SYNTHESIS OF SUPERFINE NANOMETRIC FOOD POWDER OF POTATO (*SOLANUM TUBEROSUM*) BY ECO-FRIENDLY APPROACH, PHYSICAL PROPERTIES MEASUREMENTS AND TOXICITY ANALYSIS FOR ITS APPLICATION IN FOOD, HEALTH, AND AGRICULTURAL SECTOR AS A NEW FUNCTIONAL FOOD NANOMATERIAL

Pallavi Singh[a], Rakesh Kr Singh[b], Naman Kumar Naik[c], Nishant Kumar[d], Ghufran Ahmed[e], Shyam Narayan[f]

[a,b,c,d] Aryabhatta Centre for Nanoscience & Nanotechnology, Aryabhatta Knowledge University, Patna, India.

[e,f] Division of Microbiology, Indian Council of Medical Research, Rajendra Memorial Research Institute of Medical Sciences, Agamkuan, Patna, India.

*Corresponding Author

Rakesh Kr Singh, Aryabhatta Centre for Nanoscience & Nanotechnology, Aryabhatta Knowledge University, Patna, India

Email: rakeshsinghpu@gmail.com

Abstract

Nanometric potato (*Solanum tuberosum*) food powder was synthesised by an eco-friendly approach, using high-energy ball mill and kitchen-based mixer grinder. A portion of the grounded powder was labelled as 0 hours, after which the potato powder was subjected to the ball mill equipment for time interval of 2.5 hr, 5 hr and 7.5 hr respectively. Optical properties of superfine potato powder include changes in the colour from dark grey to lightest grey which is due to characteristic changes in physical properties and crystal structure occurring due to transition of electrons. The average crystallite size of 12.53nm of the synthesised material was confirmed by HRTEM. FTIR measurements reveal a slight shift in wave number position, while no changes in the functional groups was observed with increased milling hours. SEM measurements show that the surface reactivity and agglomeration increased with increasing milling time. Rough amorphous grain was observed on the surface of ordered amorphous samples which is also evident from the result of XRD and SEM. UV Vis NIR spectrum result showed absorbance at 400nm and 270nm respectively indicating that the functional group are remaining same with changes in their crystal structure. Experiments were performed to evaluate the biocompatibility of superfine nano powder of potato against murine-derived (hepatocytes and splenocytes) and human-derived (THP-1) cells of in-house prepared superfine potato nano-particles using MTT assay and Trypan blue exclusion assays. 250µg/ml was the optimum dose concentration which showed increased biocompatibility and lessened cytotoxicity in the cells under analysis and exhibiting 85% cell viability. In this approach, eco-friendly and physical method was used to produce superfine potato powder

with novel properties and enhanced efficacy post superfine grinding for its applications in food sector, health sector, biotechnology sector and other related sectors or arenas.

Keywords: Potato, Nanoparticle, Superfine, HRTEM, Biocompatibility, Eco-Friendly, Cytotoxicity

1. Introduction

Nanotechnology has proven to be a fundamental advanced technology that enables efficient development, contributes to the food, agriculture, and medical sectors, and has long-term sustainable impact due to its potential to impart unique features to material when they are brought down to nanometric size. Nanomaterials have immense potential to enable the qualitative and quantitative production and preparation of healthier, highly safe, and high-quality functional foods. The nano functional foods may be decomposable or short-lived in nature and are considered superior to traditional food which finds place in our regular day-to day life. Since ages medicinal plants and their parts have been extensively used for treating a range of diseases and disorders. It has been reported by World Health Organisation that approximately 80% of the world population is entirely dependent on plant, plant parts or their extracts for providing therapies in various ailments[1,2].Potato plants are herbaceous perennials plants that often grow to a height of about 60 cm (24 in) high approximately, which is starchy in nature [3]. *Solanum tuberosum* is the scientific name of the plant. It is a root vegetable which is considered native to America, and falls in the family of Solanaceae [4]. Vitamins and minerals are in the storehouse of the potato tuber which provides many benefits to the human body [7-9]. This is also a store-house of antioxidants, including certain types such as flavonoids, carotenoids, and phenolic acids [5]. Previous research has shown that antioxidants can prevent certain types of lethal diseases such as heart disease, diabetes, and cancer [5]. A test-tube study investigated that the antioxidants in potatoes may reduce the growth of certain types of cancer, especially colon and liver cancer. [7-9].

Nanomaterials have showcased superiority in nature and varied properties than their own bulkier size counterparts due to generation of large surface area with respect to volume and quantum confinement behaviour. The size of the nanomaterial was found to be in nanometric scale which is in between 1 nm to 100 nm. Considerable changes in pretext to increased surface area and internal bond breaking and remaking of the nanomaterial were observed due to various pressure grinding techniques which have been observed to significantly change the size of crude food powder from micro level to nanometric range. Ball milling technology, which operates on the principle of friction, impact, shear, collision, or other mechanical action, is a green technology, mainly used these days around the world to produce superfine food powders without altering their inherent properties. Ball milling considerably alters the structure and properties of the food powder. Hence it has opened a new door for research and development and, also for deriving and gaining potential medicinal benefits of naturally found food material [10].

Superfine grinding, which is crucial for material pre-treatment during food processing, involves fast pulverization of material particles to nanometric level with mechanical equipment. In comparison to bulk or granular materials, superfine powder possesses a potential physical and chemical characteristic that are superior in terms of high rate of

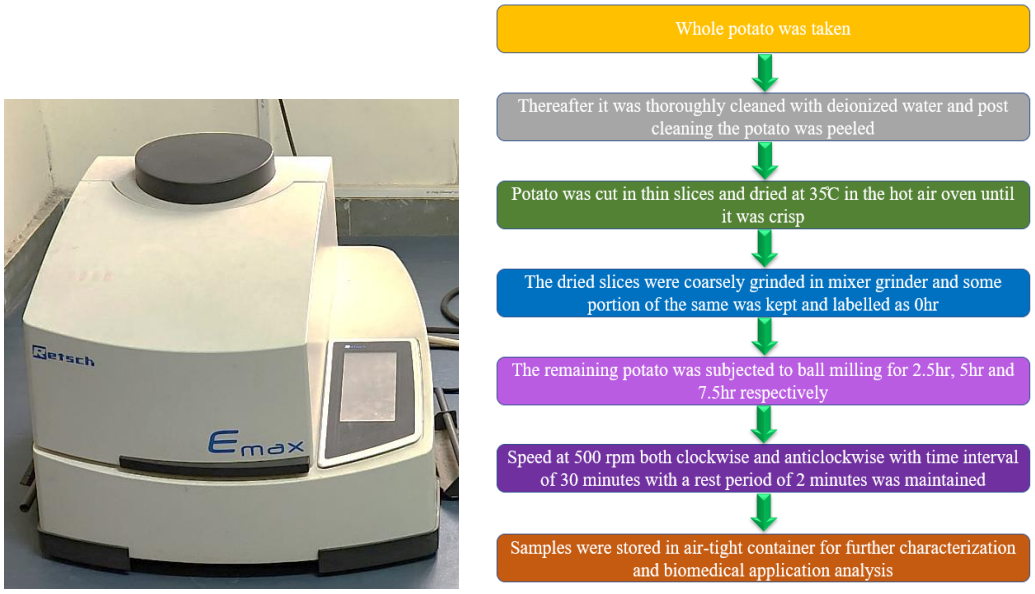
fluidity, adsorption, chemical activity, simple nutrient dissolution, and retention of biological activity. In addition, it is globally used in different industries, mainly in cosmetics, health sector, nutrition, and aerospace, due to its ability to enhance the biological mechanisms in the body and make the most of natural resources. Different researchers worldwide have published similar research works [11-13].

