International Journal of Agriculture and Animal Production
ISSN: 2799-0907
Vol: 04, No. 05, Aug-Sept 2024
http://journal.hmjournals.com/index.php/IJAAP
DOI: https://doi.org/10.55529/ijaap.45.1.8

Chemical Profile and Molecular Structure of Proteins in Air Dried and Ensiled Watermelon Rind (Citrullus Lanatus)

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Received: 29 March 2024 **Accepted:** 17 June 2024 **Published:** 01 August 2024

Abstract: ***The structure of protein in feedstuffs determines their quality and rate of responsiveness to digestible enzymes and utilization. This study was to investigate the magnitude of the differences in protein structure at the molecular level as well as the nutritive value of dried (control) and ensiled watermelon rind for use as protein source in livestock diets. Protein chemical profile and both primary and secondary molecular structure of dried (control) and ensiled watermelon rind samples were determined by standard procedures of AOAC and FTIR molecular spectroscopy, respectively. The results showed significant (P<0.05) differences in chemical profile parameters of crude protein and crude fibre as ensiled samples recorded higher crude protein (13.8%) than control (6.72%). Dry matter content however, recorded similar values. Results from protein molecular structure, showed that ensiling significantly (P<0.05) increased the spectral intensity of primary protein features of amide I area, amide II area, amide I height, amide II height and Protein secondary structure of α-helix and β-sheet height compared to control. The ratio of α-helix to β-sheet height decreased significantly (P<0.05) when compared to the control. Vibrational molecular spectroscopy could therefore, be used to detect inherent structural protein make-up characteristics of feedstuffs toward predicting protein quality of feeds.***

Keywords: Molecular Spectral, Protein, Nutrient Variation, Polypeptide, Chemical Profile.

1. INTRODUCTION

The cost of most conventional feedstuffs, which are major sources of energy and protein in livestock diets, have continued to rise due to their short supply [1]. This has resulted in the high cost of production of livestock and feeds [2]. There is therefore, the need to continue to source for alternative feedstuffs that are not likely to face competition and demand as the conventional

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feedstuffs. Such feedstuff should be able to supply similar nutrients, more cost effective and have limited domestic and industrial use yet nutritionally balanced [2, 3]. Utilizing agro-industrial by-products and domestic wastes have been proposed [3, 4, 5] to have ability to counter increase in prices of livestock production as a result of feed cost. Evidence of the use of agro-industrial by-products as replacement for conventional feedstuff exist in literature [7, 8, 9].

One of such readily available agro-industrial is watermelon rind. According to Gladvin et al. [11], watermelon rind is an excellent source of amino acids, carbohydrate, micro minerals, vitamins and bioactive that serves as antioxidants. It contains 8 % Crude protein, 6% Crude fibre, 63% nitrogen free extract, 4% ash and 5% Ether extract [9, 10]. Like most unconventional feedstuffs, watermelon rind is high in fibre content and in citrulline, an anti-nutritional factor that inhibit the release and availability of protein [10]. These restrict its use in diets of Monogastric animals [5, 10]. Processing through steaming, toasting, sun drying and ensiling has been reported to denature these compounds [2]. Agbana et al.[3] reported a significant reduction in values of crude fibre and citrulline content of watermelon rind subjected to ensiling process.

The use of unconventional feedstuff however, should be backed up by assessment of the nutrient composition [11, 13]. The readily available and fast means of determining this is by proximate composition [13, 14] or feed evaluation [15]. Wet chemistry analysis mostly employed in feed evaluation, alters the inherent structure of feed samples by changing the nutritive value, conformation, and molecular structure make-up of feed [13]. Proximate composition also, may not really give the true value of available nutrients as it rely only on estimations [2, 11, 13]. Vibrational spectroscope is not only capable of detecting molecular structure of materials alone, but, also reveal the molecule structural changes in different feed type. It can further be harnessed for studying the effect of feed processing on protein and carbohydrate related molecular structure [14, 15].

