Wild rice-wheat noodles fortified with purple sweet potato mitigate AAPH-induced oxidative damage in Caco-2 cells

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Background

Oxidative stress arises from an imbalance between the generation of reactive oxygen species (ROS) and the body's capacity to neutralize them with antioxidants, leading to cellular damage and the development of various chronic diseases (Pizzino et al., 2017). Regular consumption of plant-based foods rich in antioxidant phytochemicals can mitigate oxidative stress and lower the risk of related chronic conditions (Girgih et al., 2024).

Noodles, generally produced from wheat flour, exhibit low levels of phenolic compounds due to the removal of the grain's outer layers during milling (Žilić, 2016). Incorporating ingredients rich in phytochemicals can enhance their nutritional and functional properties.

Wild rice (WR) and purple sweet potato (PSP) are nutrient-dense foods known for their high phenolic content along with their associated health benefits. Their inclusion in noodle formulation represents a promising prospect for innovative food products with health benefits.

Wild rice (*Zizania aquatica*, WR)

Excellent source of carbohydrates, proteins, dietary fiber, vitamins, minerals, and phenolic compounds
- ✓ Ferulic acid
- ✓ Flavonoids
- ✓ Proanthocyanidins

Klensporf-Pawlik & Aladedunye, Wild Rice in Gluten-Free Ancient Grains, Elsevier Ltd, 2017.

Purple sweet potato (*Ipomea batatas L.*, PSP)

High levels of anthocyanins
- ✓ derivatives of cyanidin and peonidin

PSP powder

Health benefits

- **Anti-inflammation** — Teow et al., *Food Chem.* 2007, **103**, 829–838. Peres et al., *J. Cereal Sci.*, 2023, **113**, 103732.
- **Antioxidant** — Im et al., *Antioxidants*, 2021, **10**, 1–17. Qiu Y, Liu Q, Beta T. *J Agric Food Chem.* 2009, **57**, 7543–7551.
- **Anti-diabetes** — Hwang et al., *Nutr. Res.*, 2011, **31**, 896–906.
- **Reduce the risk of cardiovascular disease** — Surendiran et al., *Nutr. Rev.*, 2014, **72**, 227–236.
- **Anti-obesity** — Han et al., *Food Chem. Toxicol.* 2012, **50**, 2263–2269. Ju et al., *J Med Food* 2011, 14 **(10)**, 1097–1106.

Experimental design

❖ Noodles production and Chemical analysis

Table 1. Formulations for noodles preparation

Ingredients	WH Noodles	WR-WH Noodles	20% PSP Noodles
Wheat flour (g)	50	50	24
Wild rice flour (g)	-	50	24
PSP powder (g)	-	-	12
Water (ml)	18	45	30

WH: noodles made with 100% wheat flour,
WR-WH: noodles made with 50% WR flour and 50% wheat flour,
20% PSP: noodles made with WR-WH fortified with 20% PSP powder

- Determination of Total phenolic compounds (TPC)
  - ✓ Folin-Ciocalteu method (Apea-Bah et al., 2022)
- Antioxidant activity Assays (Apea-Bah et al., 2022)
  - ✓ DPPH
  - ✓ ABTS
  - ✓ FRAP