The main goal of this current study is to analyse the structural changes and changed surface texture of the synthesized superfine potato powder that changed with increased milling time, and how to apply the aforesaid changes to the pharmaceutical, nutraceutical, biotechnology and other related industries. Changes in physical and chemical parameters after milling were studied in details with the help of characterisation tools like XRD, SEM, TEM, FTIR, UV-Vis-NIR. A distinct change in crystal structure, enhanced surface reactivity and change in properties in terms of its inherent physical and chemical characteristics were observed in the new synthesised functional food nanomaterial of superfine potato nano powder, by grinding the food material with a ball mill [14-15].

2. 2. Materials and Method

2.1 Material

Potatoes were purchased from a local market in Patna, Bihar, India for the research purpose. To form a superfine potato powder, the potatoes were first thoroughly washed with deionized water, peeled, and thinly sliced. Thereafter the thinly sliced potato was placed inside a hot air oven at temperature of 35°C. Drying of the thinly sliced potato was done in the oven until they turned crisp in nature. After drying, the slices were grinded through a home-based kitchen-based mixer grinder to coarsely grind the potato slices, followed by pressure grinding in a high-energy ball mill to synthesize nanoscale potato powder. It was then stored in an airtight container for further analysis. Flowchart has been curated to represent the experimental section of synthesis in figure 1(a-b)



(a) (b)

Fig. 1 (a-b) Ball Milling Equipment and Representation of synthesis process in form of a flowchart

2.2 Methodology employed

2.2.1 Synthesis of Potato (*Solanum tuberosum*) superfine nano powder

Fig. 1 (c) –Pictorial representation of the synthesis process of superfine nano powder of potato

The steps used for synthesis are briefly highlighted in Figure 1(c). In the high-energy planetary ball mill equipment, the milled sample is held in two 50 ml capacity stainless steel jars along with 20mm stainless steel balls. The sample to balls weight ratio was maintained at 1:20 in a 50ml stainless steel jar. The sample and balls together occupy 1/3rd of the volume in the stainless-steel jar. The balls rotate horizontally in the jar at a revolution speed of 500 rpm for 2.5, 5 and 7.5 hours. The direction of rotation of the ball in the stainless-steel jar changes every 30 minutes with an interval of 2 minutes. A temperature of less than 25°C was maintained throughout the milling process. Additionally, the temperature was also kept in control by air conditioner to combat the heat generation by the ball mill equipment. The same method was used to produce turmeric, ginger, bitter gourd, and other food powders [16]. Samples was then analysed using modern scientific tools such as transmission electron microscope, X-ray diffractometer, Fourier transform infrared microscopy, Scanning electron microscope and UV-Vis-NIR spectrophotometer. The prepared nanoscale powder of potato and their optical/ visual images are depicted in figure 1(d–g). Such color changes were observed due to electronic transition of electrons which produces a remarkable change in the internal structure of the crystal.

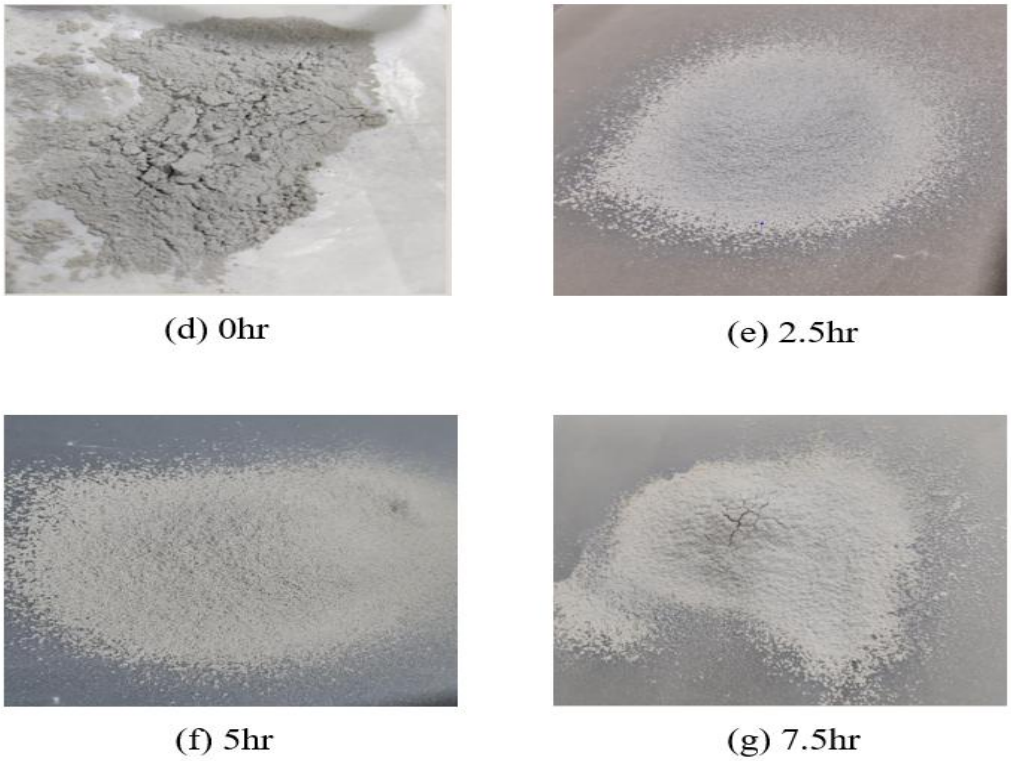


Fig. 1 (d-g) – Optical images of potato superfine powder after grinding; Colorimetric differences of potato nano powder milled at 0hr, 2.5hr, 5hr and 7.5hr

