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Comparative study on nutritional and bioactive composition of Pone-Yee-Gyi, Myanmar traditional food and its raw material, horse gram (*Macrotyloma uniflorum* L.)

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Abstract

One of the Myanmar geographical indication products, Pone-Yee-Gyi – paste and powder types, and their raw material (horse gram) were comparatively investigated in relation to the nutrient and bioactive contents, as well as the total antioxidant activity. The results showed that the paste product exhibited the highest amounts of phenolics, flavonoids and tannins compared to its raw material and the powder product. The methanolic extract of the paste product maintained the highest antioxidant activity as measured by ferric reducing antioxidant power (FRAP) assay and free radical scavenging activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method compared to horse gram and powder product. The thermal profile of horse gram was studied using the DSC technique. The presence of specific functional groups in the three investigated samples was studied using the FT-IR spectroscopy. The hereby found favouring nutritional and bioactive composition of Pone-Yee-Gyi paste product recommends it as good candidate for health promoting effects and for food ingredients production.

Keywords: Pone-Yee-Gyi, horse gram, antioxidant activity, FT-IR, DSC

1. Introduction

Pone-Yee-Gyi is one of the Myanmar traditional food products, manufactured from horse gram (*Macrotyloma uniflorum* L.) either in the form of paste or powder, respectively. Horse gram – also known as "Pe-bazat" Myanmar name – has been processed in these products in particular in dry and hot regions of Middle Myanmar, for instance Bagan region where the best Pone-Yee-Gyi product is produced. Quality, taste and flavour may vary in relation to geographical area, although same processing is applied. Most probably, the natural microbial flora influences the production of flavoured Pone-Yee-Gyi products through fermentation of horse gram even though it still needs more research to give accurate elucidation.

The main and only ingredient of Pone-Yee-Gyi is horse gram.

Horse gram is a food legume having ethno-medicinal values in Asian countries. It is consumed as whole seeds or used as raw material for food processing. It represents an underutilized legume with great potential for human nutrition, in particular for rural communities, being grown in temperate and sub-tropical regions (J.A.O. MAGBAGBEOLA & al. [1]).

Horse gram is considered a good source of proteins and some minerals, such as iron, molybdenum and calcium, but is low in fat and sodium content (A. BHARTYIA & al. [2]).

Some antinutritional compounds, for example enzyme inhibitors, phytic acid and tannins may restrict consumption, but these compounds, in particular those of polyphenolic structure (tannins), are currently considered as antioxidant compounds with potential health benefits (R. AMAROWICZ [3]). Other authors showed that tannins, in particular tannic acid, exhibit antimicrobial properties (S. MENTEŞ ÇOLAK & al. [4]). However, the content in antinutritional compounds might be reduced through processing technologies of horse gram (S.S. KADAM & al. [5]). Eight phenolic acids (*p*-coumeric, *p*-hydroxy benzoic, 3,4-dihydroxy benzoic, vanillic, caffeic, ferulic, syringic and sinapic acids) were identified in horse gram (S.M.A. KAWASAR & al. [6]). The ethanolic extract of horse gram seeds showed good antioxidant activity, as determined by nitric oxide radical scavenging assay, hydroxyl and phosphomolybdate methods (S. K. PRASAD & al. [7]).
Horse gram, in the form of various extracts, has been considered beneficial for several diseases, exhibiting antimicrobial, antihelmintic, anti-inflammatory, anti-hyperglycemic and anti-hypercholesterolemic properties (A.K. TIWARI & al. [8], A. BHARTYIA & al. [2]).
The aim of the present paper was to investigate the nutritional and phytochemical content of Myanmar horse gram and its two Pone-Yee-Gyi products, as well as the total antioxidant activity by three assays (total phenolics, DPPH free radical scavenging activity and FRAP ferric reducing antioxidant power) and thermal behaviour by differential scanning calorimetry (DSC). For the first time, the extracts of the two products were characterized through their bioactive content, which may become useful for further application of such products as ingredients in functional foods.