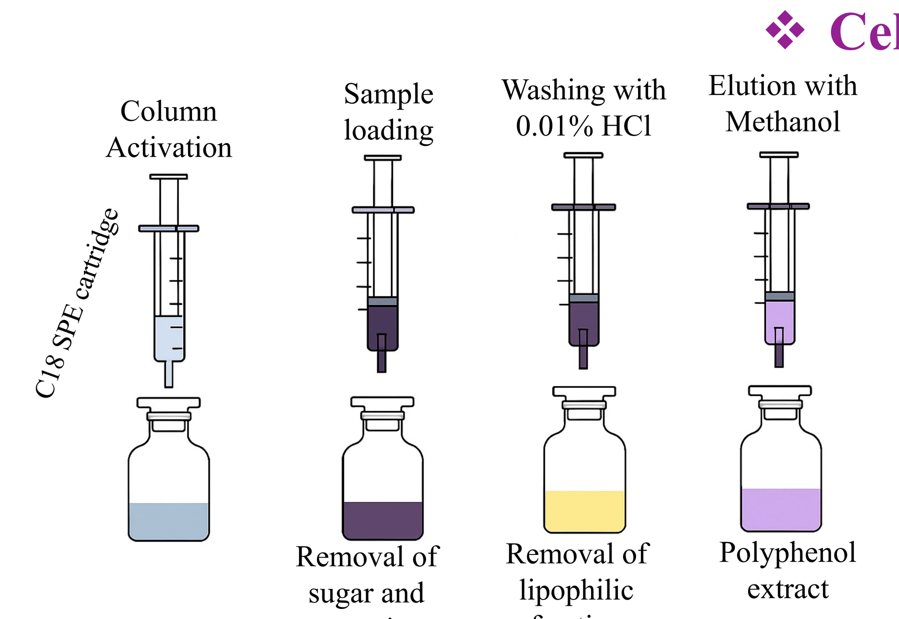
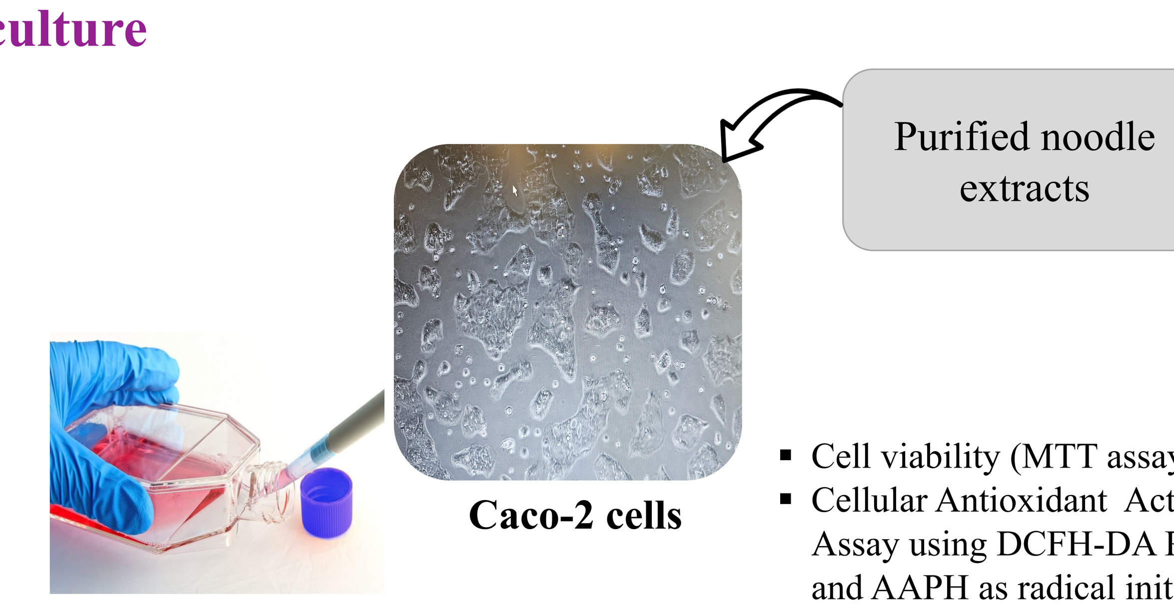


Fig.1 Purification steps of noodle extracts

❖ Cell culture



- Cell viability (MTT assay)
- Cellular Antioxidant Activity Assay using DCFH-DA Probe and AAPH as radical initiator

