 minutes. The resulting peak samples were subjected to particle size determination. Zetasizer (Malvern, ZETA Sizer, nano383 issue 5.0, England) was used (dynamic light scattering) apparatus to study the average particle diameter (nm) of *Garcinia indica* and *Garcinia cambogia* rind powder. About 1 mg power was suspended in 1 ml of ethanol. The suspension of powder was sonicated at 25 °C using the digital ultrasonication bath (Labman Scientific Instruments, LMUC-2.8L, India) for 15 min. After sonication, the sample was centrifuged using a high-speed high centrifuge (MPW Med. Instruments, MPW-350R, Poland) at 1000 rpm for 10 min. The prepared sample of powder suspension was filled in disposable cuvette up to ¾th of volume and the cuvette was placed in a dynamic light scattering chamber.

Result and Discussion

The rind samples of *G. indica* and *G. cambogia* were briefly air-dried and then systematically the moisture content was reduced to around 2.5 per cent using a hot air oven. Oven dried rind samples were ground using a Multi-function disintegrator (2000g) for different time periods at the full speed of the machine (25000 rpm), which a routine and conventional devise. Resulting powder was analyzed for the size of the particles using electron microscope. In *Garcinia indica* samples, the analysis of variance revealed significant differences in particle size of the powders resulting from different durations of grinding. Size intensity of the particles was determined for each sample. A set of three major peaks were noticed with varying particle size and intensity. The analysis of variances revealed significant differences among the different durations of grinding. There was a significant difference in particle size resulting from 5 minutes of grinding when compared to rest of the treatments (Table 1). The first peak corresponded to bulk of the particles followed by other size fractions in the sample. In *G. cambogia* significant differences were observed for all the peaks except peak 1 size, peak 3 size and peak3 intensity (Table 2).

In *G. indica*, result of peak1 size and intensity corresponding to peak 1 revealed significant difference between five and ten minutes of grinding, while the particle size and intensities resulting from the 10, 15, 20, 25, 30, 45 and 60 minutes of grinding hardly significantly different. However, the minimum size of the particle, 175.8 nm, was recorded in 60 minutes of grinding while the maximum particle size of 1172.03 nm was recorded at five minutes of grinding. It is imperative that longer diration has resulted in finer particle size compared, as also reported by Xu *et al.* (2021). The intensity was calculated and it was found that 25 minutes of grinding resulted in over 70.63 per cent of ultra-fine particle size (Table 1). The size fractions other than peak 1 did not differ significantly across different durations of grinding.

The results of peak1 size in *G. cambogia* rind powder recorded non-significant differences among different duratins of grinding. . However, in peak1, the particles sizes resulting from 5, 10, 15 and 20 minutes of grinding numerically differed with that of 25, 30, 45 and 60 minutes of grinding. The minimum particle size of 461.01nm was recorded at 60 minutes of grinding. However, there was no significant difference between duration of grinding for intensity of different sized particles. The different particle size from 48.13nm to 3705.63 nm was recorded in powder resulting from different durations of grinding of rinds of both species (Plate 1)

As the duration of grinding increased the size of the particles resulting from the rind reduced as high levels of disintegration is achieved due to repeated friction and Figure 1& 2, (Zainala *et al.,* 2013). The results revealed significant differences among the treatments- duration of grinding, wherein the minimum particle size observed in *G.*

indica was 48.13 nm in peak 2 which was ground for 60 minutes followed by 77.03 nm in peak 2 with 45 minutes of grinding and maximum particle size was 1853 nm in peak3 in which the rind was ground for 5 minutes. The results of the zetasizer revealed that the average particle diameter of *Garcinia* rind powder was in the nanoparticle size range of 48 nm. However, the relative intensity of this size fraction was much low.

The particle size of *G. cambogia* powder varied across the duration of grinding the rind. The minimum size recorded was 52.2 nm in peak3 in which the rind was ground for 60 minutes followed by 78.53 nm in peak3 with 20 minutes of grinding. Further, a maximum particle size of 3705.63 nm was in peak3 to which the rind was subjected to 5 minutes of grinding.

The variation in particle size was probably due to the change in different times of grinding and product temperature during grinding (Zainala *et al.,* 2013; Shah *et al.*, 2015;Sindhura *et al.*, 2015). During the initial grinding process, not only does the fineness of powder increase but also its particle distribution widens. The particle size of the grinding product gradually decreases with an increase in grinding time and temperature, as the temperature enhances breakage. The degree to which finer particle-particle is reduced depends on the grinding speed and time (Li *et al.,* 2021). These results point at possibilities of increasing the relative proportion of nano-size particles of rind by increasing the duration of grinding. This low cost approach to produce a small proportion of ultra-fine powder will be of practical importance in *Garcinia indica* and *Garcinia cambogia* for their extended utility in food and pharmaceutical applications.

