Extraction and Characterization of Dietary Fiber, Starch and Cellulose Microfibers from Field Bean and Cowpea Pods

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ABSTRACT

In today's modern world, there are lot of food losses and wastages during processing of foods, both at household and industrial level. These food processing wastes are rich in essential nutrients like carbohydrates, protein, fiber and phytochemicals which are very essential for maintenance of health and treatment of diseases like CVD, DM, Cancer etc. A study on field bean peel and cowpea peel was carried out to check its nutrient composition, phytochemical composition, in vitro anti-oxidant assessment and functional properties. Fiber and starch extracted from peels was studied for their functional properties. The sources proved to be a good source of fiber and starch which was also proved by the results of functional properties. The cellulose microfibers were extracted and characterized by FTIR Spectrophotometer and result showed absence of lignin in extracted CMF and it can further be used in developing biodegradable packaging material.

Keywords: Field bean pods, cowpea pods, Fiber, starch, Phytochemicals, anti-oxidants, cellulose microfibers.

INTRODUCTION

Food is a basic necessity which leads a healthy living. It nourishes our body with nutrients. Modern world has led to many food processing industries, which are also sources of food processing waste is observed. In India, Food and Agricultural Organization (FAO) observed that around 40% of the food produced is wasted[1]. Moreover, fruit and vegetable losses were estimated to be 12-21 million tons by The Ministry of Food Processing Industries (MOPFI) India.[2] The waste generated are at different stages like agriculture and farming, processing, manufacturing, distributing, etc. Higher amount of waste is being observed from fruit and vegetables contributing to 42%. Fifty percent of the cultivated fruit and vegetables are wasted before consumption.[3]

Studies show presence of high number of phytochemical constituents, bioactive compounds, phytochemicals, phenolic compounds etc.,[4] in food processing wastes like peels, rind, seeds and pomace of fruits and vegetables processing industries.[5] When these wastes are utilized properly it leads not only to sustainability but also reduces a burden to the environment. Recent studies have shown that phenolic compounds have come to light, due to their anti-inflammatory, antidiabetic, anticlotting, anti-oxidant and antimutagenic properties. They help in reducing risk of some deadly diseases like cancer, and cardiovascular diseases.[6]

Vegetable peel waste contain a high amount of phytochemicals and other essential nutrients like carbohydrate, fiber[6], protein etc. This study was carried out with an objective of analyzing the nutrient content, phytochemical content of field bean and cow pea peels which are widely available but unused further. Extracting fiber, starch and cellulose micro fibers and analysing their functional properties.

MATERIALS AND METHODS

Procurement and processing of samples

Field Bean peel and Cowpea peel were collected from cottage industry in Anantapur. Peels were checked and cleaned thoroughly for the removal of any damage and deteriorated peels. Good-quality peels were selected for drying. They were dried using a solar dryer and ground into fine powder. The powder was stored in air tight containers for further studies.

Proximate analysis of Field bean peel powder and Cowpea peel powder

The proximate analysis of Field bean peel powder (FBPP) and Cowpea peel powder (CPPP) was carried out by adopting standard procedures. Moisture content, Fat content, Ash content[7], Total carbohydrates[8], Protein (Tulin's KjelTRON based on kjeldhal principle), Fibre content (Tulin's FibroTRON), Iron and calcium content[9] and Phosphorus[7] were estimated.

Extraction of active components from FBPP and CPPP

Methanol was taken as a solvent for extraction. The extracts were concentrated in Superfit RotaVap and were further tested for different activities.

Phytochemical analysis

Both the samples FBPP and CPPP extracts were assessed for total phenolic content[10], total flavonoids[11] and total flavonols[11]

In vitro antioxidant assessment of FBPP and CPPP

To assess the antioxidant potential of the sample extract, 2, 2- diphenyl-1-picyl hydrazyl (DPPH) radical scavenging activity[12,13] was carried out. DPPH, a stable organic nitrogen radical, exhibits longevity and, when dissolved in a solvent, produces a deep purple solution. Upon interaction with antioxidants, it undergoes a color change from purple to yellow, forming 2,2-diphenyl-1-picrylhydrazine[14.]

Anti-nutrient analysis

Extracted samples were assessed for antinutrients, Tannin[15] and oxalates[9] based on the standard methods.

Extraction of Fibre

Estimation of fibre was done by neutral, acid and total dietary fibre methods. Total dietary fibre was extracted and the sample was stored in an air tight container[16].