Fig.2 Cell experimental setup

Results

(1) Effect of WR and PSP on noodles' appearance

(A) **(B)** **(C)**

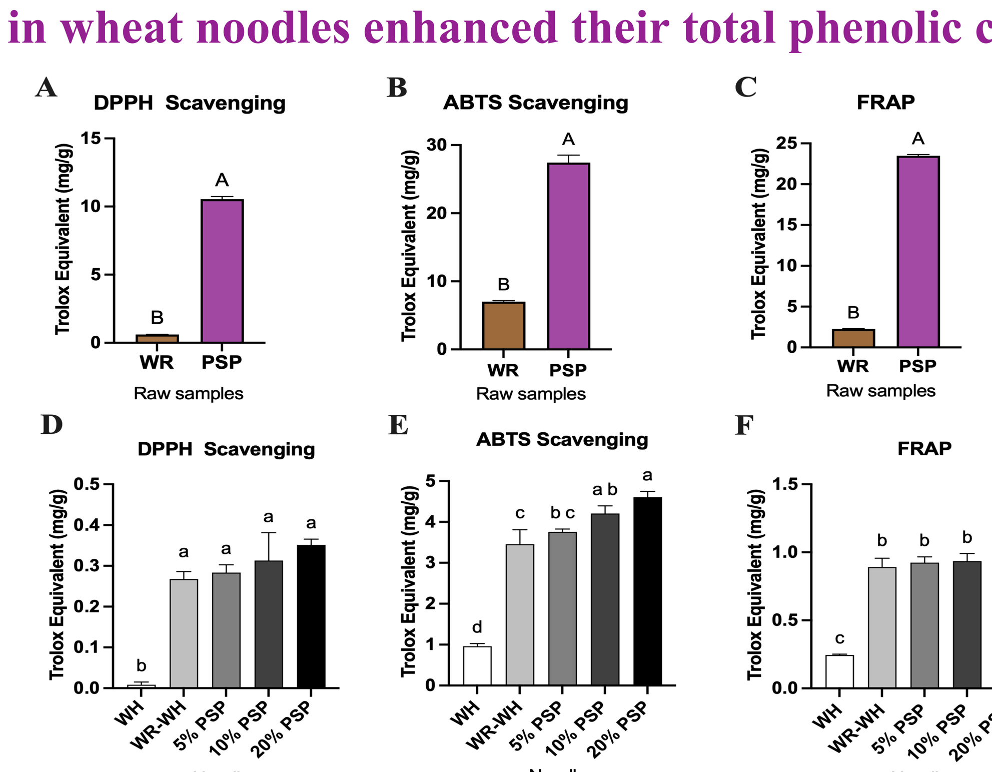
Fig. 3 Visual appearance of noodles made from 100% wheat flour **(A)**, 50% wild rice flour and 50% wheat flour (WR-WH) **(B),** and WR-WH fortified at 20% PSP powder **(C)**

(2) The incorporation of WR and 20% PSP in wheat noodles enhanced their total phenolic content and antioxidant capacities

Table 2. Total phenolic content of the raw and noodle samples

	Sample	TPC (mg GAE/g DW)
Raw	WR	1.07 ± 0.03
Raw	PSP	12.27 ± 0.19☆
Noodles	WH	0.52 ± 0.04ᶜ
Noodles	WR-WH	0.95 ± 0.02ᵃ
Noodles	20% PSP	0.85 ± 0.01ᵇ

WR: wild rice
PSP: purple sweet potato
WH: noodles made with 100% wheat flour,
WR-WH: noodles made with 50% WR flour and 50% wheat flour,
20% PSP: noodles made with WR-WH fortified with 20% PSP powder



Results are expressed as mean ± SD. Significant differences ($p < 0.05$) are indicated by different lowercase letters on the means, as processed by one-way ANOVA followed by Tukey's multiple comparisons test.

Fig. 4 Antioxidant activities of the raw ingredients and the noodles obtained through DPPH (A, D), ABTS (B, E), and FRAP (C, F) assays

