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Microscopic Analysis on Structural Morphologies of Total Protein Isolates from Two Varieties (DAS and BS) of Nigerian Cultivated Solojo Cowpea (Vigna Unguiculata L. Walp)

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ABSTRACT

Structural morphologies of total protein isolate of two varieties of solojo cowpea were studied by scanning electron microscope (SEM). The morphological properties of raw and germinated protein isolates of DAS and BS are presented in Figures. 4.42 – 4.53. Results showed the apparent structural differences between them. The Raw DAS showed plate like structure with relatively smooth surface topography with large cavities while Raw BS showed flaky structure like that of Albumins. This pattern is similar to that of albumin shapes of the Great Northern Bean, field pea protein and that of Crotalaria pallida. The SEM micrographs results of DAS isolates revealed smooth lamella with flake-like structures on it, having large particle sizes and irregular geometry shapes. Large vacuum spaces were also observed. After six hours of modification, a change in morphology of the original isolate was observed with disappearance of the plate-like and flake-like structure and the vacuum space; DAS 24 h protein isolates then exhibited a spongy plate like structure. Rise in the germination period to 36 h led to disappearance of the cloudy mass and formation of smooth non homogeneous mass. Further increase in the germination time to 48 and 72 h led to smoother mass with a few dispersed particles with cracked surface. The observation of the microstructure presentation from 36 h is also comparable to that obtained for bitter vetch (Vicia ervilia) protein films strengthened by microbial transglutaminase, in this case, the images of BVPC films containing mTGase clearly indicate a more compact microstructure, with evident continuous zones. The BS isolates morphology which presented a thin wafer-like structure like that of albumin, possessing wide surface area could in part justify the great solubility of albumins in neutral environment, enabling better access to water molecules. The structure was observed to become more homogeneous and compact, with no cavities as germination proceeded. The DAS protein isolates presents micrograph similar to those of white bambarra (WB) and black bambarra (BB) surfaces which presented cracking. The micrograph of BS 6 h germinated protein isolate, which presents a flaky plate like structure is comparable to that of commercial textured Glycine max protein.

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Introduction

Cowpea botanically known as Vigna unguiculata, an affiliate of the class fabaceae and genus Vigna, identified by several names for example; southern pea, beans, crowder pea and black-eye pea, is a dicotyledon plant. It is planted extensively in Africa, especially the dry savanna region, particularly the low land area and middle-altitude area. A legume of African origin, even though there is no archaeological proof for primeval cowpea development, the centre of diversification is West-Africa, influencing the common agreement that this is the most probable emergence centre, and early domesticity [1].

The cowpeas crop, because of their ability to cope with warm weather and to adapt to drought situations, are properly suited to the dry territory of the tropics, situation other foods does not function well. It can grow well in soils with as much as 85% sand, low levels of phosphorous and with lower than even 0.2% organic matter [2]. This makes them special and indispensable crops especially in desert, semi-desert area where not several other crops can survive. Cowpea is sometimes grown alone but routinely grown alongside cereals such as maize, great millet, groundnut or Pennisetum glaucum [3]. They are used in crop succession to control erosion and revamp the soil properties because they help in meeting a cash crop's nitrogen needs. They also suppress weed growth and encourages beneficial insects' populations in order to defend cash crops from insect pest. The seeds of cowpea are the most economically valuable part, because of their nutrition and health (medicinal) qualities [4].

In less developed countries of the tropics, millions of relatively poor people depend on Cowpea for their livelihoods [5]. There is

no part of cowpea plant that is not useful for either food or fodder. The parts used as food are not only nutritious, they also provide proteins and vitamins, several refreshment and main meals are produced from the grains, while, the younger pods and seed can be utilized as vegetables [6]. Consumed at the seeding stage are, the tender shoot tips and leaves, while the young pods and grains are eaten at the ripen stage. Several dishes are produced from the harvested dry seeds, such as, cowpea cake or bean balls made from seeds ground into slurry, boiled, or deep fried respectively; the grains could also be heated to boiling, made into porridge or eating with stew. Plant left over part could be utilized as animal feeds [4,7,8].

