Comparative analysis of the physicochemical characteristics of kafirin proteins extracted from various grain sorghums and their distillers' grains

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INTRODUCTION

- Sorghum is a globally significant cereal crop with unique agronomic traits and potential as a source of innovative plant proteins. The protein content of sorghum varies around 6-18%, with an average of 11%. The primary storage protein in grain sorghum is kafirin.
- In U.S., sorghum is mainly used for animal feed and ethanol production. Distillers' grains (DGs) are the major co-product after ethanol fermentation. There is a need for value-added uses of the DGs.

OBJECTIVES

- Extract kafirin proteins from different types of sorghums, as well as their dried distillers' grains (DGs).
- Analyze the physicochemical and functional properties of kafirin proteins in comparison with commercial plant proteins (SPI, PPI, gluten).
- Evaluate effect of sorghum type and fermentation on protein properties.

MATERIALS AND METHODS

Materials

Varieties	Color
Red-NLM-20	Red
Red-Waxy	Red
Red-NLM-SB	Red
White-F1000	White
White-32020	White
White-4525	White
Black-NLM-16	Black

Sorghum DGs & flours → Extract proteins using glacial acetic acid → Defat the protein powders with hexane → Sorghum kafirin proteins → Evaluation

Physicochemical properties

- Protein content
- FTIR and secondary structure
- Surface hydrophobicity
- Protein *in vitro* digestibility
- SDS-PAGE
- Total tannin content (TTC)

Functional properties

- Oil/water holding capacity (OHC&WHC)
- Solubility
- Emulsion capacity and stability (EAI&ESI)

RESULTS

Protein content and color test analysis

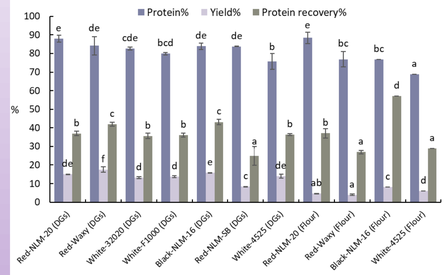


Figure 1. Protein content

Table 1. Protein color test

Sample	L*	a*	b*
Red-NLM-20 (DGs)	79.93 ± 0.19^{ef}	4.50 ± 0.08^{i}	12.84 ± 0.18^{h}
Red-Waxy (DGs)	78.61 ± 0.09^{d}	4.89 ± 0.02^{k}	11.69 ± 0.04^{d}
White-32020 (DGs)	83.94 ± 0.33^{j}	1.81 ± 0.01^{d}	11.93 ± 0.08^{e}
White-F1000 (DGs)	85.73 ± 0.16^{k}	1.57 ± 0.03^{b}	12.81 ± 0.14^{h}
Black-NLM-16 (DGs)	75.46 ± 0.17^{b}	6.27 ± 0.03^{m}	10.59 ± 0.02^{b}
Red-NLM-SB (DGs)	79.98 ± 0.05^{f}	3.96 ± 0.02^{j}	12.15 ± 0.07^{f}
White-4525 (DGs)	85.56 ± 0.04^{k}	1.67 ± 0.05^{c}	12.64 ± 0.09^{g}
Red-NLM-20 (Flour)	80.55 ± 0.06^{g}	3.71 ± 0.04^{h}	11.53 ± 0.07^{c}
Red-Waxy (Flour)	79.73 ± 0.05^{e}	3.55 ± 0.02^{g}	9.84 ± 0.06^{a}
Black-NLM-16 (Flour)	71.74 ± 0.07^{a}	5.88 ± 0.02^{l}	9.75 ± 0.04^{a}
White-4525 (Flour)	85.77 ± 0.12^{k}	1.29 ± 0.02^{a}	11.58 ± 0.02^{cd}
PPI	77.65 ± 0.02^{c}	4.91 ± 0.01^{k}	20.89 ± 0.05^{k}
SPI	82.85 ± 0.03^{i}	1.97 ± 0.01^{e}	17.59 ± 0.11^{j}
Gluten	81.87 ± 0.06^{h}	2.20 ± 0.02^{f}	16.27 ± 0.02^{i}