2. Materials and methods

Plant materials and reagents

Myanmar horse gram seeds and two commercial Pone-Yee-Gyi products ("Shwe-Myin-Pyan" paste and powder) were purchased from the local market of Myanmar. These commercial products are manufactured by naturally fermentation. The powder product is manufactured through further sun drying of the paste product.
Chemical reagents of analytical grade were used.

Nutrient composition

Nutrient content including crude protein, fat, starch, moisture and ash, was determined using standard methods (AOAC [9]). Crude protein (N x 6.25) was evaluated by Kjeldhal method. Lipids extracted with diethyl ether using the Soxhlet apparatus were determined as weight difference. Starch content was performed by Ewers' polarimetric method [10].

Extraction of bioactive compounds

All samples were initially defatted. The petroleum ether was used. A ratio of 1/10 (w/v) of sample/solvent was applied for defatting with occasional shaking for about 24 hours, at room temperature. After filtration, the residues were air-dried for about 24 hours. Extraction of phenolic compounds was further performed using 80% methanol for about 2 days at room temperature with occasional shaking.
The obtained methanolic extracts were analyzed for phenolic compounds content and for total antioxidant activities.

Assay of total phenolics

The total phenolics content of methanolic extracts were determined by the Folin-Ciocâlteu assay (V. L. SINGLETON & al. [11]) and expressed as mg gallic acid equivalents on dry mass basis (mg GAE $100g^{-1}$ DM).

Assay of total flavonoids

The total flavonoids content of methanolic extracts were determined by the colorimetric method (G.C. BAG & al. [12]) and expressed as mg quercetin equivalents on dry mass basis (mg quercetin $100g^{-1}$ DM).

Assays of total tannins

The condensed tannins content of methanolic extracts was determined by the colorimetric method (R.B. BROADHURST & AL. [13] and expressed as mg catechin equivalents on dry mass basis (mg catechin $100g^{-1}$DM).

Assays of total antioxidant activity

DPPH Free radical scavenging activity

The antioxidant activity of methanol extracts was determined by the free radical scavenging activity using DPPH method (W. BRAND-WILLIANS & al. [14]).

Ferric reducing antioxidant power (FRAP) assay

The antioxidant activity of methanolic extracts was determined by ferric reducing antioxidant power (FRAP) assay (I.F.F. BENZIE & al. [15]).

Differential scanning calorimetry (DSC)

For thermal analysis of horse gram, the SDT Q600 differential scanning calorimeter (TA Instruments) was used. The samples were prepared by mixing with distilled water in the ratio 1/2 sample/solvent (w/v), the mixture being let to stand for about 2 hours, as described by SÁNCHEZ-ARTEAGA et al. [16]. Between 6.8 and 8.1 mg of sample was weighted in Tzero aluminum pans and sealed. Sample was heated in the range of 40–100°C with a rate of 5°C/min, under nitrogen atmosphere (50 mL/min). The empty sealed Tzero aluminum pan was used as reference. The TA instruments universal analysis software (TA Instruments Inc., New Castle, USA) was used.

Fourier transform infrared (FT-IR) spectra

The presence of functional groups in the investigated samples (horse gram, paste and powder products) was investigated by Fourier transform infrared spectroscopy using the ALPHA FT-IR spectrometer (Bruker, Germany) with the combined QuickSnap™ sampling modules and ZnSe ATR (attenuated total reflection).

Statistical analysis

All measurements were performed in triplicate. The results were expressed as mean values. The correlation between variables was done by calculating the Pearson's correlation coefficient.

3. Results and discussions

Nutrient Composition

The nutrient composition of Myanmar horse gram and its Pone-Yee-Gyi paste and powder products is presented in Table 1.

Table 1. Nutrient composition of Myanmar horse gram and Pone-Yee-Gyi paste and powder products (mean values).