Structural morphologies of total protein isolate of the two varieties of solojo cowpea were studied by scanning electron microscope (SEM). This has been established to be a valuable tool for examining the microstructures of cereal, pseudo cereal, grains and products produced from them as well. The SEM is a powerful and versatile analytical instrument for material characterization. It is a microscope that generate images pertaining to a specimen by browsing the surface using a concentrated ray of electrons. It is very useful for small material analysis. It uses electrons for imaging. It is used to determine the morphological property of flour, concentrates and isolates of protein. It has been established as a valuable instrument for analyzing the micro-structures of cereal grains, imitation cereal seeds as well as allied produce. SEM has also been utilized in the determination of the internal microstructure of beef products, soybean foods, dairy products by respectively [9-12]. It has also been utilized in the investigation of the characteristic framework of starch, flours and proteins of legumes such as vigna subterranean [13-16]. The internal microstructure, shapes, dimensions and distinguishing facial characteristics have also been investigated for foods in powdery form.

Methodology

Materials

Two varieties of the underutilized cowpea (V. unguculata) found in South west region of Nigeria where it is called 'solojo' were used (Figure 1: Brown Solojo Cowpea and Figure 2: Dark-Ash Solojo Cowpea).

Figure 1: Brown Solojo Cowpea

Figure 2: Dark-Ash Solojo Cowpea

Methods

Preparation of flours: The grains were segregated to remove the spoilt ones; then dry dehulled with a mechanical dry dehuller (fabricated in FIIRO), dried at 40°C and later milled dry to powder then sifted using 80 µm mesh. The flour was stored in flexible bags and preserved at 4°C preceding utilization in a refrigerator freezer.

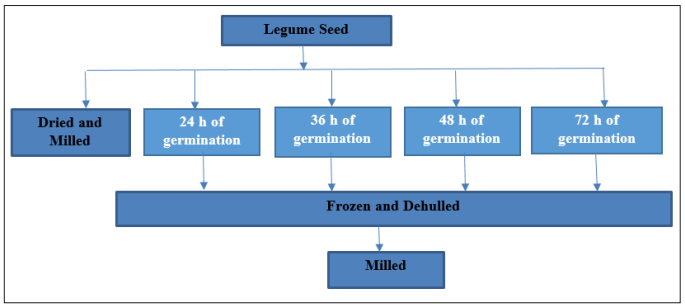

Figure 3: Preparation of Beans Flour/ Schematic representation

6 h Soaked flour: The seeds were segregated to remove the unwholesome ones, then immersed for 6 h in the ratio (1:10 w/v) (seed/water). The grains were then frozen to prevent germination from setting in, then the hull was removed manually, dried for 48 h at 40°C later milled dry to smooth powder prior to sieving using 80 µm mesh screen. The resulting flour was packaged in plastic pack and preserved in a fridge freezer at 4°C pending utilization.

Germination of seed: This was implemented by the method of Mubarak AE with minor adjustment. The seeds for germination were disinfected by soaking in 0.07% sodium hypochlorite for30 mins, then, it was rinsed painstakingly. The solojo seeds were then immersed for 6 hours in distilled water at ambient temperature (1:10w/v) (~25°C), then placed in a colander and germinated under subdued light in an open laboratory for, 24 h, 36 h, 48 h and 72 h (Figure 3). The process of germination was terminated by freezing; the seeds were manually dehulled, dried in a draught oven at 40°C for 48 h, cooled, milled and packaged in an air tight plastic bag in the refrigerator pending analysis.

Scanning Electron Microscopy (SEM) of Solojo Cowpea

Freeze dried raw and sprouted Solojo isolates and their morphological properties were analyzed using Scanning Electron Microscope (SEM) (Model No. JSM 6610-LV) at magnification of 1.00K [16]. All experiment was replicated, one-way analysis of variance (ANOVA) was carried out to calculate significant differences in treatments. Differences in mean values were determined using Duncan's multiple range test at ($p< 0.05$) (95% confidence level) was used to separate means (SAS1999).

The SEM is a microscope that generate images pertaining to a specimen by browsing the surface using a concentrated ray of electrons. SEM require running a strong energy ray of primary electrons over the exterior of an aggregate sample that inspires discharge of subsidiary electrons. The released trapped electrons are electronically transformed to a representation of facial topography, exhibited through a cathode beam tube in raster form. The electrons react with atom in the material, generating varied signs containing facts concerning the surface area and structure of the material [14]. The image produced by SEM is due to the signal obtained by the interaction at varied depth within the sample of the electron ray with atoms at these varied levels. The signals produced comprise of; Secondary electrons (SE), characteristic X-rays and light (cathodoluminescence) (CL), reflected or back-scattered electron (BSE), transmitted electrons and absorbed current (specimen current). SE detectors are part of the standard

equipment in all Scanning Electron Microscopic instruments [10].