2.3 MTT and Trypan blue exclusion assay-Biomedical application: Determination of cell cytotoxicity

To determine the biomedical applications of nanomaterials, cytotoxicity assays and cell viability assays are considered vital indicators for assessing the health of a cell. Assaying of cytotoxicity of in-house prepared potato nano powder synthesised at different time intervals (0hr, 2.5hr, 5hr, and 7.5hr) was measured against mouse-derived liver and spleen cells (hepatocytes and splenocytes). The trypan blue exclusion assay was performed simultaneously along with the 3-(4,5-dimethylthiazol 2-yl)-2,5-diphenyltetrazolium bromide (Sigma-Aldrich)- MTT assay method [17–18]. Hepatocytes (murine derived liver cells) and splenocytes (murine derived spleen cells) were processed to obtain purified single-cell suspensions along with confluence of THP-1 cells (Human monocyte leukaemia cells). Post obtaining the purified cell suspensions the cells were subjected to twice washing with PBS-sterile phosphate-buffer saline by centrifuging the above purified cell suspensions at a revolution speed of 500 rpm for a time interval of 15 minutes temperature of 40°C. The purified cell suspension was resuspended in RPMI-1640 growth medium which had been supplemented with 25µg/ml gentamicin, 10% foetal bovine serum (FBS), 50U penicillin and 50µg streptomycin. The counting of the cell number is a crucial step. With the aid of the Neubauer cell counting chamber the cell number were maintained at 1×10^6 cells/ml. Seeding of the cell suspension was done in a cell culture plate in 12 wells. Post seeding nanoparticles at different concentrations of 25µg/ml, 50µg/ml, 100µg/ml, 250µg/ml, 500µg/ml, 1000µg/ml were added to each well of the culture plate. Incubation of 48 hours at a temperature of 37°C

were imparted to the culture plate.5% CO_2 and 95% humidity was maintained in the CO_2 incubator. After incubation, aliquots (100µl) of cell suspension were taken from all wells of 96-well culture plates. After seeding it in a 96-well culture plate, 10% MTT reagent was added to each well and cultured for another 4 hours in a CO_2 incubator. An equal volume of MTT solubilizer (Sigma-Aldrich MTT assay kit, provided in the catalog) was then added to each well and mixed gently with a pipette to completely dissolve the formazan crystals. Finally, absorbance was recorded using a spectrophotometer (BioRad) at 570 nm wavelength range. During the experimentation the wells which were not imparted any type of treatment were considered as controls and according to the given formula the cell viability percentage was calculated.

$$\text{Cell viability } \% = \frac{(\text{Mean absorbance of treated samples})}{(\text{Mean absorbance of untreated samples})} \text{ X } 100$$

Subsequently, for distinction between the viable and non-viable cells a trypan blue exclusion assay was performed [19]. For the aforesaid experimentation 50µl of cell suspension was taken and rigorously washed by centrifugation. Post washing 0.4% of the trypan blue dye was mixed with the cell suspension. Darker dyed cells are considered highly viable as compared to their lighter counterpart. A binocular light microscope (Nikon) was then used to assess the number of viable cells.

3. Results and Discussion

Physical-Chemical Property Measurement

3.1 X-ray Diffraction Measurement (XRD)

A D8 Advance, Bruker Germany, make, X-ray diffractometer equipment was used to analyse the surface texture and crystallite size of the synthesised nanometric potato food powder. For the determination of 2θ diffraction angles, scanning from 10^0 to 90° was performed. 2°/min scanning rate was maintained for analysis. XRD analysis was determined for nanometric potato food powder pulverised for 0hr, 2.5hr, 5hr and 7.5hr, as shown in figure 2.

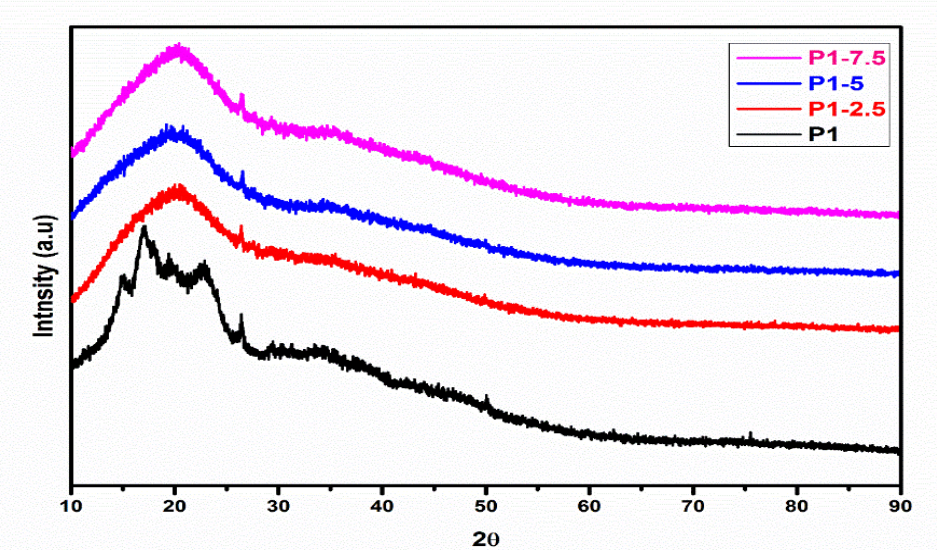
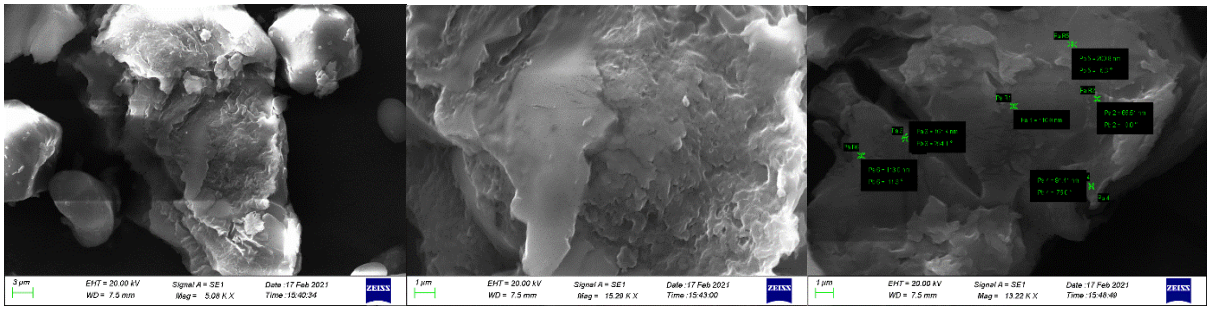


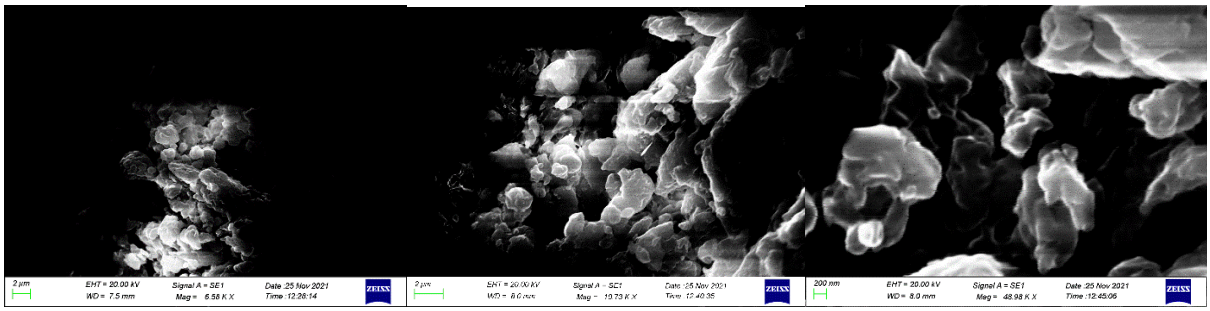
Figure 2 –XRD spectra of Potato superfine powder (P1)

The XRD spectrum indicates that small size of the particles and information on crystallite size can be confirmed using Transmission Electron Microscopy. The results were in accordance with TEM images. Remarkable changes in the bioactive compounds structure of superfine nano powder of potato were observed which is due to the changes and structural distortion of cell wall of the superfine nanometric powder of potato. No distinct peak was observed in the XRD spectrum which clearly indicates that the synthesized superfine powder of potato is amorphous in nature.