Constituents (%)	**Horse gram (raw material)**	**Paste Product**	**Powder Product**
Moisture	7.40	43.80	5.30
Ash*	3.77	12.92	4.41
Protein*	21.62	23.66	24.00
Fat*	0.37	3.84	3.23
Starch*	33.05	26.35	37.55

**Values expressed as a percentage of dry mass*

The nutritional value of the investigated horse gram was found similar to that described by other authors (S.K. PRASAD & al. [7], A. BHARTYIA & al. [2]).
To our knowledge there are no reported results on the nutrient composition of the paste and powder horse gram products obtained through natural fermentation processing. However, food processing caused an increase in the content of crude protein, fat and starch in both types of products compared to the raw material.
The nutritional values reported by different authors vary within quite large limits, depending on genetic and climatic factors. GEETHA & al. showed that the protein content varies between 18 and 29% in horse gram, which may explain the differences found for the present investigated products (K. GEETHA & al. [17]). KHETARPAUL and CHAUHAN have found that by fermenting pearl millet, the protein content either decreased or remained constant, while the fat content increased (N. KHETARPAUL & al. [18]). An increase in the fat content and ash in horse gram fermented with *Penicillium camemberti* was also found by other researchers (M. DWIVEDI & al. [19]).

Comparative studies on phenolics content in horse gram and its products
The content of total phenolics, flavonoids and tannins of methanolic extracts prepared from horse gram and Pone-Yee-Gyi products initially defatted are shown in Figure 1.

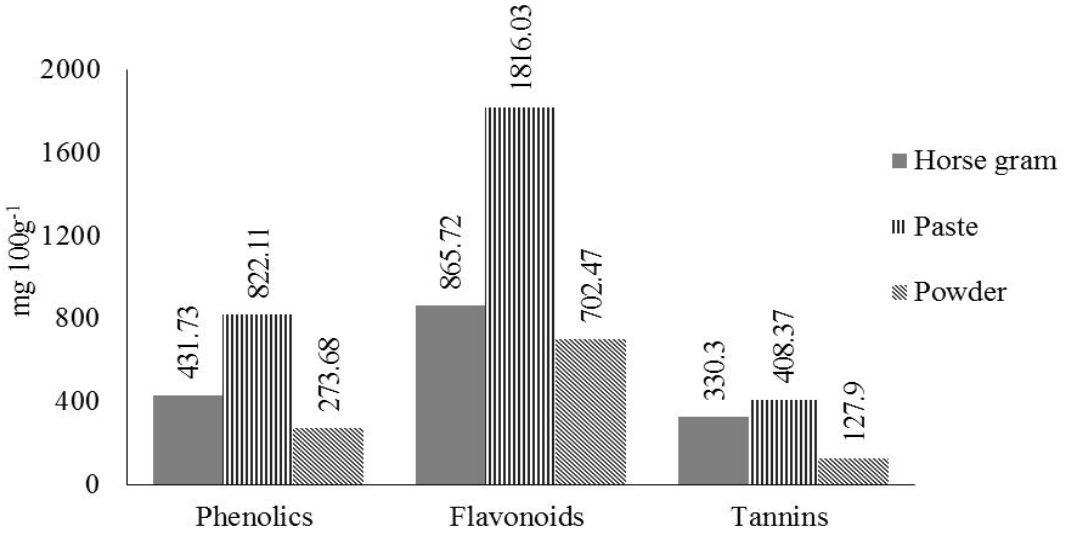


Figure 1. The content of total phenolics (mg GAE $100g^{-1}$ DM), flavonoids (mg quercetin $100g^{-1}$ DM) and tannins (mg catechin $100g^{-1}$ DM) in horse gram and its fermented products.

As observed in Figure 1, the content of bioactive compounds of polyphenolic structure increased during food processing in the paste product, compared to the raw material, and decreased in the powder product, probably due to the effect of sun drying involved in the powder product processing (effect of temperature and light). The increase in bioactives during processing might be due to the generation of new compounds through natural fermentation. However, the tannins content was found less than 5% in all the investigated samples. High concentration of tannins is considered to have antinutritional effects. Thus, it can be assumed that the flavour of the investigated products was not too much astringent.