Results and Discussion

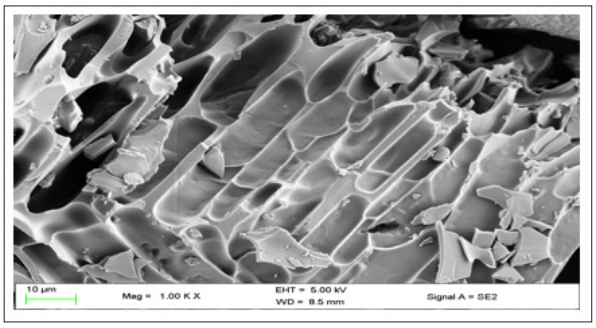

Figure 4: DAS Raw Isolate /Scanning Electron Microgram

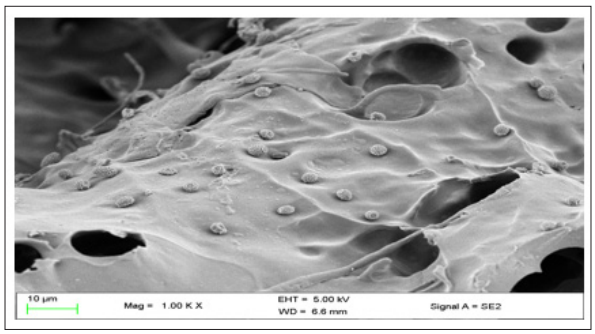

Figure 5: DAS 6 h Isolate/ Scanning Electron Microgram

The structure was observed to become more homogeneous and compact, with no cavities as germination proceeded. This is like the image found for the outer and inner depths of chorizo stuffed in natural and synthetic casings before and after 5 days of smoking. Both protein isolate presented irregular, rectangular-shaped particles, which were agglomerated and had a thick mass with limited pores.

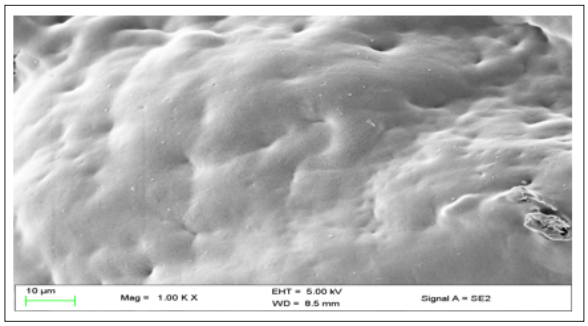

Figure 6: DAS 24 h Isolate/ Scanning Electron Microgram

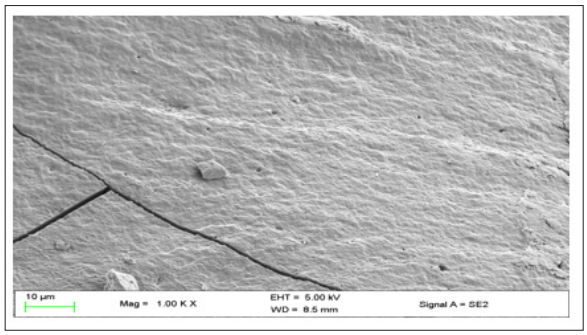

Figure 7: DAS 36 h Isolate /Scanning Electron Microgram

The likeness between the particles in these micrographs is in agreement with that of loss of albumin proteins from the isolate extracted at pH 9. This isolate is envisioned to have most of the features of globulins. The patterns with increase in germination time clearly indicate a more compact microstructure, with evident continuous zones.