Extraction of Starch

Starch from the sample was extracted using a method that involved suspending samples in an alkaline solution followed by homogenizing, filtering and sedimentation. The starch was then washed and dried at 40-45°C for 14-18 hours. The resulting starch powder was finely ground and sifted and stored at ambient temperatures, until further analysis. The process ensured the purity of the starch[17].

Analysis of physical and functional properties of extracted fibre and starch

Fibre fractions and starch were tested for bulk density[17], color (Konika Minolta [R-10]), water activity (LabMASTER-a_w system-Novasina), water holding capacity and oil holding capacity[17]. Starch was assessed for swelling power and least gelatin concentration[18.].

Extraction of cellulose microfibers from field bean and cowpea pods powder

The cowpea and field bean pods were washed, dried and ground into fine powder. Dewaxing was done to remove impurities. The samples are bleached to remove lignin. The mixture solutions of sample were boiled and washed with distilled water to remove complete lignin. The resulted portion is a solution is cellulose. This extracted cellulose is dried in hot air oven at 100-105^{0}C for 24 hours to get cellulose microfibers[19].

Characterization of Cellulose microfibers using FTIR

Fourier transform infrared spectroscopy studies on raw and extracted cellulose microfibers of FBPP and CPPP were carried out by dispersing the powdered raw and cellulose microfibers samples separately on KBr pellets and using a Spectrum Two, Perkin Elmer FTIR spectrophotometer. All the samples were recorded between the 4000–400 cm1 region[19].

Statistical Analysis

The analysis of samples was carried out in triplicate. The results obtained were subjected to two-way analysis of variance ANOVA and the significance between the means was calculated. The data analysis was done using Microsoft excel software 2016.

Results and Discussion

Proximate analysis

Proximate analysis is the quantitative analysis done to estimate macromolecules that are present in foods. Under nutrient analysis, moisture, total carbohydrates, fiber, protein, fat, ash, and mineral contents of FBPP and CPPP were estimated.

Moisture, total carbohydrates, fiber, protein, fat, and ash of FBPP was estimated to be 61.3%, 59.6%, 9.53%, 1.26% and 3.16 % respectively and in CPPP it was found to be 62%, 60%, 55.6%, 2.9 %, 1.8 %, and 3.5 % respectively.

Total Phenolic Content

The principal compounds in plants are phenols which have hydroxyl functional group and are responsible for antioxidant activity. It was measured using Folin-Ciocalteau reagent and the results were expressed in Gallic acid equivalents (GAE). The total phenolic content of the field bean peel was 850.33mg GAE/100gm and cowpea peel was 600.83 mg GAE/ 100 gm as shown in Fig.1.

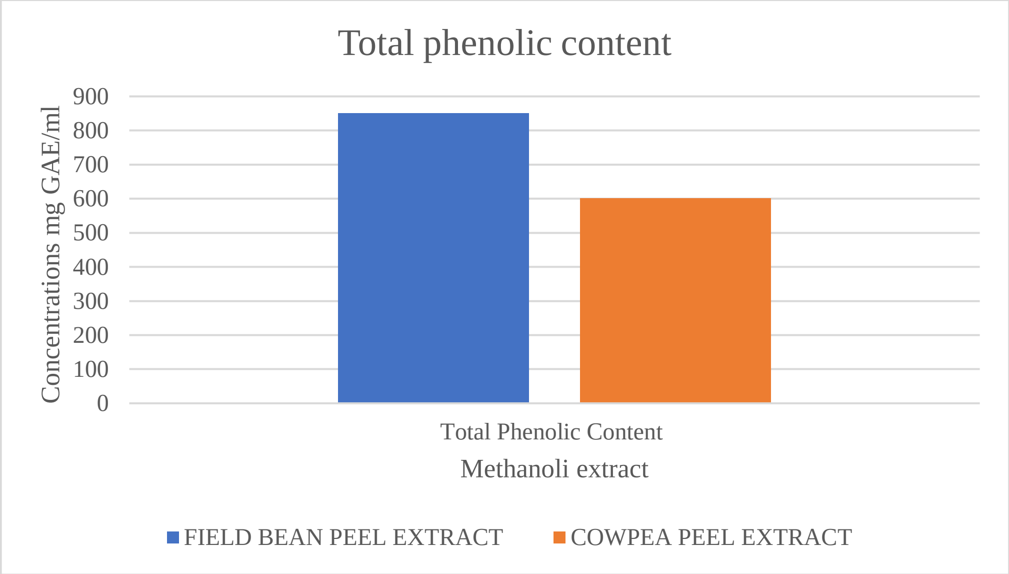

Figure:1 Total phenolic content of FBME and CPME

Flavonoids

They are secondary class metabolites which have importance due to low molecular weight that have polyphenolic structure. Flavonoids content is expressed in terms of quercetin equivalents[20,21]. FBME have the highest flavonoid content of 20.91mg QE/ 100 gm when compared to CPME 18.72 mg QE/100 gm.