2. RELATED WORKS

Several studies have been documented in literatures on the chemical, nutrient profile, protein molecular structure and protein value of major feed stocks employing Fourier transform infrared spectroscopy (FTIS) technique [13], [14], [15]. Similarly, Yu [23] proposed the use of synchrotron techniques as better way for analyzing inherent structure of biological tissues. This method examined scanned picture of changes of protein structure in the intrinsic protein structures of alpha (α)-helix to beta (β)-sheet intensity and their ratio in samples. Ismael et al. [14] reported a spectra region of ca. 400 -800 cm $^{-1}$ and protein structure baseline regions (ca. 1,715 – 1,480 cm $^{-1}$) for soybean seed. [23] evaluated the spectral region of hulless barley as ca. 355 – 890 cm $^{-1}$. Furthermore, Theodoridou and Yu [21] also reported varying protein secondary structure in Canola seeds.

Though, [12], [13], [16], and [18] determined the chemical components and their variations in most non-conventional feedstuffs, for use in Monogastric animal production. The detailed

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study of the differences in processing methods of watermelon rinds on protein structure is rare. This study therefore, divulged and compared the protein profile and protein molecular structure (α-helix to β-sheet intensity, their ratio, amide I and amide II intensity and their ratio) of air dried and ensiled watermelon rind from Fourier transform infrared spectrometry (FTIR).

3. MATERIALS AND METHODS

Study Area

Chemical profiling of air dried and ensiled watermelon rind samples was performed at the Biochemical laboratory, Department of Animal Health and Production Technology, Kogi State Polytechnic, Itakpe Campus. Itakpe lies on Latitude 7.6384 ^{0} N and Longitude 6.335 ^{0} E within the Guinea Savanna and has a total land mass of 32 km^2 [map-satellite image of Itakpe. https//www.mapland.cpm/Nigeria/Kogi/okehi/itakpe, accessed on 28/02/2024]. The protein molecular structure was determined at the biomolecular spectroscopy laboratory, Forte Hare University, Alice, South Africa.

Sample Collections and Processing.

Watermelon rinds were collected from a fruit vendor at Abobo, Okehi Local Government Area, Kogi State. The rinds were sorted, washed and sterilized in warm water to remove suspected bacteria and afterward, the rinds were chopped into tiny cubes and divided into two portions. The first portions were air dried under shade (unprocessed) and the other portion was kept in an air tight black polyethene bag to ferment for 3 days and afterward air dried. The dried samples were grounded in a 0.42 mm screen hammer mill. Then, bagged separately for chemical profiling in triplicates. Samples for molecular spectroscopy test were crushed in a Wiley mill of 2.5 mm screen.

Chemical Profiling

The samples were analyzed for dry matter (DM), crude protein (CP), ash, ether extract (EE) according to AOAC methods [6]. The total carbohydrate / NFE content was estimated as 100-EE-CP- Ash according to standard procedure [12].

Protein Molecular Spectra Profile and Analysis.

The protein molecular spectral features for air dried and ensiled watermelon rind samples were collected using mid infrared microspectroscopy (Lambda FTIR-7500) following standard procedures. Spectra were generated randomly in transmission mode at mid-IR (5500 - 850 cm $^{-1}$) portion of the electromagnetic spectrum by scans and spectral resolution of 4 cm $^{-1}$. Detected spectral for protein secondary structures was then analyzed with an OMNIC 8.5 (Thermo-N, Madison, USA) software.

Statistical Analysis

All values obtained from chemical analysis and molecular structure spectral parameters were analyzed using the one way ANOVA procedure of SPSS and Significant means were compared and separated using the Duncan Multiple Range test (DMRT) as outlined by Steel and Torrie [20].

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4. RESULTS AND DISCUSSION

Results

Chemical Profile of Unprocessed and Ensiled Watermelon Rind.

Table 1 shows the result of the chemical composition of dried (Control) and ensiled watermelon rinds. Ensiling recorded a significant (P<0.05) improvement in Crude protein content of watermelon rind as it increased the crude protein content to 13.82 % compared to Control (6.72 %), respectively. Similarly, ensiling technique (6.97%) caused a significant (P<0.05) increase in ash content compared to 4.81% recorded for dried (Control). Ensiling however, has no statistical (P > 0.05) effect on ether extract and Nitrogen free extract contents. Crude fibre content decreased considerably (P< 0.05) following processing and was least in ensiling (2.78).