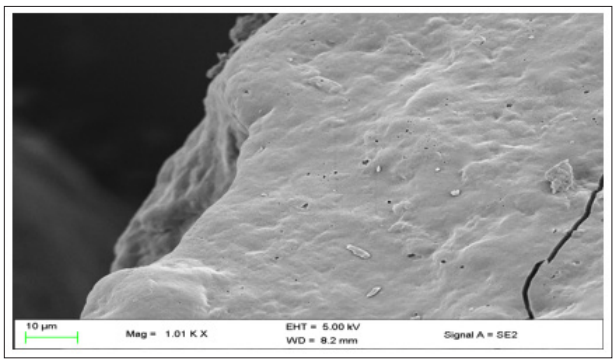

Figure 8: DAS 48 h Isolate /Scanning Electron Microgram

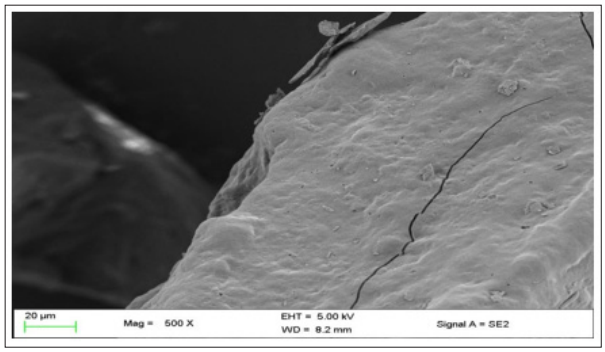

Figure 9: DAS 72 h Isolate /Scanning Electron Microgram

The DAS protein isolates presents micrograph similar to those of white bambarra (WB) and black bambarra (black bambarra) surfaces which presented cracking. The micrograph of BS 6 h germinated protein isolate, which presents a flaky plate like structure is comparable to that of commercial textured Glycine max protein.

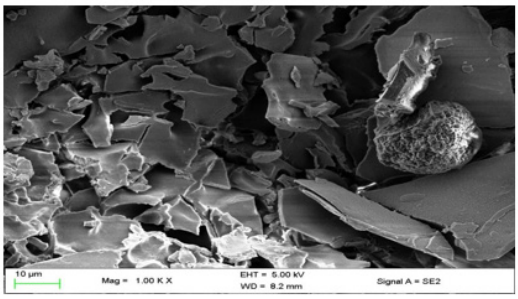

Figure 10: BS Raw Isolate /Scanning Electron Microgram

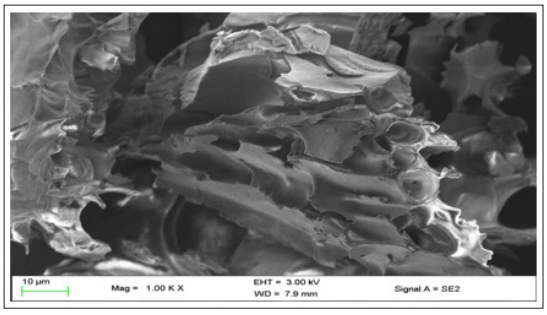

Figure 11: BS 6 h Isolate /Scanning Electron Microgram

It was also observed that the micrograph of freeze-dried pea protein isolate presented an irregular shaped structure, with denser mass and some pores which was observed to form a more compact structure with increase in germination time.

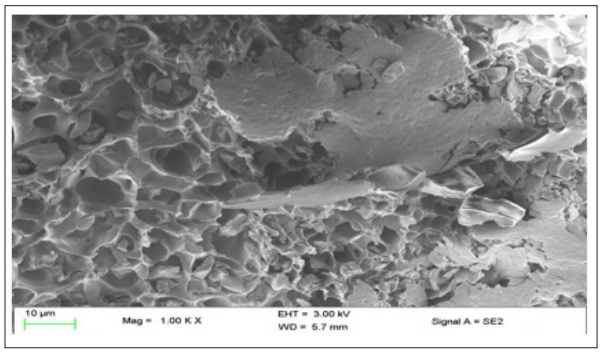

Figure 12: BS 24 h Isolate/Scanning Electron Microgram

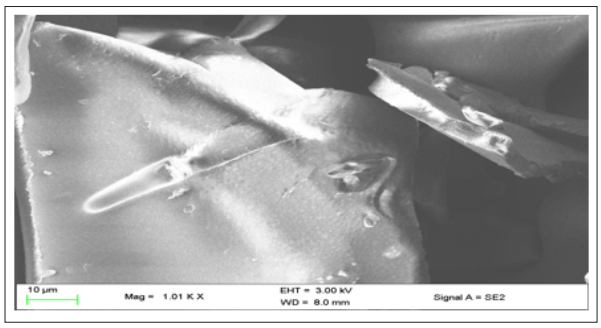

Figure 13: BS 36 h Isolate /Scanning Electron Microgram

The mode of drying was also found to affect the surface morphology as observed in the case of freeze drying and spray drying.