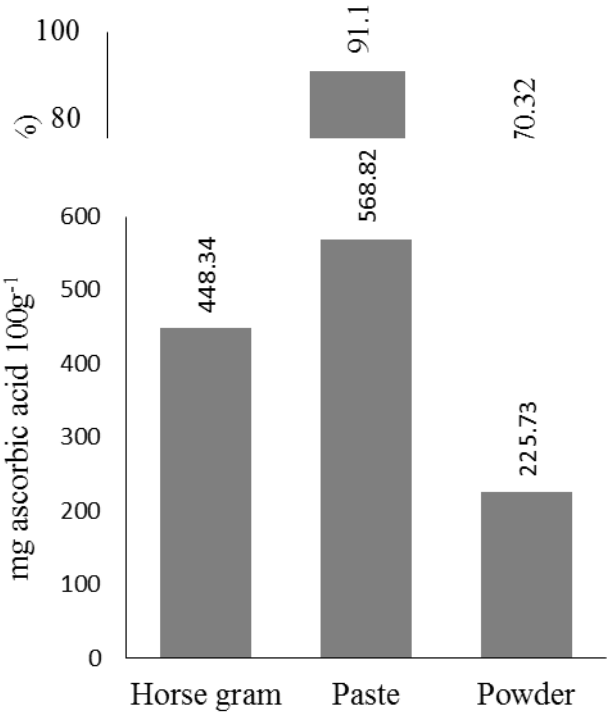
The content of extractable phenolics of the paste product was the highest (822.113 mg GAE $100g^{-1}$ DM) almost doubled than the raw material, while in the powder product it decreased by 66.6% compared to that of the paste product, and by 36.5% compared to the raw material.

Regarding the flavonoids, their content was the highest in the paste product (1816.026 mg quercetin $100g^{-1}$ DM), while in the powder product it decreased by 68.6% compared to that of the paste product, and by 61.2% compared to the raw material.

Based on significant differences linked to original region of samples, extraction solvent, reference compounds and analytical technique, it is difficult to have an accurate comparison of the results of bioactive compounds content. However, the results obtained by other authors (U.M.A. SUNDARAM & al. [20]) regarding the phenolics and flavonoids content of south Indian horse gram, showed higher amounts of phenolics (1670 mg GAE $100g^{-1}$), but lower amounts of flavonoids (38 mg quercetin $100g^{-1}$) compared to those of Myanmar horse gram. Regarding tannins, the results obtained by the mentioned authors were expressed as tannic acid equivalents (101 mg tannic acid 100 g^{-1}) while our results were of 303.3 mg of catechin equivalents 100 g^{-1} DM.

Comparative studies on antioxidant activity in horse gram and its products

Antioxidant activity of horse gram and its products was measured using three assays: total phenolics by Folin-Ciocâlteu, ferric reducing antioxidant power (FRAP) and free radical scavenging assay by 2,2-diphenyl-1-picrylhydrazyl (DPPH). The total phenolics content was already presented in the previous section.



PH of methanolic extracts.

t power (FRAP) of methanolic extracts.

ıd in the paste horse gram product, by st amounts of bioactive compounds cts from both types of products (91.10 higher than that of raw material (52.26 d Pone-Yee-Gyi extracts, expressed as ed that the paste product exhibited the orbic acid $100g^{-1}$ DM) than the raw

ı and its two Pone-Yee-Gyi products ant activity were calculated and are

nvestigating samples based on the bioactives (phenolics, flavonoids, tannins) and antioxidant activity.

Pearson's correlation coefficient	Horse gram	Paste products	Powder products
Horse gram	1		
Paste products	0.967716	1	
Powder products	0.950556	0.992466	1

The results indicate that technological processes involved in horse gram products similarly influences the recovery of bioactive compounds of phenolic structure and antioxidant activity. When considering the content in bioactive compounds for calculation of the Pearson's correlation coefficients, there was a high positive correlation between Pone-Yee-Gyi paste and powder products, all values being above 0.99. Good positive correlation was found between bioactives (phenolics, flavonoids, tannins) and antioxidant activity by FRAP. DPPH mainly correlate with flavonoids compared to the other bioactives.