Table 1: Chemical Composition of Un-processed and Ensiled watermelon rinds.

Parameters (%)	Control	EWMR	SEM	Level of Signif.
Organic matter	88.70	88.83	0.52	NS
Crude protein	6.75^{b}	13.87^{a}	0.50	*
Crude fibre	5.37^{b}	2.75^{a}	0.20	*
Ash content	4.81^{b}	6.95^{a}	0.02	*
Ether extract	9.95	10.05	15.0	NS
NFE	70.00	69.83	0.15	NS

^{a,b,c} Mean values on the Same row with different superscript are significantly (P<0.05) different.
*= Significant, NS- non Significant, SEM-standard error of mean
Control-Air Dried Watermelon rind; EWMR-Ensiled Watermelon Rind; NFE- Nitrogen free extract

Changes in Protein Molecular Structure Spectral Features

Table 2 reveals the results for the effect of processing on the protein molecular structure spectra characteristics of watermelon rind samples. All parameters of primary protein structures were significantly different (P<0.05) among treatments and better in ensiled samples except, amide I to amide II height ratio that recorded similar influence. Amide I area, amide I peak height, amide II area, Amide II peak height, alpha-helix and beta-sheet heights in the control (dried watermelon rind meal) were significantly lower compared to ensiled watermelon rind meal (EWMR). Alpha (α) -helix to beta (β) - sheet height ratio in ensiled watermelon rind (EWMR) were significantly (P<0.05) lower compared to the control (air dried).

Table 2: Effect of processing on protein molecular Structure spectral features of watermelon rinds.

Parameters	Control	EWMR	SEM	P-Value
Primary structure				
Amide I area	3.30	4.35	0.042	0.010
Amide II area	1.80	3.14	0.041	0.010

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Amide I Peak Height	0.03	0.05	0.091	0.010
Amide II peak height	1.04	1.06	0.020	0.010
Area ratio Amide I:II	2.42	2.56	0.080	0.020
Height ratio I:II	1.70	1.52	0.021	0.200
Secondary structure				
α-helix height	0.06	0.04	0.001	0.100
β-sheet height	0.02	0.04	0.001	0.100
Ratio: α/β	1.20	0.97	0.003	0.100

Control-air dried watermelon rind
EWMR-ensiled watermelon rind

Discussion

Chemical Profiles of Processed Watermelon Rinds.

The significant increase in crude protein content of ensiled watermelon rind could be attributed to the activities of extracellular enzymes (protein) secreted by fermenting organisms (fungi) during their metabolic activities on the rinds whose proliferation, according to Akindahusi [5] form a complex protein that contributes to the nutrient content and protein value of the mash. This corroborates the finding of Erukainure et al.[10] who reported an increase in Crude protein content of watermelon rinds subjected to Saccharomyces solid media fermentation. The observed crude protein contents of ensiled watermelon rinds compared with major conventional protein sources like Cowpea (12.97 %), Groundnut cake (14.62 %) and Soya bean (13.80 %). Hence, it may serve as protein substitute in diets of livestock. Similarly, a surge in ash content of ensiled watermelon rinds over dried (Control) could suggest an increase in mineral contents of samples possibly as a result of the enrichment of the watermelon rinds mash by minerals present in the fruiting bodies of Fungi grown on the fermenting substrate. The result recorded in this sudy was similar to that reported by Bentil et al. [7] who documented a richer supply of minerals up to 10 % ash on dry matter basis for fermented mash of cocoa seed shell. This contradicts the result of Gladvin et al. [11], who observed no significant difference in ash content of watermelon rinds subjected to certain processing methods. The difference in results could be as a result of differences in cultivar of watermelon used for study or climatic conditions, variations in edaphic factors, harvesting stage of plant and laboratory analysis.