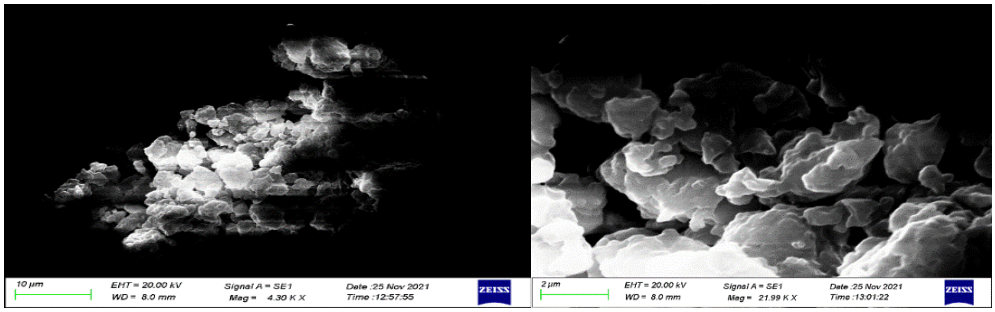
3.2 Morphological Characterization



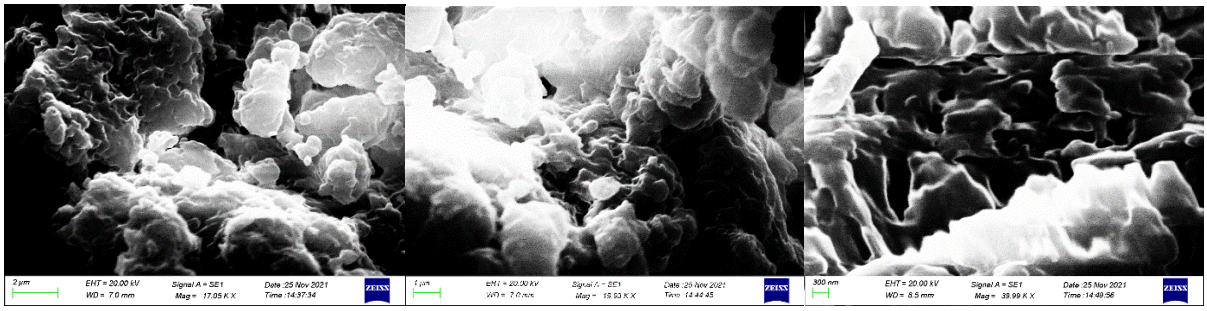
(a) Surface morphologies of 0hr superfine potato powder at different magnification



(b) Surface morphologies of 2.5hr superfine potato powder at different magnification



(c) Surface morphologies of 5hr superfine potato powder at different magnification



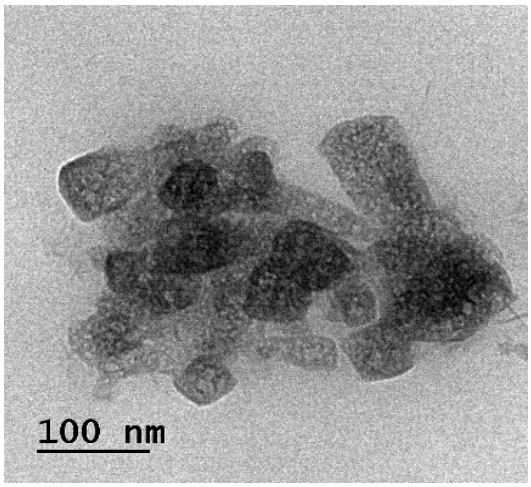
(d) Surface morphologies of 7.5hr superfine potato powder at different magnification

Figure3 (a-d) –Superfine nanometric food powder of potato milled at 0hr, 2.5hr, 5hr, and 7.5hr- SEM images

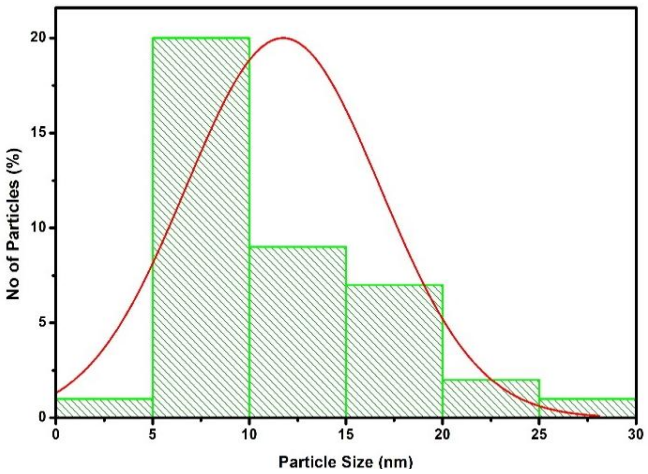
The superfine potato powder produced was examined using an EVO 18 Research Zeiss, UK, make SEM- scanning electron microscope. The accelerating potential of the SEM equipment was 10 KV. Mounting of the sample was done over a circular aluminium stud. The aluminium stud possesses double-sided tape which is instrument-specific. Post mounting of the sample sputtering is done. 10 nm gold film is coated over the nanoparticle in the sputtering process to increase the conductivity of the sample. Thereafter the sample is scanned with the aid of SEM equipment. Figure 3 (a–d) show the surface morphology of the prepared superfine potato powder. SEM images show that the outer surface morphology and aggregation of the particles indicate changes in molecular structure in form of an irregular and/or chaotic shape with different particle sizes as evident from the images. It was observed that by varying the milling time, the shape and structure of the agglomerates also changed considerably. Thus, it was observed that pressure milling proved to be an effective green method for altering the intermolecular bonding and original surface structure of the synthesized superfine potato powder. Different milling times significantly change the surface morphology and this is because the surface area to volume ratio changes. Increased milling time can have a significant impact on physical and chemical properties and can be used for a variety of applications. Pressure milling converts materials from ordered and/or ordered states to disordered and/or disordered structures by strongly breaking the intermolecular bonds. By milling, the bulky particles are effectively divided into various types and small size fractions, exhibiting agglomeration due to increased surface reactivity, and exhibiting characteristically different shapes, as shown from figure 3(a-d). The increased ratio of surface area with respect to its volume of any sample also implies that the sample exhibits a high degree of interaction at the surface, which in turn promotes the characteristic aggregation phenomenon. Therefore, it improves the physical and chemical properties of superfine food powder. Various research groups have also reported similar behavior in food powders of cinnamon, turmeric, ginger etc which were synthesised using high-energy ball milling [16,20].