Conclusion

The rind dried using sun light followed by an oven could get grind leading to ultra-fine powder in the scale of micrometers. The ulta-fine powder with has multiple applications in case of *Garcinia indica* and *Garcinia cambogia*. Differtent durations of grinding could yield ultra fine powder of rind sample sof both species. The ultra-fine powder in the range of nanooptics was recorded in 60 minutes of grinding. Great proportion of ultra-fine powder was recorded from the rind samples of both species. This simple methodology could further prospect to fine-tune the protocol. These results hold promise to further fine tune the procedure to maximize the yield of ultra-fine powder from the rind samples of *Garcinia indica* and *Garcinia cambogia*.

References

Antony, B. Spectrophotometric determination of Hydroxycitric acid. *Indian J. Pharm. Sci*. 1998;15 (4): 316-317.

Anu, A. P., Menon, L. N. and Rameshkumar, K. B. Structural diversity of secondary metabolites in *Garcinia* species, *In Diversity of Garcinia species in the Western Ghats: Phytochemical perspective.* (Ed) Rameshkumar, K. B., JNTBGRI, Kerala. 2016; 196-201.

Ashish, G. R., Parthasarathy, U., Zachariah, J. and Kokkat, G. C. A comparative estimation of (-) - Hydroxycitric acid in different species of *Garcinia. The Hort. J.* 2008; 21(1): 26·29.

Chaudhry, Q., Scotter, M., Blackburn, J., Ross, B., Boxall, A., Castle, L., Aitken, R. and Watkins, R..Applications and implications of nanotechnologies for the food sector, *Food Additives and Contaminants*. 2008; 25(3): 241–258.

Duodu, K.G.; Nunes, A.; Delgadillo, I.; Parker, M.L.; Mills, E.N.C.; Belton, P.S.; Taylor, J.R.N. Effect of grain structure and cooking on sorghum and maize in vitro protein digestibility. J. Cereal Sci. 2002; 35, 161–174.

Gogoi, A., Gogoi, N., and Neog, B. Estimation of (-) - Hydroxycitric acid (HCA) in *Garcinia lanceaefolia* using novel HPLC methodology. *Int. J. Pharm. Sci. Res.* 2014;, 5 (11): 4995-4999.

Han, K. H., Seo, J. A., and Yu, J. H. Regulators of G-protein signalling in *Aspergillus nidulans*: RgsA downregulates stress response and stimulates asexual sporulation through attenuation of GanB (Gaaaa) signaling. *Mol.Microbiol.*2004; 53(2): 529 – 540.

Hegde, I., Kokum (*Garcinia indica)*- its status, problems and prospect of cultivation and processing. *Int. J. Agri. Sci.* 2019; 11(7): 8239-8241.

Lewis, Y. S. and Neelakantan, S., (-) - Hydroxycitric acid-the principal acid in the fruits of *Garcinia cambogia*. *Phytochem.* 1965; 4 (4): 619-625.

Li, B., Deng, R., Shi, F., He, Z., Ku, J. and Zuo, W. Effect of feed quantity on breakage degree of ore particles subjected to high voltage pulses. Minerals Engineering, 2021; 160, p.106693.

Mahmoud, M.B. Nanotechnology in food industry; advances in food processing, packaging and food safety, *Int. J. Curr. Microbiol. App. Sci.* 2015; 4 (5): 345-357.

Mathew, G. E., Mathew, B. and Nyanthara, B. Diuretic activity of leaves of *Garcinia cambogia* in rats. *Indian J. Pharm. Sci.* 2011; 73(2): 228–230.

Nagaraja, B. C, Raj, M. B, Kavitha, A, Somashekar, R. K., Impact of rural community harvesting practices on plant biodiversity in Kudremukh National Park. India. *Int. J. Biodivers. Sci. Ecosystem Serv. Manag.* 2011*;* **7**(1):69-74.

Niu, M.; Hou, G.G.;Wang, L.; Chen, Z. Effects of superfine grinding on the quality characteristics of whole-wheat flour and its raw noodle product. J. Cereal Sci. 2014; 60, 382–388.