Differential scanning calorimetry (DSC)

There are several studies regarding the thermal analysis used to highlight the protein denaturation and starch gelatinization in flour samples obtained from various sources. No separation of proteins and starch has been done, as sample preparation may influence the obtained values.

The thermal denaturation of Myanmar horse gram was studied by DSC. The thermogram is presented in Figure 4.

Figure 4. DSC thermogram of horse gram.

The thermogram showed two endothermic peaks. The onset temperature of the first endothermic curve was 45.22°C, while the peak temperature was 52.15 °C. This is probably due to the denaturation of protein molecules in the sample. The other endothermic curve with the onset temperature of 75.16°C and the peak temperature of 82.34°C was assumed to be due to the starch gelatinization. SÁNCHEZ-ARTEAGA & al. have obtained similar values for the gelatinization of starch from common beans (H.M. SANCHEZ-ARTEAGA & al.[16]).
Regarding the DSC analysis of the horse gram products (paste and powder), no exothermic or endothermic reactions were detected, probably due to the denaturation of biomolecules during food processing at high temperature.

FT-IR spectroscopy

The FT-IR spectra of solid samples of horse gram and Pone-Yee-Gyi products showed the presence of various functional groups as described in Table 3 and Figures 5-7. The interpretation of the obtained FT-IR spectra was realized by comparison with literature (J. COATES [21]).

Table 3. FT-IR spectra of raw material horse gram and its two Pone-Yee-Gyi (paste and powder) products.

Absorption range (cm^{-1})			**Types of Vibration**	**Functional groups**
Horse gram	*Paste*	*Powder*		
3500 - 4000	-	3500 - 4000	N-H stretch O-H stretch	Amines

3274.54	3348.98	3303.87 3273.82	N-H stretch O-H stretch (Phenolic OH band with broad peak)	Amides Alcohol
2929.37	2933.52	2911.13	C-H Stretch (asymmetric / symmetric)	Alkyl Alkene
2360.44 2341.47	-	-	O=C=O stretch (strong peak)	Carbon dioxide
1647.6	1632.77	1645.06	C = O stretch N-H band C=C stretch	Amide Amines Alkenyl
1652.05 – 1507.66	1451.91 1404.87	1532.98 1441.81	C=C-C stretch (symmetric / asymmetric)	Aromatic rings
1318.10 – 1006.58	1404.87 1145.99	1399.38 1318.55 1235.80 1149.77	C-N stretch C-O	Aromatic amino Ether Sulfur-oxy compounds
	1076.82 1027.60	1076.36 1025.17	C-O	Ether

The band shape of FT-IR spectra of horse gram was significantly different compared to that of paste and powder products, while band shapes of the products spectra were similar, although with some lack of peaks in case of paste. A number of sharp peaks in horse gram spectra in the region between 3853.23 cm^{-1} and 3566.49 cm^{-1} was absent in the paste spectra, but present as small absorption bands in product spectra. A string and wide stretching peak around 3348.98 cm^{1} in paste spectra that was assumed to phenolic -OH groups, was also found in product spectra as the band with two peaks at 3303.87 cm^{-1} and 3273.82 cm^{-1}, while in the horse gram it was shifted to a weak broad band with many small absorption peaks centered at 3274.54 cm^{-1}. Besides, a relatively strong absorption peak in powder product spectra at 1645.06 cm^{-1} that was supposed to be amide (C=O) stretching, was observed in the paste spectra as a medium broad peak at 1632.77 cm^{-1}, while in the horse gram spectra, a very sharp medium peak at 1647.60 cm^{-1} was found.