3.2.1 Transmission Electron Microscopy (TEM) measurement

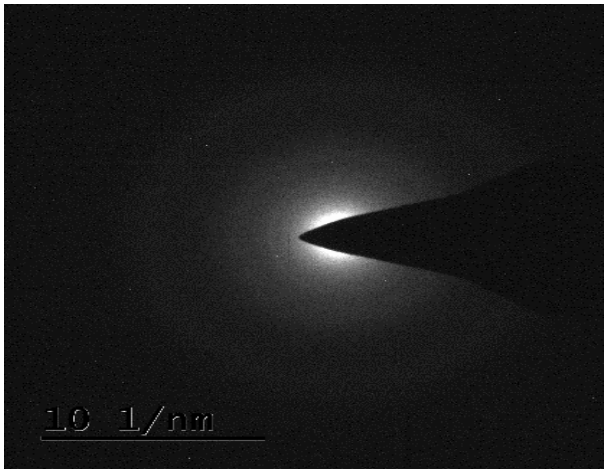
A microstructural study of a potato sample milled for 7.5hr was performed by HRTEM as shown in figure 3. The microstructure accessible in figure 3(e) clearly shows the evolution of small nanoparticles as aggregate structures. Therefore, a histogram was created using the measured nanoparticle sizes to calculate the average particle size, and the same is shown in figure 3(f). The particle size was calculated using statistical analysis of histogram plots and was found to be approximately 12.53nm. Figure 3(g) shows the selected area (SAED) electron diffraction. The SAED obtained shows that there are no bright spots and concentric rings in the pattern confirming that the synthesized superfine food powder is amorphous in nature. Hence TEM data supports and is in accordance with the XRD results.



(e)



(f)



(g)

Fig. 3 (e-g) –HRTEM images of potato nanoparticles (e) Measurement (f) Histogram (g) SAED Pattern

3.3 FTIR- Functional group measurement

The composition of the functional groups along with its inherent nature type and purity of the nanometric potato food powder was determined and analyzed with the aid of Frontier, PerkinElmer, UK make FTIR- Fourier transform infrared spectroscopy equipment. A pelletizer was used for sample preparation for FTIR. 1:20 ratio of potassium bromide to sample was used for the analysis. Figure 4(a) shows the FTIR spectrum of the synthesized potato superfine nano powder. As shown in Table 1, a table has been curated showing the functional groups present at different wavenumbers in superfine potato powder at four different milling times ranging from 0 to 7.5 hours. The FTIR spectrum of potato exhibited a broad range of absorption bands. From 4000cm^{-1} to 400cm^{-1}range absorption band was analyzed at room temperature.

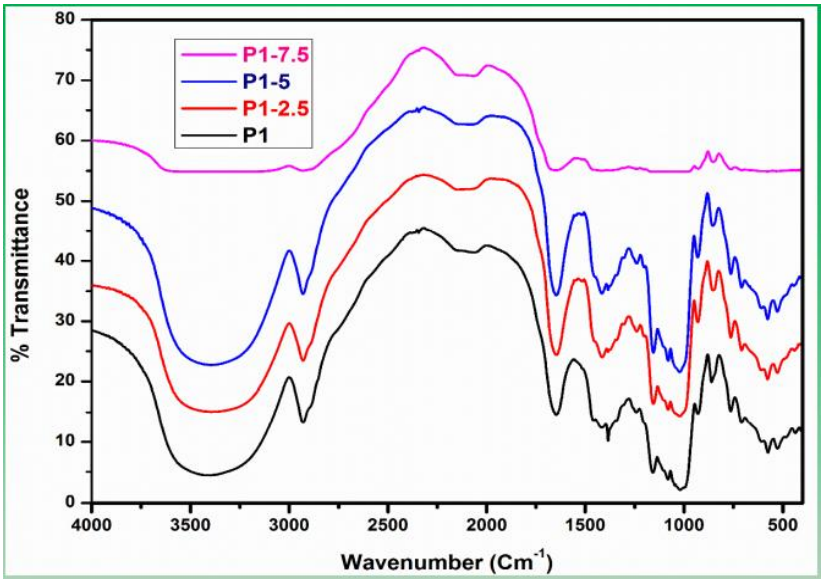


Fig. 4 – FTIR spectra of potato nanoscale powder

Table 1 – FTIR Spectra of P1 at 0,2.5,5 & 7.5hr

FTIR spectra of P1-0		FTIR spectra of P1-2.5		FTIR spectra of P1-5		FTIR spectra of P1-7.5	
Wavenumber (Cm^{-1})	Functional Group	Wavenumber (Cm^{-1})	Functional Group	Wavenumber (Cm^{-1})	Functional Group	Wavenumber (Cm^{-1})	Functional Group
3395 Cm^{-1}	O-H	3390	O-H	3390	O-H	3390	O-H
2976 Cm^{-1}	N-H	2970 Cm^{-1}	N-H	2968 Cm^{-1}	N-H	2965 Cm^{-1}	N-H
2165 Cm^{-1}	Alkynes	2162 Cm^{-1}	Alkynes	2162 Cm^{-1}	Alkynes	2161 Cm^{-1}	Alkynes
1645Cm^{-1}	C=C	1642 Cm^{-1}	C=C	1642 Cm^{-1}	C=C	1641 Cm^{-1}	C=C
1478 Cm^{-1}	CH_3	1476 Cm^{-1}	CH_3	1476 Cm^{-1}	CH_3	1476 Cm^{-1}	CH_3
1251 Cm^{-1}	C-O	1249 Cm^{-1}	C-O	1249 Cm^{-1}	C-O	1249 Cm^{-1}	C-O
1193 Cm^{-1}	C-O	1191 Cm^{-1}	C-O	1191 Cm^{-1}	C-O	1190 Cm^{-1}	C-O
997 Cm^{-1}	CH_2	995 Cm^{-1}	CH_2	995 Cm^{-1}	CH_2	995 Cm^{-1}	CH_2
515 Cm^{-1}	C-Br	514 Cm-1	C-Br	514 Cm^{-1}	C-Br	514 Cm^{-1}	C-Br