Shah, R. K., Boruah, F. and Parween, N. Synthesis and characterization of ZnO nanoparticles using leaf extract of *Camellia sinesis* and evaluation of their antimicrobial efficacy. *Int. J. Curr. Microbiol. App. Sci*, 2015; 4(8): 444-450.

Shameer, P. S., Rameshkumar, K. B., Sivu, A. R., Sabu, T., Pradeep, N. S. and Mohanan, N. Morphological, chemical and molecular taxonomy of a new *Garcinia* species-*Garcinia pushpangadaniana*, *In Diversity of Garcinia species in the Western Ghats: Phytochemical Perspective.* (Ed) Rameshkumar, K. B., JNTBGRI, Kerala. 2016; Pp.196-201.

Shivakumar, S., Sriraman, S., Subhasree, N. and Dubey, G. P. *In vitro* assessment of antibacterial and antioxidant activities of fruit rind extracts of *Garcinia cambogia* L. *Int. J. Pharm. Pharm. Sci.* 2013; 5(2):254-257.

Sindhura, K. S., Prasad, T. N. V. K. V., Selvam, P. and Hussain, O. M. Biogenic synthesis of Zinc nanoparticles from *Thevetia peruviana* and influence on soil exo-enzyme activity and growth of peanut plants. *Int. J. Appl. Pure Sci. Agr.* 2015; 1(2): 19-32.

Skrabanja, V.; Kreft, I.; Golob, T.; Modic, M.; Ikeda, S.; Ikeda, K.; Kosmelj, K. Nutrient content in buckwheat milling fractions. Cereal Chem. 2004; 81, 172–176.

Wu, G.C.; Zhang, M.; Wang, Y.Q.; Mothibe, K.J.; Chen, W.X. Production of silver carp bone powder using superfine grinding technology: Suitable production parameters and its properties. J. Food Eng. 2012, 109, 730–735.

Wu, Z.; Ameer, K.; Jiang, G. Effects of superfine grinding on the physicochemical properties and antioxidant activities of sanchi (*Panax notoginseng*) Flower Powders. J. Food Sci. Technol. 2020; 58, 62–73.

Xu.,Q., Zheng, F., Cao .,X Yang ,P., Xing., Y, , Zhang.,P Hon Liu.,P Zhou.,G, Liu.,X and Bi.,X. Effects of Airflow Ultrafine-Grinding on the Physicochemical Characteristics of Tartary Buckwheat Powder *Molecules* 2021; 26, 584.1

Zainala, N. A., Shukor, S. R. A., Wabb, H. A. A. and Razakb, K. A. Study on the effect of synthesis parameters of silica nanoparticles entrapped with rifampicin. *Chem. Eng.* 2013; 32(7): 432-44.

Zhang, M.; Chen, H.; Li, J.; Pei, Y.; Liang, Y. Antioxidant properties of Tartary buckwheat extracts as affected by different thermal processing methods. LWT-Food Sci. Technol. 2010; 43, 181–185.

Zhao, X.Y.; Ao, Q.; Yang, L.W.; Yang, Y.F.; Sun, J.C.; Gai, G.S. Application of superfine pulverization technology in biomaterial industry. J. Taiwan Inst. Chem. Eng. 2009; 40, 337–343.

Zhu.,F. Chemical composition and health effects of Tartary buckwheat, Food Chem 2016; 203,231-245.

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	4912	Peak 1:	141.8	100.0	1.907e-6
PdI:	1.000	Peak 2:	0.000	0.0	0.000
Intercept:	1.05	Peak 3:	0.000	0.0	0.000
Result quality	Refer to quality report				