Flavanols

They are the sub class of flavonoids in which the major functional group is alcohol. They also have the potential to scavenge free radicals. Total flavanol content of field bean pod and cowpea pod were found to be 47.25 mg RE/ 100 gm and 43.97 mg RE/ 100 gm respectively.

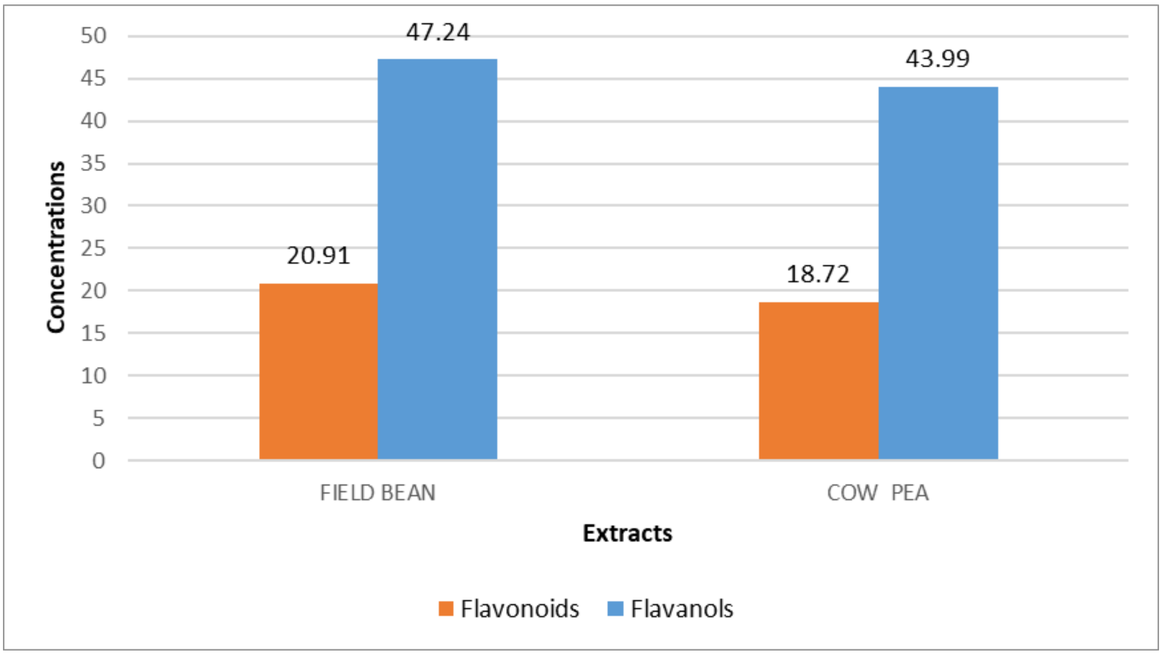

Figure:2 Total Flavonoids and Flavanols content of FBME and CPME

Assessment of in vitro anti-oxidant activity of extracts

Reactive oxygen species with unpaired electrons cause oxidative stress, leading to food degradation and undesirable reactions such as lipid oxidation, resulting in rancidity[21].

DPPH radical scavenging activity is a calorimetric method which helps observing the radical scavenging activity of anti-oxidants. The DPPH activity of the extracts are shown in the figure 3.