At 2909-3698cm-1, 1700-2700cm^{-1}, 1190-1645cm^{--1}, and 514-997cm^{-1} [20-21] broad IR absorption band are seen [20-21]. O-H, N-H, H=C=H, HO-C=O, and C-H groups creates a strong broadband region corresponding to the region 2909cm^{-1} to 3698cm^{-1}. The broad band absorption in the aforesaid region can be attributed to the stretching of the aromatic rings present in the bioactive components of the sample under consideration. Alkynes present in the sample are represented by the absorption band present in 2161-2165cm^{-1} region. The absorption band from 1700 to 2700cm^{-1} indicates the presence of C=O, C≡C, C=N groups. The absorption band in the aforesaid region may be due to vibrational stretching of aldehydes, ketones, esters, nitriles and alkynes. Vibrational stretching of the similar type of aromatic compounds is observed in the region from 1190-1645cm^{-1}. CH_2 and C-Br groups exhibit vibrational strains between the region 514cm^{-1} 997cm^{-1}. Below 1000cm^{-1} absorption

band distinct in the fingerprint region was observed. The different functional groups inherent in superfine nanometric potato food powder is depicted in table-1. Pressure milling did not create any new functional group which is distinctly evident from the result obtained. Certain considerable changes were observed in terms of transmittance and/or wavenumbers, while there are no changes in the functional groups, which indicates that superfine milling did not significantly break the intermolecular hydrogen bonds. Milling changes the surface texture of an ordered sample of amorphous nature. By pulverizing potato powder into superfine powder, the intermolecular bonds are broken and reformed, creating a new functional superfine potato powder with a new amorphous structure and functionality. Changes in surface area, nature of bond and the internal cohesion were analysed by the results FTIR data. This is also confirmed by the latest scientific measurements using SEM, TEM, FTIR, UV-Vis-NIR, and XRD data which are consistent with the findings above. Similar methods have been used by different research groups to showcase their FTIR data in pretext to various food materials [20-23].

3.4 UV VIS NIR Measurement

To obtain and analyse the UV spectrum of superfine nanometric potato powder milled at different time intervals (0hr, 2.5hr, 5hr, 7.5hr), it was subjected to Lambda 950, PerkinElmer, UK, make, UV/VIS/NIR spectrophotometer as depicted in figure -5. The reading was recorded between 200nm to 800nm wavelength. Spectra were recorded by analysing the four different food powder materials that were milled at different time span. The absorbance maxima for all four samples are approximately at the same position around 400 nm, with a slight peak observed at 270 nm. The observed peak at said wavelengths indicates the presence of structures related to protein in the synthesised samples. Characteristic peaks at 400nm and 270nm is mainly due to the presences of tryptophan, tyrosine, and cysteine amino acids as depicted in figure 5.

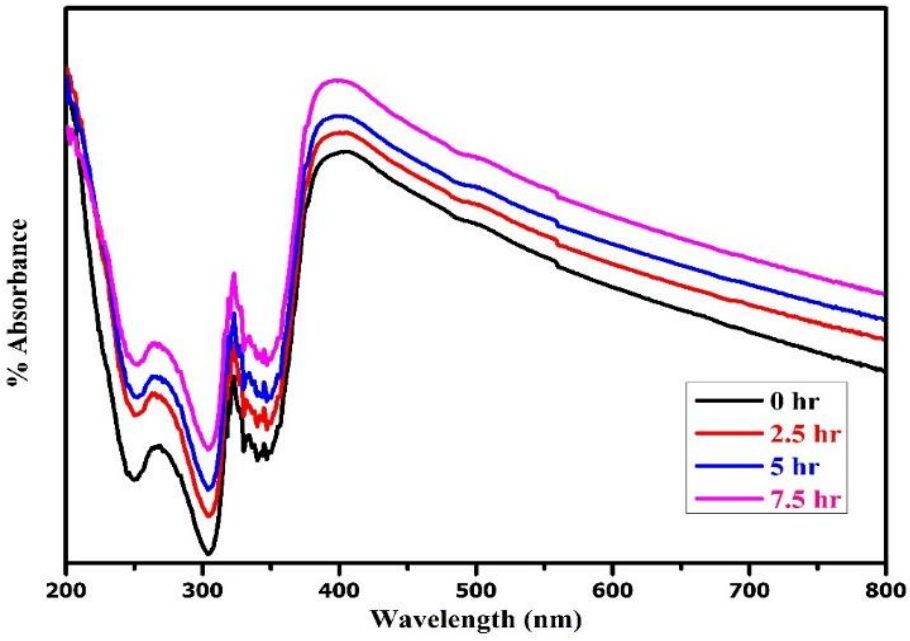
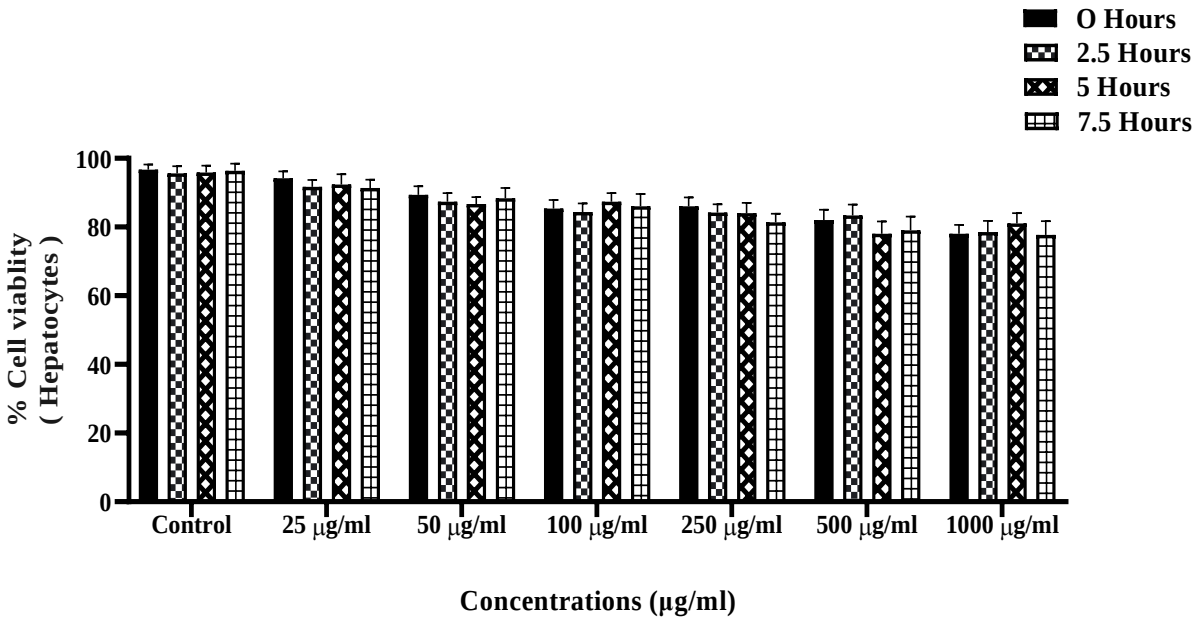


Figure 5 – UV VIS NIR spectrum of superfine potato powder at 0hr, 2.5hr, 5hr and 7.5hr