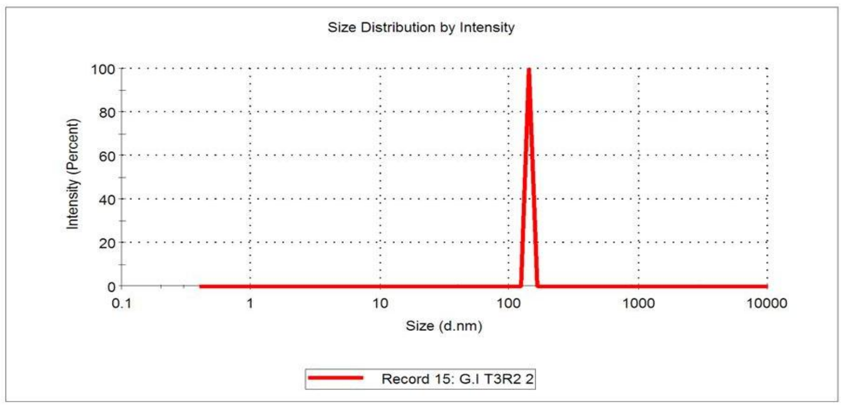

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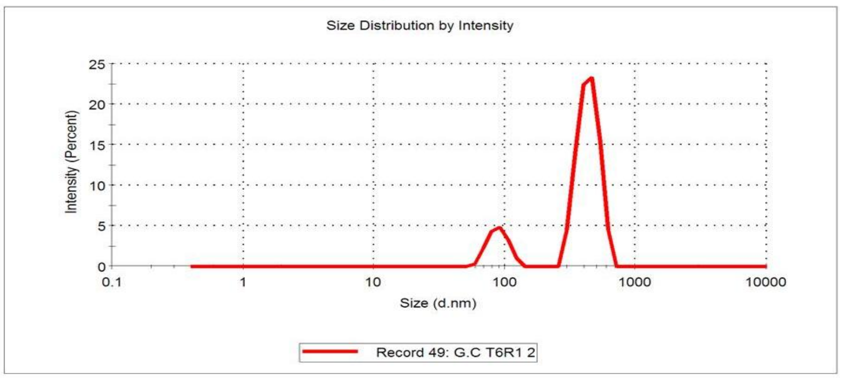
Figure 1: Particle size distribution. Y intensity of the rind powder of *G. indica* prepared by grinding for 60 minutes in a Multi-function disintegrator (2000g)

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	1314	Peak 1:	435.7	84.1	80.57
PdI:	0.897	Peak 2:	89.10	15.9	15.32
Intercept:	0.791	Peak 3:	0.000	0.0	0.000
Result quality	Refer to quality report				



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Serial Number : MAL1080873

File name: kokam samples(Garcinia indica)
Record Number: 49
06 Aug 2021 11:06:36

Figure 2: Particle size distribution. Y intensity of the rind powder of *G. cambogia* prepared by grinding for 60 minutes in Multi-function disintegrator (2000g)

Table 1: Particle size and their relative intensity of powder of *Garcinia indica*

Treatment	Peak 1 size (nm)	Peak 1 intensity %	Peak 2 size (nm)	Peak 2 intensity %	Peak 3 size (nm)	Peak 3 intensity %	Average
5MG	1172.03	72.1	968.66	19.13	1853.33	0.5	921.50
10MG	857.51	74.56	185.06	23.5	1853.33	0.76	777.93
15MG	747.43	65.26	163.56	22.06	1731.66	4.46	745.30
20MG	686.8	86.06	198.28	6.2	0	0	739.00
25MG	630.63	70.63	130.5	20.76	1853.33	1.46	721.36
30MG	467.23	72.4	126.99	21.1	4284	5.96	717.16
45MG	433.9	77.5	77.03	9.8	1729.66	1.76	633.10
60MG	175.8	81.5	48.13	7.53	1853.33	1	475.76
Mean	**646.41**	**75.0042**	**237.28**	**16.26**	**1894.83**	**1.99**	**707.64**
S.Em.±	102.09	8.78	303.02	7.29	1823.59	1.96	11.56
C.D. (5%)	309.68	-	-	-	-	-	-

5MG-5 Minutes grinding **10MG**= 10Minutes grinding **15MG**= 15Minutes grinding **20MG**= 20Minutes grinding
25MG= 25Minutes grinding **30MG**= 30 Minutes grinding **45MG**= 45 Minutes grinding **60MG**= 60 Minutes grinding

Table 2: Particle size and relative intensity of powder of *Garcinia cambogia*

Treatment	Peak 1 size (nm)	Peak 1 intensity %	Peak 2 size (nm)	Peak 2 intensity %	Peak 3 size (nm)	Peak 3 intensity %	Average
5MG	1119.7	76.3	240.8	15.93	3705.63	0	1258.00
10MG	1000.9	72.93	174.43	17.4	3690.03	6.96	1136.00
15MG	862.13	59.06	173.53	26.3	1853.33	11.76	1160.83
20MG	859.93	54.13	143.36	29.46	78.53	9.86	1040.96
25MG	830.7	86.43	129.96	13.56	0	0	985.03
30MG	738.1	76.13	112.96	15.93	0	0	988.83
45MG	668.13	74.5	105.13	13.36	0	1.83	827.70
60MG	461.01	57.36	104.9	11.4	52.2	6.56	728.03
Mean	**817.55**	**69.6**	**148.08**	**17.92**	**1172.49**	**4.72**	**1015.67**
S.Em.±	219.92	4.57	21.83	2.88	1153.68	4.07	73.5170
C.D. (5%)	-	14.18	66.21	8.74	-	-	222.9003