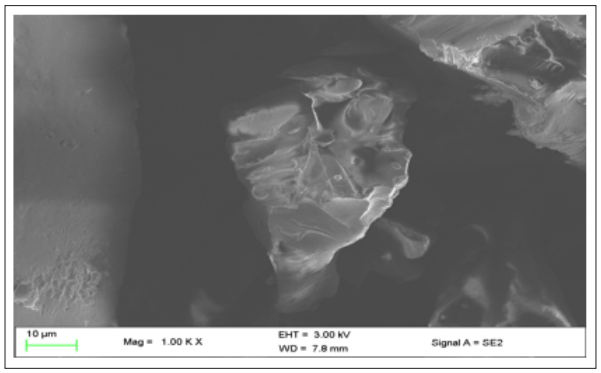

Figure 14: BS 48 h Isolate /Scanning Electron Microgram

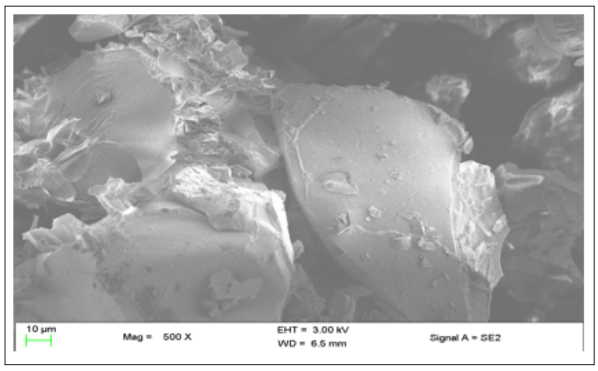

Figure 15: BS 72 h Isolate/ Scanning Electron Microgram

The observations made in the SEM micrographs of the modified protein isolates is likely attributed to degradation of the stored proteins (synthesized during seed development) to small peptides or amino acids during germination, the higher molecular weight amino acids being broken down to lower molecular weight amino acids.

Conclusion

The amount of the crude protein of the full fat ranged between 24.82 and 31.00% for FFDAS and 24.90 to 30.14% for FFBS while that of DFDAS and DFBS was between 25.86 to 34.62%, and 25.64 to 31.80% respectively. This is expected because the removal of oil due to defatting reduces the competition of the oil with protein in the flour during analysis. The protein content of the isolates too was observed to increase with germination. The NFE of the FFDAS was also found to be higher than that of DFDAS; this was due to the removal of the fat.

Fat, a major component, which is also an avenue of production of nutritional and biologically active compounds such as fatty acids of the mono- and polyunsaturated class, tocopherols and phytosterols, reduced with germination time for both the flour and the isolate. This degradation of fat is as a result of the germination process. The decrease in fat content is equally very good for shell life stability. The germinated flour and isolate will be able to last longer on the shelf than the un-germinated samples.

Energy for germination was obtained through the oxidation of fatty acids to carbon dioxide and water. This reduction in oil content on malting, may be connected to its utilization as a source of energy in malting process.

The crude fibre of germinated FFDAS, FFBS and DFDAS generally reduced with germination, except for 72 h for all of them and 24 h FFDAS. While DFBS had its crude fibre increasing with germination except that of 36 h which reduced. The experienced reduction is probably due to degradation of fibre into simple sugars brought about by endogenous enzymes.

Recommendation

The solubility of protein at both high and low pH observed with germination in this research study is an advantage because protein solubility is a useful guide for the conduct of protein in the food system.

This research work also shows that biochemical modification (germination/malting/sprouting) had an enormous impact on the nutritional composition, functional properties, mineral bioavailability, and amino assay of solojo bean; thus, it could be used as protein supplement in infant, young children and geriatric foods. More efforts should be geared towards promoting the cultivation of this legume plant. Also, the consumption and industrial application of this under-utilized legume should be encouraged by the government, especially in the south-western region where it can survive the rain fall level. Large scale production of this legume should be encouraged in order to fight the menace of malnutrition in developing countries where animal protein price is exorbitant. This will ensure food security and also create jobs in different aspects of the production process thereby reducing the rate of unemployment and also prevent the crop from going into extinction.