Secondary structure

Table 2. Secondary structure composition

Sample	β-Sheet (%)	β-Turn (%)	α-Helix (%)	Random coil (%)
Red-NLM-20 (DGs)	26.86 ± 1.89^{de}	19.53 ± 9.12^{cd}	53.59 ± 7.23^{d}	0 ± 0^{a}
Red-Waxy (DGs)	18.99 ± 1.68^{bc}	10.02 ± 1.19^{ab}	71.00 ± 2.87^{g}	0 ± 0^{a}
White-32020 (DGs)	21.26 ± 6.56^{cd}	20.79 ± 7.71^{cd}	57.95 ± 1.15^{de}	0 ± 0^{a}
White-F1000 (DGs)	14.83 ± 2.43^{abc}	24.86 ± 4.40^{de}	60.31 ± 6.82^{def}	0 ± 0^{a}
Black-NLM-16 (DGs)	11.67 ± 1.49^{ab}	6.27 ± 0.43^{a}	82.06 ± 1.92^{h}	0 ± 0^{a}
Red-NLM-SB (DGs)	22.01 ± 1.19^{cde}	10.45 ± 0.74^{ab}	67.54 ± 0.45^{fg}	0 ± 0^{a}
White-4525 (DGs)	28.84 ± 0.14^{e}	15.86 ± 1.76^{bc}	55.30 ± 1.90^{d}	0 ± 0^{a}
Red-NLM-20 (Flour)	19.07 ± 2.15^{bc}	9.99 ± 1.42^{ab}	37.69 ± 5.06^{c}	33.25 ± 1.49^{b}
Red-Waxy (Flour)	17.95 ± 5.41^{bc}	9.80 ± 0.03^{ab}	72.25 ± 5.38^{g}	0 ± 0^{a}
Black-NLM-16 (Flour)	12.97 ± 0.65^{ab}	20.83 ± 2.37^{cd}	66.20 ± 3.02^{efg}	0 ± 0^{a}
White-4525 (Flour)	9.38 ± 0.41^{a}	31.84 ± 0.57^{e}	58.77 ± 0.16^{def}	0 ± 0^{a}
PPI	53.88 ± 4.74^{f}	5.88 ± 0.28^{a}	11.13 ± 0.70^{a}	29.11 ± 3.77^{b}
SPI	17.92 ± 4.19^{bc}	7.70 ± 4.04^{ab}	24.64 ± 2.94^{b}	49.73 ± 19.11^{c}
Gluten	27.66 ± 1.89^{de}	5.12 ± 0.29^{a}	14.79 ± 5.48^{a}	52.44 ± 6.21^{c}

SDS-PAGE

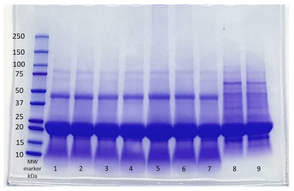


Figure 2. SDS-PAGE of kafirin proteins (reducing)

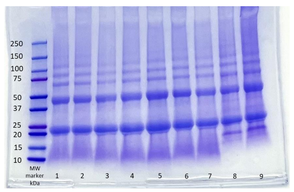


Figure 3. SDS-PAGE of kafirin proteins (non-reducing)

1: Red-NLM-20 (DGs)
2: Red-Waxy (DGs)
3: White-32020(DGs)
4: White-F1000 (DGs)
5: Black-NLM-16 (DGs)
6: Red-NLM-SB (DGs)
7: White-4525 (DGs)
8: Red-NLM-20 (Flour)
9: White-4525 (Flour)