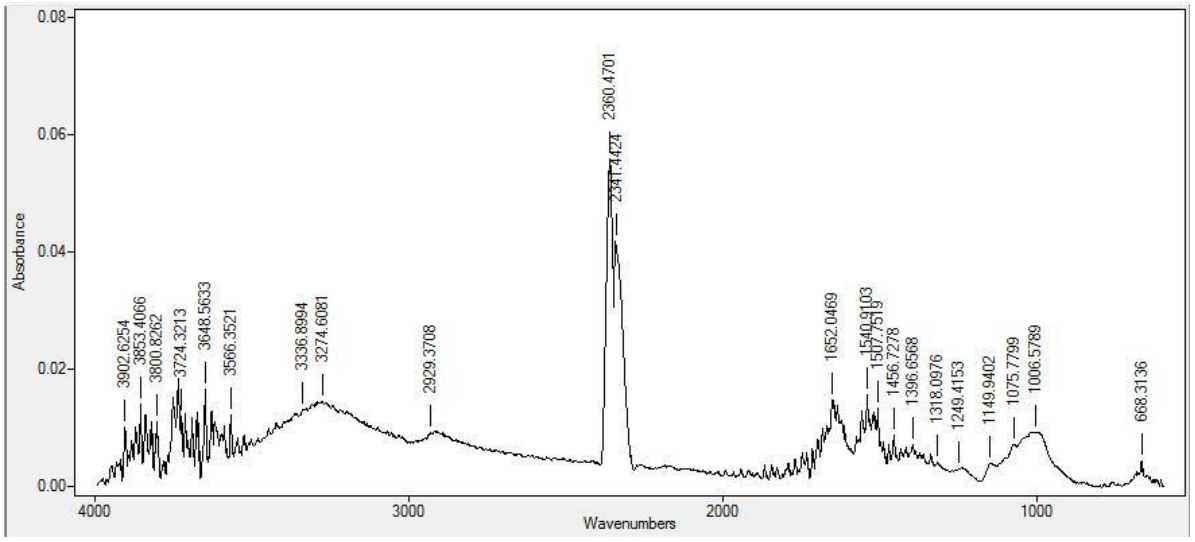


Figure 5. FT-IR spectra of horse gram.

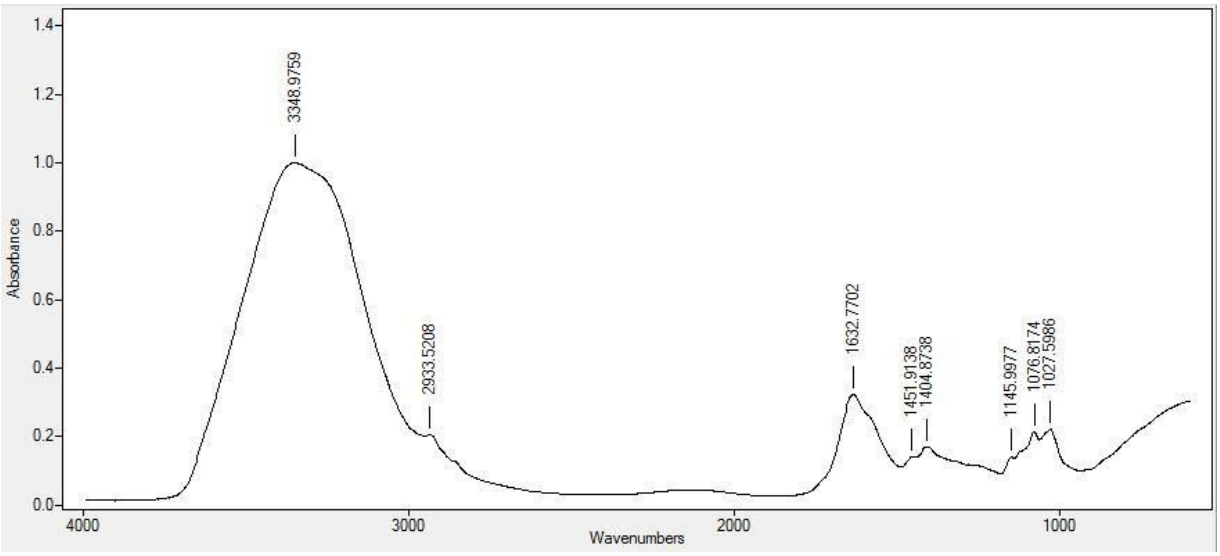


Figure 6. FT-IR spectra of paste horse gram product.