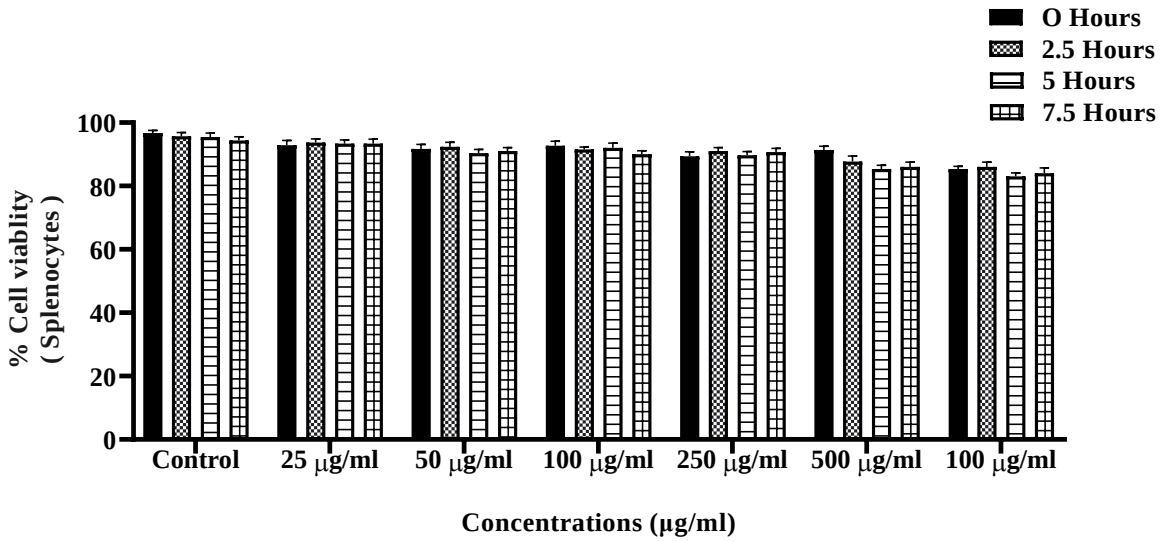
4. Biomedical Application

Cell Viability Assay

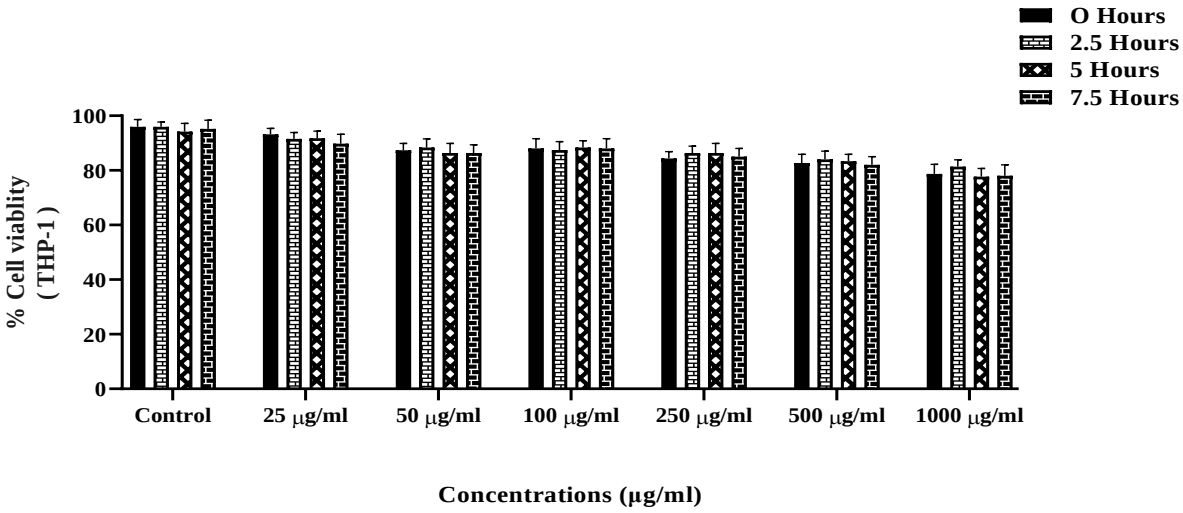
Cell viability and cell toxicity of superfine potato powder were used to investigate the biocompatibility and cytotoxicity of the synthesised superfine potato food powder for determining the applications as a new functional food nanomaterial. The superfine potato powder can be used as applications in fields of biotechnology, food sciences, and pharmaceutical industries, etc. To assess the biocompatibility of in-house produced potato superfine nanoparticles with mouse (hepatocytes and splenocytes) and human (THP-1)-derived cells, MTT assay and trypan blue exclusion assay were used. An experiment was conducted by taking three types of cells - mouse derived liver cells and spleen cells and THP-1 cells which is a human derived monocyte leukaemia cell. 250µg/ml is the optimum dose concentration observed in vitro to be non-cytotoxic and readily biocompatible to the cells under consideration. At 250µg/ml dose treatment 85% of cell viability has been observed. When the cells under considerations are treated with higher concentration of nanoformulation dose i.e. 500µg/ml and 1000µg/ml, significant decrease in cell viability was observed. The changes can be attributed to depletion of the nutrient medium or due to presences of high drug pressure. With the increase in superfine behavior of the powder with respect to the increasing time interval from 0, 2.5, 5 and 7.5 hours respectively the cell health index is seen to increase and was observed maximum at 250µg/ml. The results of the observed study are shown in Figure 6 (a–c).



(a)



(b)



(c)

Fig. 6 (a-c) – Cell viability and biocompatibility evaluation on human and murine derive cell against prepared superfine potato powders. The observed percentage viability was expressed through bar diagram for (a) splenocytes, (b) hepatocytes, (c) THP-1

Hence, the change in size of superfine nanoparticle seems not only to increase the bio activities, but is also safe for various biological application even at very higher doses (500, and 1000μg/ml). This study is concomitant with the previously published studies by various research groups about the superfine food powder of cinnamon, ginger, turmeric, and bitter gourd for different functional behavior compared to normal crude food powder [16, 24-25]. Change in the crystal structure at atomic and molecular level and change in surface to volume ratio is the main cause of the aforesaid phenomenon. Hence production of such evidence based superfine potato powder as a new functional food material in large scale may be one of best practices for a self-reliant society.

In nut shell, we can conclude from the present research that the MTT assay for cell viability studies on superfine potato food powder, increases with the change in time intervals of 0hr, 2.5hr, 5hr and 7.5hr milling. The synthesised powder showed enhanced biological activities, and biocompatibility. In biomedical science, pharmaceutical sector, food sector, and biotechnology field the current research work can be further analysed to tap the maximum potential of the synthesised superfine potato food powder. The measurements done by the modern scientific instruments of XRD, FTIR, UV-Vis-NIR, SEM and TEM support the changes in physical properties of the synthesised superfine powder of potato and hence proves that a new functional food nanomaterial is synthesised which can be further analysed for its application in various sectors.