5MG-5 Minutes grinding **10MG**= 10Minutes grinding **15MG**= 15Minutes grinding **20MG**= 20Minutes grinding
25MG= 25Minutes grinding **30MG**= 30 Minutes grinding **45MG**= 45 Minutes grinding **60MG**= 60 Minutes grinding

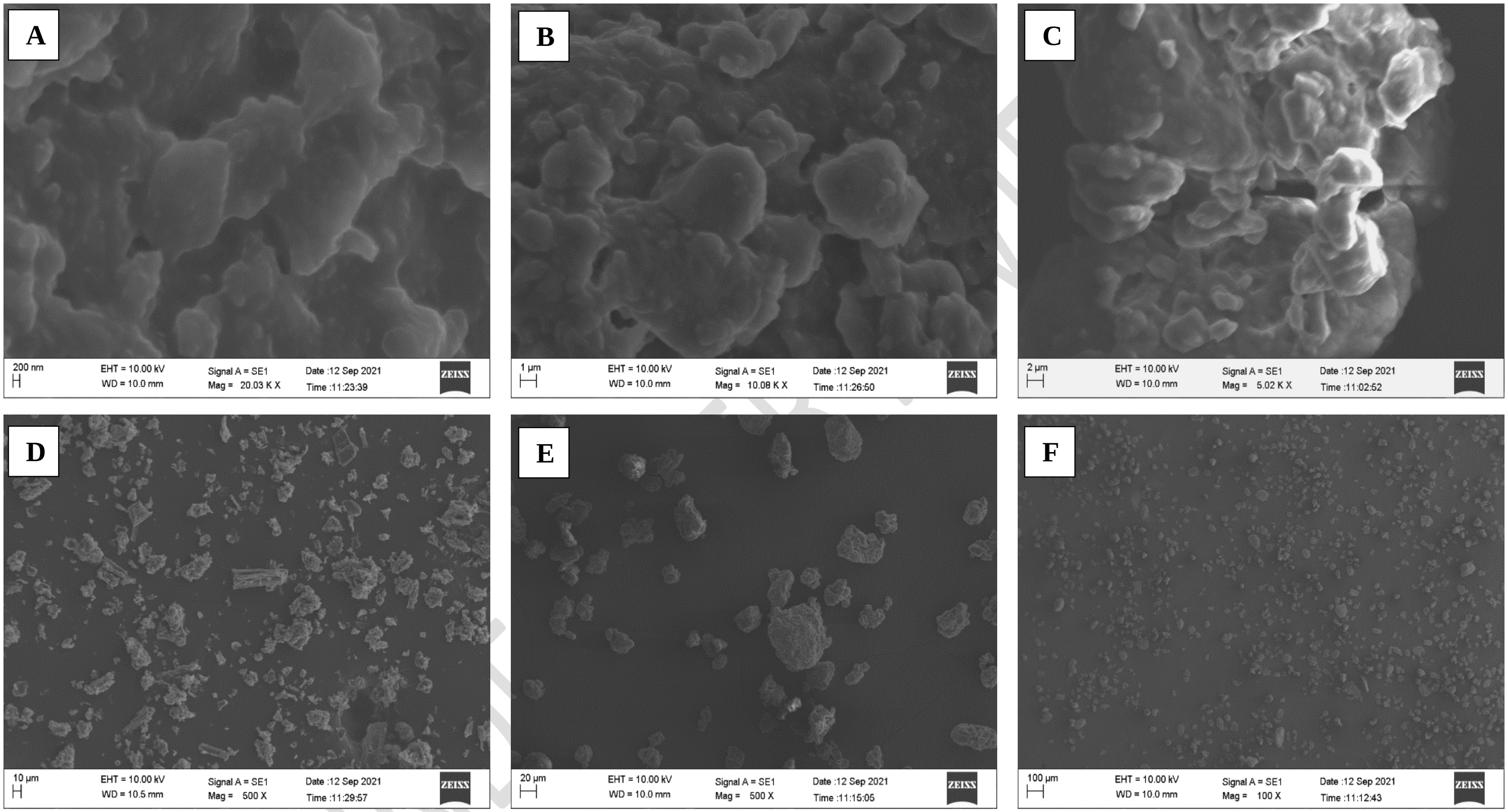

Plate 1: **Electron microscopy view of *Garcinia indica* rind powders developed from different duration of grinding using Multi-function disintegrator**