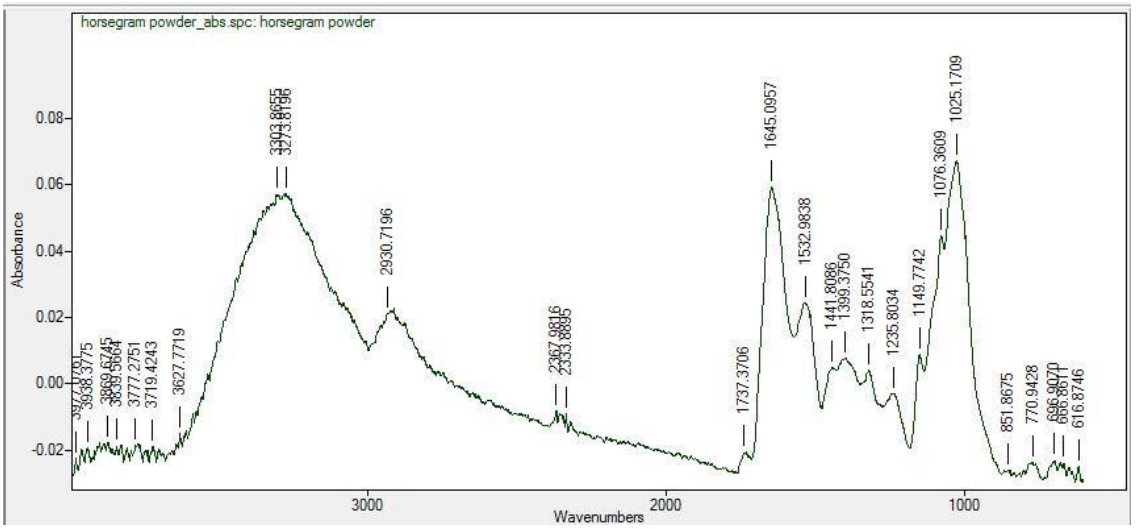


Figure 7. FT-IR spectra of powder horse gram product.

4. Conclusions

The nutrient content of Myanmar horse gram and its Pone-Yee-Gyi paste and powder products was not significantly different.

The phenolics, flavonoids and tannins content determined by UV-Vis spectrophotometric methods was higher in the paste product compared to the powder product and raw material (horse gram seeds).

The antioxidant activity as measured by FRAP assay was almost two-fold higher in the paste product compared to horse gram. The free radical scavenging activity by DPPH assay of paste product was found higher than that of horse gram, while the inhibition was lower in the powder product, probably related to the decrease caused by drastic processing conditions.

The results on FT-IR spectroscopy applied to horse gram and its products showed that the technique may become useful to fingerprint such products.

The DSC analysis highlighted that the main nutrients (proteins and starch) are denatured during hydrothermal treatment, in both paste and powder products.

The hereby found favouring nutritional and bioactive composition of Pone-Yee-Gyi paste product makes it a good candidate for health promoting effects.