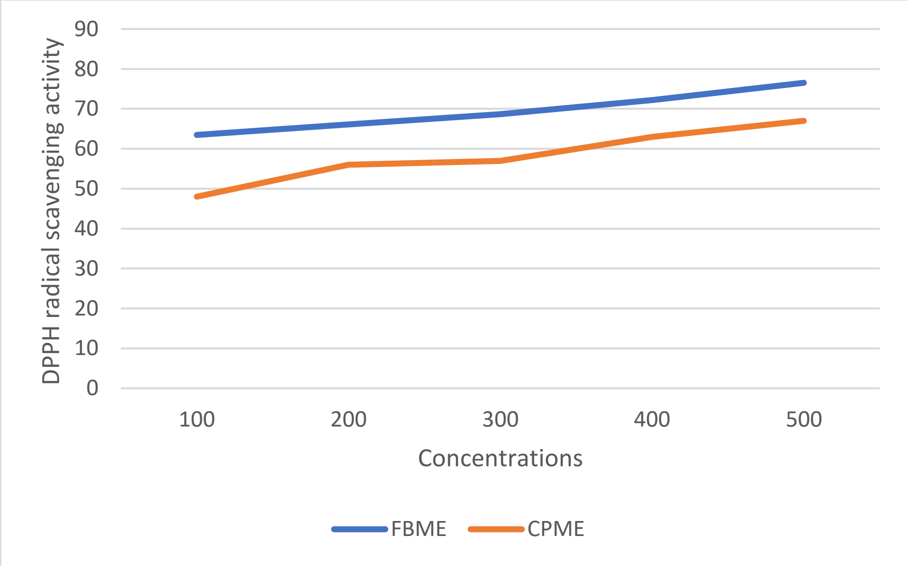

Figure:3 DPPH radical scavenging activity of FBME and CPME

Yield of Starch

Legume pods are good sources of carbohydrates; hence they were tested by boiling in an alkaline solution. The starch obtained ranged from 54% and 53.83% in FBP and CPP respectively. Further study can be conducted on the application in food and packaging industries.

Functional properties of dietary fiber and starch

Water holding capacity

It is ability of carbohydrates to retain water or capacity to absorb water per gram. It is also important in formulations of food. There can be liquid loss during processing[22,]. The amount of water retained back by the known weight of the sample in specific temperature, time soaked and during centrifugation is WHC. The water holding capacity were 24.28% and 23.85% respectively for FBPF and CPPF and for starch samples of FBP and CPP 25.7% and 22.5% were obtained.

Oil holding capacity

Oil holding capacity resembles to the amount of oil a sample can absorb per unit weight. If a sample is containing high oil holding capacity, it can be used to increase the texture of a product. The oil holding capacity for FBP and CPP were 3.45% and 2.84% and for starch samples 4.2% and 3.2% respectively.

Physical characteristics of fiber and starch

Bulk density

Bulk density of the samples of fiber and starch were examined. Loose bulk density of FBP and CPP fibers were 0.18 and 0.2 and for starch 0.2 and 0.2. The tapped bulk density for fiber and starch were 0.22 and 0.238; 0.9 and 0.82 for FBP and CPP respectively.

Functional characteristics of starch

Swelling Power

The swelling power is the ratio of the volume occupied by the wetted material after equilibration to the actual sample weight. It affects the technological and physiological attributes of the food. The swelling power of the FBP and CPP were 2.44% and 2.17% respectively.

Least gelation concentration

It indicates gelation capability where the lowest concentration at which gel stayed in the inverted tube. The lower least gelation concentration indicates a higher gelling capacity. Samples were observed at 0.5%, 2.5%, and 4.5% and CPP was consistent at 2.5% and both the samples were found to be consistent at 4.5% which indicates that CPP has high gelling power.

FTIR analysis

FTIR spectra of FBPP and CPPP raw and extracted cellulose microfibers(CMF) are shown in Figure 4 and Figure 5 respectively. The most elevated peak of all fibers is observed at 1730 to 1740 cm-1 where the stretching of acetyl ester and carbonyl aldehyde group (C = O) of lignin and hemicellulose[19]. According to Figure 4, of FBPP, it was noted that the absorption band observed at 1731 cm-1 and 1230 cm-1 for the Raw FBP and as per Figure 5 of CPPP, shows that the absorption band observed at 1736 cm-1 and 1213 cm-1 has disappeared in the cellulose microfibers. This indicates the absence of hemicellulose and lignin in both FBPP and CPPP.