The observed similarity in values of Ether extract among treatments implies that the specie of Watermelon rind employed in this experiment is rich in fat. However, the recorded values are higher than the range recorded for most conventional protein sources such as Ground nut cake (3.63 %), Soybean (4.50%) and Cowpea (8.45%). Thus, watermelon rind could be examined for use as oil source. The significant reduction in Crude fibre content following ensiling may possibly suggest microbial enzymes degradation of complex polysaccharides in watermelon rind meal during fermentation process. This result is in consonance with the report of Yissa et al. [22] who opined that processing reduces crude fibre values in Legumes. The crude fibre values obtained in this study however, are lower compared to those recorded for conventional fruits like tomatoes (5.90 %) and jack fruit - 7.01 % [8, 23]. The recorded lower crude fibre

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value for watermelon peel meal therefore, could be advantageous to monogastric animals that have little ability to digest fibre.

Protein Molecular Structure Spectral Characteristics of Processed Watermelon Samples. The observed differences in protein molecular structure of dried and ensiled watermelon rind revealed variations in the sequence of amino acids in polypeptide chains and in the bonding of peptides to other nutrients in the samples to atoms that binds up molecules in plant cells [21]. The nature of bonding determines how weakened or strongly packed the nutrients are in the lattice [22]. Investigations revealed that amide I region (1600 - 1700 cm $^{-1}$) resulted from C=O stretching vibration and C-N stretching vibration of polypeptide bond which is directly related to or a determinant in the backbone conformation and hydrogen bonding pattern of sequence of peptides. Similarly, the region of amide II (1510 - 1580 cm $^{-1}$) has been opined [13] to be associated with N-H in-plane bending vibration and either or both C-N and C-C stretching vibration could determine the conformational nature of side chains of peptide and Hydrogen bonding [23]. This important information on primary structure (amide I, II) bands are requisite to evaluate protein concentration, protein conformation and quality in feedstuffs [16]. Differences in protein molecular structure therefore, can assist animal Nutritionist to predict protein degradation and digestion levels that may influence protein solubility, accessibility to microbes, proteolytic enzymes and livestock.

The recorded values for spectral features of amide I area and amide II area in this study inferred sensitivity to changes in blends, as it increased for ensiled watermelon rind meal further suggesting, self-structural stabilizing element of peptide bonds and sequence of non-polar amino acids in ensiled treated watermelon rind over the control [21]. The difference may also reveals variations in in-vivo biological tissues, better protein conformation and suggest high crude protein molecular values attributed to fermentation [18, 20]. The results was in accord with the findings from Wet chemistry analysis [16]. The ensiled watermelon rind meal that recorded significant increase in heights of amide I and amide II compared to the dried watermelon rind meal (control) indicates better protein molecular structure stability [14,15], as the peptide bond and weak hydrogen bonding could not be destroyed by enzymatic action of fermenting microbes and Fungi. This result corroborates the observation of Jackson et al. [15].

The differentials values obtained for ratio of amide I to amide II confirmed variations in influence of fermentation of feed [18]. Previous studies also asserted that amide I and II ratio had a positive correlation with metabolizable protein [13] as such, based on our results, the high amide I to II ratio of ensiled watermelon rind blend could be a consequence of the high levels of protein in ensiled watermelon rind blend. This was in agreement with the findings of Liu et al.[16] who reported a higher digestible protein content in co-product of carina meal (3.2 % CP).

The observed values for β (beta)-sheet and α (alpha)-helix (secondary structures) and their ratio suggest information about structural protein molecular makeup of feedstuff. Thus, the ensiled watermelon rind samples showed changes in secondary molecular protein structures

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compared to the dried blend. The alteration in the β-sheet and α-helix by ensiling therefore, are possibly related to the denaturation of β-sheet and α-helix during fermentation [13, 16]. The current results showed that the ratio of α-helix to β-sheet decreased following ensiling, which could reflect an increase in metabolizable protein supply in ensiled blend. The result was similar to that reported by Guevava et al.[13] who found out that metabolizable protein supply reduced with decreasing inclusion level of co-products of canola meal in bio-fuel processing.

5. CONCLUSION

Our investigations revealed that ensiling process (microbial fermentation) has the greatest effect in reducing crude fibre content and enhancing Crude protein content of watermelon rinds. Similarly, the highest values observed for self-stability in peptide bond and conformation in amino acid sequence in both primary or internal structural construction and secondary protein molecular structures for ensiled watermelon rind could suggest better protein characteristics, quality and nutritive value over dried samples. This could be employed by Animal nutritionist in providing cheaper protein feed stuffs for livestock.

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