SDS-PAGE

1: PPI
2: SPI
3: Gluten

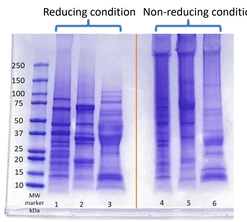


Figure 4. SDS-PAGE of PPI,SPI and gluten

Protein digestibility, TTC and surface hydrophobicity

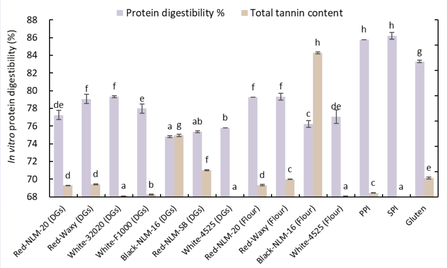


Figure 5. *In vitro* protein digestibility and total tannin content

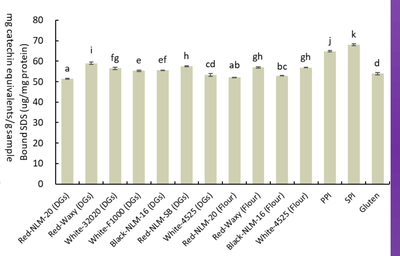


Figure 6. Surface hydrophobicity

RESULTS

Solubility, OHC&WHC, EAI&ESI

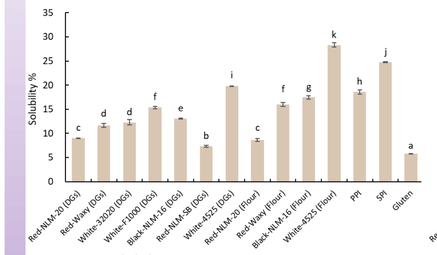


Figure 7. Solubility

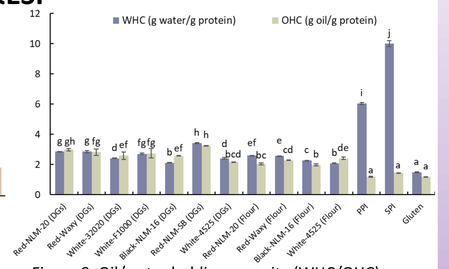


Figure 8. Oil/water holding capacity (WHC/OHC)

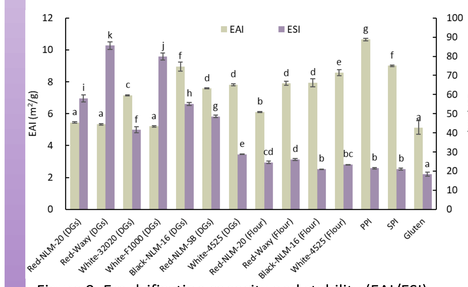
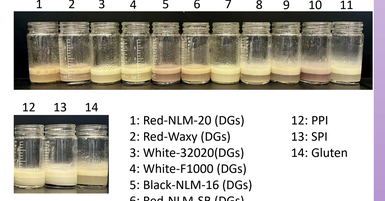


Figure 9. Emulsification capacity and stability (EAI/ESI)



1: Red-NLM-20 (DGs)
2: Red-Waxy (DGs)
3: White-32020(DGs)
4: White-F1000 (DGs)
5: Black-NLM-16 (DGs)
6: Red-NLM-SB (DGs)
7: White-4525 (DGs)
8: Red-NLM-20 (Flour)
9: Red-Waxy (Flour)
10: Black-NLM-16 (flour)
11: White-4525 (flour)
12: PPI
13: SPI
14: Gluten

DISCUSSIONS (Cont.)

- SDS-PAGE results showed that the kafirins from different types of sorghums had similar band profiles, while new bands in the range of 10-15 kDa were observed for the kafirins from the DGs.
- The kafirin from black sorghum DG had slightly lower *in vitro* protein digestibility (around 74%) compared to that from other sorghum DGs (75-79%), which may be due to the much higher total tannin content (5.6 mg catechin equivalents/g sample) compared to others (0-2.4 mg catechin equivalents/g sample).
- The surface hydrophobicity of the kafirins from different types varied between 50-60 μg SDS/mg protein, and the fermentation process further altered the surface hydrophobicity to some extent.
- The extracted protein from White-4525 flour had the highest solubility of 28.3%, higher than the commercial PPI and SPI.
- The OHC and WHC values of the extracted protein ranged from 1.97-3.24 g oil/ g protein and 2.08-3.42 g water/ g protein, respectively. The EAI ranged from 5.2-8.9 m^2/g. Black-NLM-16 DGs protein had the highest EAI among all the kafirins, similar to the commercial SPI (9 m^2/g).

DISCUSSIONS

- The extracted kafirins had protein content ranging between 75-85%, and the protein extracted from Red-NLM-20 had the highest protein purity (87.91%). Based on the color test, L* value was significantly higher for white sorghum proteins compared to others.
- From FTIR results, all the extracted sorghum proteins had similar composition of β-sheet, β-turn, and α-helix, except that the Red-NLM-20 protein contained 33.25% of random coil structure.

CONCLUSIONS

- In summary, the protein composition (SDS-PAGE) and secondary structure (FTIR) generally remain similar for the kafirins extracted from different types of sorghum or their DGs.
- The protein in vitro digestibility was correlated with the total tannin content of the protein (r = -0.42).
- The protein functional properties varied to some extent among different sorghum types and with fermentation.

ACKNOWLEDGEMENTS

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