5. Conclusions:

With the aid of high-energy ball mill nanometric superfine potato powders with different crystal arrangements and morphologies was effectively synthesized. The average crystallite size of approximately 12.53nm of the synthesised material was calculated with the help of histogram and the absences of any bright spots or rings confirmed that the newly synthesised superfine powder is amorphous in nature, which was confirmed by TEM. FTIR measurements reveal a slight shift in spectral position, while no changes in the functional groups was observed. SEM measurements show that the surface reactivity and agglomeration increased with increasing milling time, with a rough amorphous grain present on the surface of ordered amorphous samples which is also evident from the result of XRD and SEM. UV Vis NIR spectrum result showed absorbance at 400nm and 270nm respectively indicating that the functional group are remaining same with changes in their crystal structure. This in-turn is altering their properties and behaviour at nanometric range which is in coherence with structural analysis. The MTT assay for cell viability studies against murine-derived (hepatocytes and splenocytes) and human-derived (THP-1) cells showed that the superfine behaviour increases with the time intervals 0, 2.5, 5 and 7.5hr. Increase in biological activities, is evident with increased cell viability and this causes change in properties of prepared food powder. The present research finding reveals increase in the biological activities with superfine behaviour can further be studied for tapping its maximum potential for suitable applications in biomedical science, pharmaceutical sector, food, and biotechnology field. Physical and chemical behaviour of superfine potato nanometric food powder can be considered as a new functional food nanomaterial. Hence, large scale production of such evidence based nano functional food nanomaterial can be considered as one of the best practices for the development of self-dependent and reliable society.

Acknowledgement

The authors are highly indebted an obliged towards the Ministry of Education, Government of Bihar and Aryabhatta Knowledge University (AKU), Patna, Bihar, for their assistance in establishing and operating the Centre for Nanoscience and Nanotechnology at Aryabhatta University of Knowledge, Patna, Bihar, India.

Declaration of Competing Interest

The authors state that there is no competing financial interests or personal ties that may have influenced the research presented in this study.

Conflicts of interest/Competing interests –The authors do not show any conflicts of interest.

References

[1] Nanoscale Adv. 2019, 1, 937

[2] A. Sofowora(1999) Spectrum books Ltd.,2 8-76.

[3] V. Amador; J. Bou; J. Martínez-García; E. Monte; M. Rodríguez-Falcon; E. Russo; S. Prat (2009) "Regulation of potato tuberization by daylength and gibberellins" (PDF). International Journal of Developmental Biology (45): S37–S38.

[4] S.Turner(2016) Potatoes and Related Crops: Role in the Diet, Encyclopaedia of Food & Health, 2nd edition, 452-457.

[5] F. Hesam et al; (2012) Avicenna J Phytomed, "Evaluation of antioxidant activity of three common potato (*Solanum tuberosum*) cultivars in Iran"; Spring; 2(2): 79–85.

[6] H. Mange et al.; (2014) World J Cardio; "Antioxidants, inflammation and cardiovascular disease"; 6(6): 462–477.

[7] Q. Wang et al.; (2011) Nutr Cancer, "Inhibitory effect of antioxidant extracts from various potatoes on the proliferation of human colon and liver cancer cells";63(7):1044-52.

[8] J.A Higgins.; (2004); "Resistant starch: metabolic effects and potential health benefits";J AOAC Int, 87(3):761-8.

[9] H.S. Holt et al., (1995), "A satiety index of common foods"; Eur J Clin Nutr, 49(9):675-90.

[10] N.Tkacova, Stevulova, Freiberger. AForsch(1998) (Partik.), 30 14–25.

[11] J.Hu,L. Ma, X. Liu,H.Li,M.Zhang, &Z. Jiang, et al. (2022)."Superfine grinding pretreatment enhances emulsifying, gel properties and in vitro digestibility of laccase-treated α-lactalbumin",. LWT, 157.

[12] Q. Xu,R. Huang, P.Yang, L.Wang,Y.Xing, &H. Liu, et al(2021)."Effect of different superfine grinding technologies on the physicochemical and antioxidant properties of tartary buckwheat bran powder", RSC Adv., 11(49), 30898-30910.

[13] T.Zhang,S. Y. Xiao, Z. H.Ding, &Y. T. Song,(2021). "Effect of superfine grinding on physicochemical and morphological structure of coix seed powder," Journal of Cereal Science, 102, 103361.

[14] Z.Q.Huang, X.L. Xie, Y. Chen, J.P. Lu, Z.F. Tong,(2008)."Ball-milling treatment effect on physicochemical properties for cassava and maize starches", C. R. Chim. 11 73–79.

[15] G.Li, W. Guo, X. Gao, Y. Sun S Wang,(2020)Effect of superfine grinding on physicochemical and and antioxidant properties of soyabean residue powder, Food Sci Nutr, 8, 1208–1214.

[16] A.K. Aman, R.K Singh, R. Kumar, A. K. Ghosh; (2018). "Effect of High Energy Ball Milling grinding on Physico-Chemical, Morphological and Optical properties of Curcuma longa Nanoparticles", IJPSR; 0975-8232.9672-77.

[17] G.Ahmed, A.K Thakur, S.K Chaturvedi,P. Shivam, F Jamal,M.K Singh,S. Bimal, Singh, S.K.Choudhary, P.Das, S.Narayan (2019)."Modulation of the immune

response and infection pattern to Leishmania donovani in visceral leishmaniasis due to arsenic exposure: an in vitro study", PLoS ONE 14(2):e0210737.

[18] N.Kumar,R.K Singh,V. Kumar,S.B Das,G. Ahmed,S. Narayan,R. Kumari (2021). "Physical properties of Pr-substituted Li/Ni ferrite magnetic materials at nanometric scale for its multifunctional applications in industries/environment and their cytotoxicity, lymphocyte studies as nanomedicine.", Applied Nanoscience.;11(12):2847-59.

[19] W. Strober Trypan Blue Exclusion Test of Cell Viability. Curr. Protoc. Immunol. Appendix 3: Appendix 3B.

[20] Archana, R.K. Singh, A.K. Aman, N. Kumar, B. Prasad(2021)."Preparation of superfine cinnamon bark nanocrystalline powder using high energy ball mill and estimation of structural and antioxidant properties", IOP Conf. Ser.: Mater. Sci. Eng. 1126 012020.

[21] L.Hua, Q.Lizhao,H. He andL. Qing; (2021)."Preparation of starch nanoparticles via high-energy ball milling", Journal of Nano Research ISSN: 1661-9897, Vol. 40, 174-179.

[22] R. Wongsagona,S. Shobsngobb, S. Varavinita,(2005)."Preparation and physicochemical properties of dialdehyde tapioca starch", Starch-Stärke. 57 166-172.

[23] A.K. Aman,R.K. Singh,R Kumar, A.K.Ghosh, (2018)."Effect of High energy Ball milling griding on physico chemical, morphological and optical properties of curcuma longa nanoparticles powder", Int J Pharm Sci Res. 9 672–677.

[24] Archana,R.K. Singh, A.K. Aman, N. Kumar, A. Jaben(2020)."Effect of superfine grinding on structural, morphological and antioxidant properties of ginger (Zingiber officinale) nano crystalline food powder",Elsevier- Materials Today: Proceeding, 43 3397-3403.

[25] R.K. Singh, A.K. Aman, M.Kirti (2017)."Effect of high energy ball milling griding on physico-chemical, structural and morphological studies of bitter melon nanopowder", International Journal of Recent Scientific Research, 8, 19258-19263.