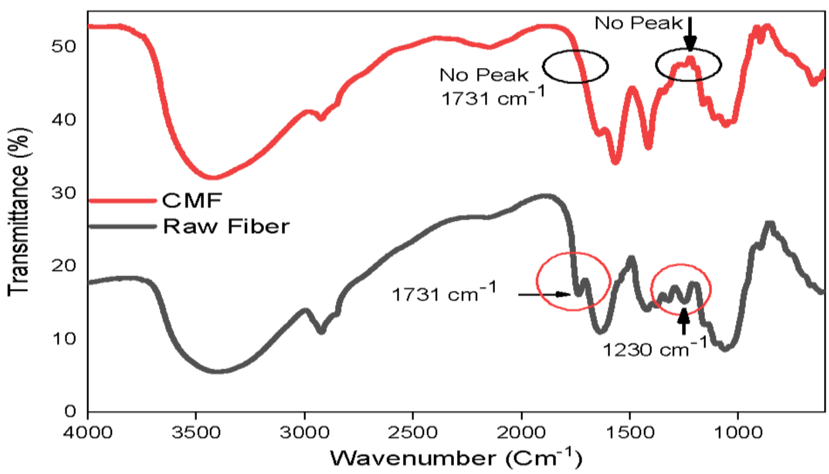

Figure:4 - FTIR Spectra of Raw FBP and extracted CMF

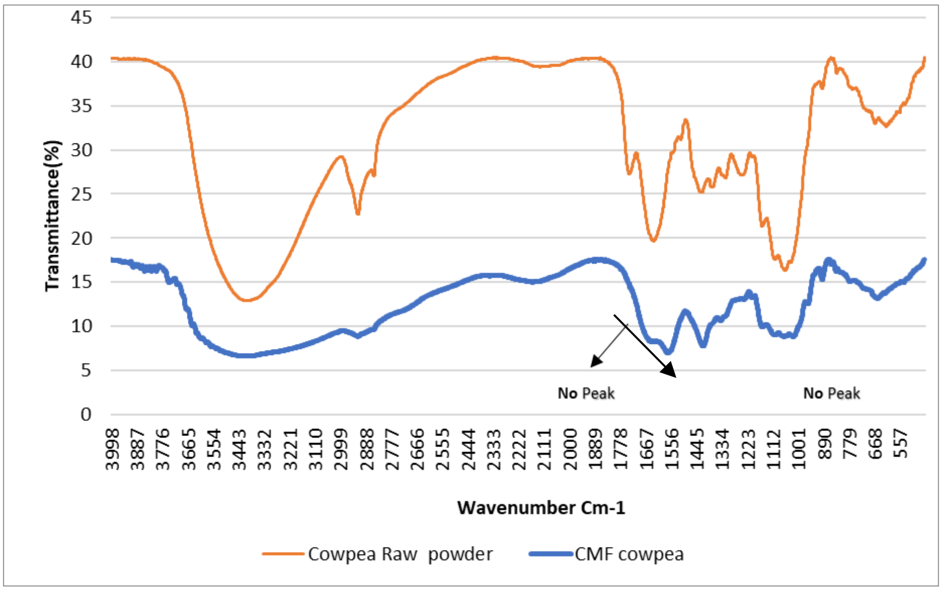

Figure:5. FTIR Spectra of Raw CPP and extracted CMF

Conclusion

The main objective was to utilize the pods of field beans and cowpeas as a good source of nutrients like carbohydrates, fiber, starch, protein, antioxidants, etc. The incorporation of fiber in crackers, etc., helps in healthy snacking which is the need of the hour will be an effective application. Further study will be conducted on starch and its application in soup mix and as a packaging material. Instead of polluting the environment with these by-products, they can be used to extract essential nutrients which helps in overcoming many diseases. The FTIR spectrum showed the absence of hemicellulose and lignin and the existence of cellulose in extracted cellulose microfibers. The results of the current study show that FBP and CPP and their extracts can further be used to develop value added products as well as in developing biodegradable polymers.

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References

1. Plazzotta, S., Manzocco, L., & Nicoli, M.C. (2017). Fruit and vegetable waste management and the challenge of fresh-cut salad. *Trends in Food Science and Technology*, *63*, 51-59.
2. Kumar, H., Bhardwaj, K., Sharma, R., Nepovimova, E., Kuča, K., Dhanjal, D. S., Verma, R., Bhardwaj, P., Sharma, S., & Kumar, D. (2020). Fruit and Vegetable Peels: Utilization of High Value Horticultural Waste in Novel Industrial Applications. *Molecules (Basel, Switzerland)*, *25*(12), 2812.
3. Hussain S, Jõudu I, Bhat R. Dietary Fiber from Underutilized Plant Resources—A Positive Approach for Valorization of Fruit and Vegetable Wastes. *Sustainability*. 2020; 12(13):5401. https://doi.org/10.3390/su12135401