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References

1. J. A. O. MAGBAGBEOLA, J. A. ADETOSO, O. A. OWOLABI, Neglected and Underutilized Species (NUS): A Panacea for Community Focused Development to Poverty Alleviation/Poverty Reduction in Nigeria. J. Econ. Int. Finance, 2(10), 208-211 (2010).
2. A. BHARTYIA, J. P. ADITYA and L. KANT, Nutritional and Remedial Potential of An Underutilized Food Legume Horse Gram (*Macrotyloma uniflorum*): A REVIEW, J. Anim. Plant Sci., 25(4), 908-920 (2015).
3. R. AMAROWICZ, Tannins: The New Natural Antioxidants?, Eur. J. Lipid Sci. Technol., 109, 549-551 (2007).
4. S. MENTEŞ ÇOLAK, B. MERİÇLİ YAPICI, A. NAİL YAPICI, Determination of Antimicrobial Activity of Tannic Acid in Pickling Process, Rom. Biotechnol. Lett., 15(3), 5325-5330 (2010).
5. S. S. KADAM, D.K. SALUNKHE AND J.A. MAGA, Nutritional Composition, Processing, and Utilization of Horse Gram and Moth Bean. Crit Rev. Food Sci. Nutr., 22, 1–26. (1985).
6. S.M.A. KAWASAR, E. HUQ, N. NAHAR, Y. OZEKI, Identification and Quantification of Phenolic Acids in *Macrotyloma uniflorum* by Reversed Phase HPLC. Am. J. Plant Physiol., 3(4) 165-172 (2008).
7. S. K. PRASAD, M. K. SINGH, Horse Gram- An Underutilized Nutraceutical Pulse Crop: A Review, J Food Sci Technol, 52(5), 2489–2499, (2015).
8. A. K. TIWARI, K. MANASA, D. A. KUMAR, A. ZEHRA, Raw horse gram seeds possess more in vitro antihyperglycaemic activities and antioxidant properties than their sprouts, Nutrafoods, 12, 47 (2013).
9. Association of Official Analytical Chemists. (1990). Official Methods of Analysis, 15th ed.; AOAC: Washington, DC.
10. Native Starch – Determination of Starch Content – Ewers Polarimetric Method, International Standard, ISO 10520:1997(E).
11. V. L. SINGLETON, J. A. J. ROSSI, Colorymetry of Total Phenolics with Phosphomolybdic-phosphotungstic Acid Reagents, Am. J. Enol. Vitic., 16, 144−158 (1965).
12. G. C. BAG, P. G. DEVI, TH. BHAIGYABATI, Assessment of Total Flavonoid Content and Antioxidant Activity of Methanolic Rhizome Extract of Three Hedychium Species of Manipur Valley, Int. J. Pharm. Sci. Rev. Res., 30(1), 154-159 (2015).

13. R.B. BROADHURST, W.T. JONES, Analysis of Condensed Tannins Using Acidified Vanillin. J. Sci. Food Agr., 48(3), 788–794 (1978).
14. W. BRAND-WILLIANS, M. E CUVELIER, C. BERSET, Use of Free Radical Method to Evaluate Antioxidant Activity. Lebensm.-Wiss. u.-Technol., 28, 25-30 (1995).
15. I. F. F. BENZIE, J. J. STRAIN, The Ferric Reducing Ability of Plasma (FRAP) as A Measure of Antioxidant Power – The FRAP Assay, Anal. Biochem., 239, 70–76 (1996).
16. H. M. SANCHEZ-ARTEAGA, J. E. URIAS-SILVAS, H. EAPINOSA-ANDREWS, E. GARCIA-MARQUEZ, Effect of Chemical Composition and Thermal Properties on the Cooking Quality of Common Beans (*Phaseolus vulgaris*), CyTA-J. Food, 13(3), 385-391 (2015).
17. K. GEETHA, A. K. MANI, M. J. HEPZIBA, R. LATHA, P. SHANTHI, Studies on Genetic Diversity among Germplasm Accessions of Horsegram *Macrotyloma Uniflorum* (Lam) *Verde*., Legume Res., 34(1), 14 – 19 (2011).
18. N. KHETARPAUL, B. M. CHAUHAN, Effect of Fermentation on Protein, Fat, Minerals and Thiamine Content of Pearl Millet, Plant Food Hum. Nutr., 39, 169, (1989).
19. M. DWIVEDI, K.Y. VASANTHA, Y.N. SREERAMA, d. J. HAWARE, R. P. SINGH, A .P. SATTUR, Kaulath, A New Fungal Fermented Food from Horse Gram, J. Food Sci. Technol., 52, 8371 (2015).
20. U. M. A. SUNDARAM, M. MARIMUTHU, V. ANUPAMA, P. GURUMOORTHI, Comparative Antioxidant Quality Evaluation of Underutilized/Less Common South Indian Legumes, Int. J. Pharm. Bio. Sci., 4(2), (B) 117 – 126 (2013).
21. J. COATES, Interpretation of Infrared Spectra, A Practical Approach, in Encyclopedia of Analytical Chemistry, R.A. Meyers (Ed.), John Wiley & Sons Ltd, Chichester, 2000, pp. 10815–10837.