References

1. Ogunkanmi LA, Taiwo A, Mogaji OL, Awobodede A, Eziashi EE, et al. (2006) Assessment of genetic diversity among cultivated cowpea (Vigna unguiculata L. Walp.) cultivars from a range of localities across West Africa using agronomic traits. Journal of Science Research and Development 10: 111-118.
2. Obatolu VA (2003) Growth pattern of infants fed with a mixture of extruded malted maize and cowpea. Nutrition 19: 174-178.
3. Agbogidi OM (2010a) Screening six ciltivars of cowpea (Vigna unguiculata (L.) Walp) for adaptation to soil contaminated with spent engine oil. Journal of Environmental Chemistry and Ecoloxicology 7: 103-109.
4. Shurtleff W, Aoyagi A (2015) History of soybeans and soyfoodsin the United Kingdom and Ireland (1613-2015): extensively annotatedbibliography and sourcebook Includes England, Great Britain, Scotland and Wales Published by: Soyinfo Center P.O. Box 234Lafayette, CA 94549-0234 USA https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=462a00ccbfc5fec135d6896bb867bb37be86ac91.
5. FAO (2002) World Agriculture: towards 2015/2030. Summary report, Rome.
6. Agbogidi OM, Egho EO (2012) Evaluation of eight varieties of cowpea (Vigna unguiculata (L.) Walp) in Asaba agro ecological environment, Delta State, Nigeria. European Journal of Sustainable Development 2: 303-314.
7. Fery RL (2000) New Opportunities in Vigna. In Trends in New Crops and New Ideas; Jamik, J., Whipkey, A., Eds.; ASHS Press: Alexandria, VA, USA 424-428.
8. Zia-Ul-Haq M, Ahmad S, Amarowicz R, De Feo V (2013) Antioxidant Activity of the Extracts of Some Cowpea (Vigna unguiculata (L) Walp.) Cultivars Commonly Consumed in Pakistan. Molecules 18: 2005-2017.
9. Horita CN, Messias VC, Morgano MA, Hayakawa FM, Pollonio MAR (2014) Textural, microstructural and sensory properties of reduced sodium frankfurter sausages containing mechanically deboned poultry meat and blends of chloride salts. Food Research International 66: 29-35.
10. Ledesma E, Laca A, Rendueles M, Díaz M (2016) Texture, colour and optical characteristics of a meat product depending on smoking time and casing type. LWT - Food Science and Technology 65: 164-172.
11. Chen Y, Ye R, Yin L, Zhang N (2014) Novel blasting extrusion processing improved the physicochemical properties of soluble dietary fiber from soybean residue and in vivo evaluation. Journal of Food Engineering 120: 1-8.
12. Zhou L, Wang J, Li D, Li Y (2014) An electrochemical aptasensor based on gold nanoparticles dotted graphene modified glassy carbon electrode for label-free detection of bisphenol A in milk samples. Food Chemistry 162: 34-40.
13. Kaptso KG, Njintang YNG, Nguemtchouin MM, Scher J, Hounhouigan J, et al. (2014) Physicochemical and micro-structural properties of flours, starch and proteins from two varieties of legumes: Bambara groundnut (Vigna subterranea) Journal of Food Science and Technology DOI: 10.1007/s13197-014-1580-7.
14. Yeboah-Awudzi M, Lutterodt HE, Kyereh E, Reyes V, Sathivel S, et al. (2018) Effect of bambara groundnut supplementation on the physicochemical properties of rice flour and crackers. Journal of Food Science and Technology 55: 3556-3563.
15. Zhang S, Xia C, Dong Y, Yan Y, Li J, et al. (2016) Soy protein isolate-based films reinforced by surface modified cellulose nanocrystal. Industrial Crops and Products 80: 207-213.
16. Majid I, Dar BN, Nanda V (2018) Rheological, thermal, micro structural and functional properties of freeze-dried onion powders as affected by sprouting. Food Bioscience 22: 105-112.
17. Keshri JP (2014) Use of Scanning Electron Microscope In Plant Sciences, National Workshop on Scanning Electron Microscopy https://www.researchgate.net/publication/269988725.
18. Stokes D (2008) Principles and Practice of Variable Pressure / Environmental Scanning Electron Microscopy (VP-ESEM) John Wiley & Sons, 20 Nov 2008 - Science- 234.