4. Charis M. Galanakis, (2012) Recovery of high added-value components from food wastes: Conventional, emerging technologies and commercialized applications, Trends in Food Science & Technology,26(2), 68-87,
5. Rudra, S.G., Nishad, J., Jakhar, N., & Kaur, C. (2015). Food industry waste: mine of nutraceuticals.4(1), 205-229
6. Kanchan Bhardwaj, Agnieszka Najda, Ruchi Sharma, Renata Nurzyńska-Wierdak, Daljeet Singh Dhanjal, Rohit Sharma, Sivakumar Manickam, Atul Kabra, Kamil Kuča, Prerna Bhardwaj, (2022) "Fruit and Vegetable Peel-Enriched Functional Foods: Potential Avenues and Health Perspectives", *Evidence-Based Complementary and Alternative Medicine*, Article ID 8543881, 14 pages,. https://doi.org/10.1155/2022/8543881
7. AOAC (2012) Official Method of Analysis: Association of Analytical Chemists. 19th Edition, Washington DC,
8. AOAC (2000) Official Methods of Analysis. 17th Edition, The Association of Official Analytical Chemists, Gaithersburg, MD, USA.
9. Raghuramulu, N., Madhavan Nair, K., Kalyanasundaram, S., & National Institute of Nutrition (India). (2003). *A manual of laboratory techniques* (2nd ed). National Institute of Nutrition.
10. Singleton, V. and Rossi, J. (1965) Colorimetry of Total Phenolic Compounds with Phosphomolybdic-Phosphotungstic Acid Reagents. American Journal of Enology and Viticulture, 16, 144-158
11. Chang, C.-C.; Yang, M.H.; Wen, H.M.; and Chern, J.C. (2002) "Estimation of total flavonoid content in propolis by two complementary colometric methods," *Journal of Food and Drug Analysis*: Vol. 10(3).
12. Kumaran, A., & Karunakaran, R.J. (2006). Antioxidant and free radical scavenging activity of an aqueous extract of Coleus aromaticus. *Food Chemistry, 97*, 109-114.
13. Sreejayan, N., & Rao, M. N. (1996). Free radical scavenging activity of curcuminoids. *Arzneimittel-Forschung*, *46*(2), 169–171.
14. Kaneria, M.J., Bapodara, M.B., & Chanda, S.V. (2012). Effect of Extraction Techniques and Solvents on Antioxidant Activity of Pomegranate (Punica granatum L.) Leaf and Stem. *Food Analytical Methods*, *5*, 396-404.
15. A.O.A.C. (1990) Official Methods of Analysis. 15th Edition, Association of Official Analytical Chemist, Washington DC.
16. Van Soest, P.J. and Wine, R.H. (1967) Use of Detergents in the Analysis of Fibrous Feeds. IV. Determination of Plant Cell-Wall Constituents. Journal of the Association of Official Analytical Chemists, 50, 50-55.
17. Welti-Chanes, J., Serna-Saldívar, S. O., Campanella, O., & Tejada-Ortigoza, V. (Eds.). (2020). *Science and technology of fibers in food systems*. Springer Nature.
18. Alqah, H., Alamri, M. S., Mohamed, A. A., Hussain, S., Qasem, A. A., Ibraheem, M. A., & Ababtain, I. A. (2020). The effect of germinated sorghum extract on the pasting properties and swelling power of different annealed starches. Polymers, 12(7), 1602.
19. Reddy, J.P., & Rhim J.W(2018) Extraction and Characterization of Cellulose Microfibers from Agricultural Wastes of Onion and Garlic. Journal of Natural Fibers, 15(4), 464-473.
20. Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. *Journal of nutritional science*, *5*, e47. https://doi.org/10.1017/jns.2016.41

21. Aryal, S.; Baniya, M.K.; Danekhu, K.; Kunwar, P.; Gurung, R.; Koirala, N. Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. *Plants* **2019**, *8*, 96. https://doi.org/10.3390/plants8040096

22. Shen, Y., Hong, S., & Li, Y. (2022). Pea protein composition, functionality, modification, and food applications: A review. *Advances in food and nutrition research*, *101*, 71–127. https://doi.org/10.1016/bs.afnr.2022.02.002