(3) Effect of the noodle extracts and AAPH on cells' viability

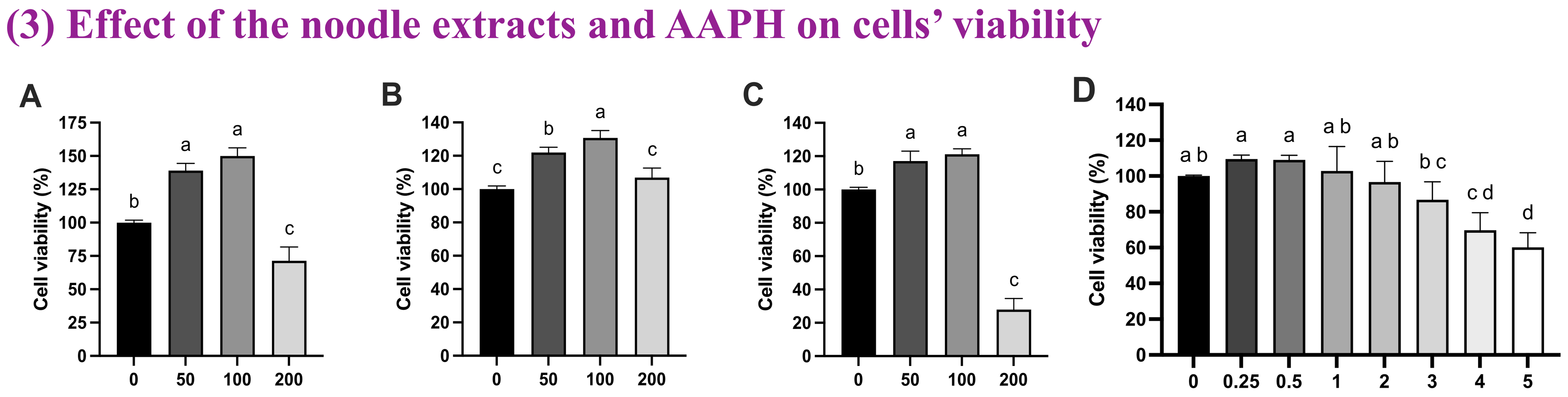


Fig. 5 Survival rate of Caco-2 cells treated for 24h

Caco-2 cells were incubated with 50, 100, and 200 µg/ml purified extracts from WH, WR-WH, and 20%PSP noodles (A, B, and C, respectively), and AAPH (D). Results are expressed as % of control cells and each bar represents mean ± SD (n=6).

AAPH is a free radical-generating compound commonly used to induce oxidative stress in cells.

(4) Noodle extracts inhibit AAPH-induced cytotoxicity

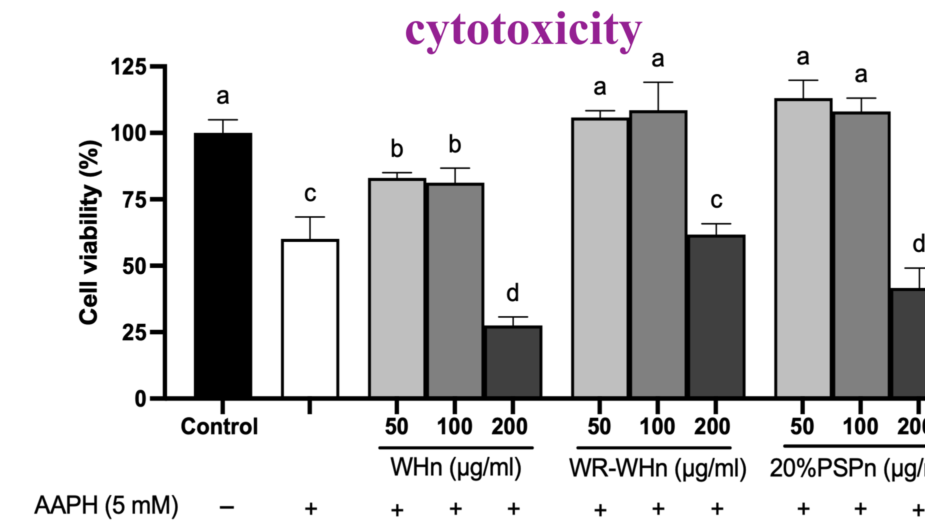


Fig. 6 Protective effect on the different noodle extracts against AAPH-induced cytotoxicity

Caco-2 cells were pretreated with 50, 100, and 200 µg/ml purified extracts from WH, WR-WH, and 20%PSP noodles for 2h, then exposed to AAPH (5 mM) for 3h. Results are expressed as % of control cells and each bar represents mean ± SD (n=6).

(5) WR and PSP enhanced Cellular Antioxidant Activity (CAA)

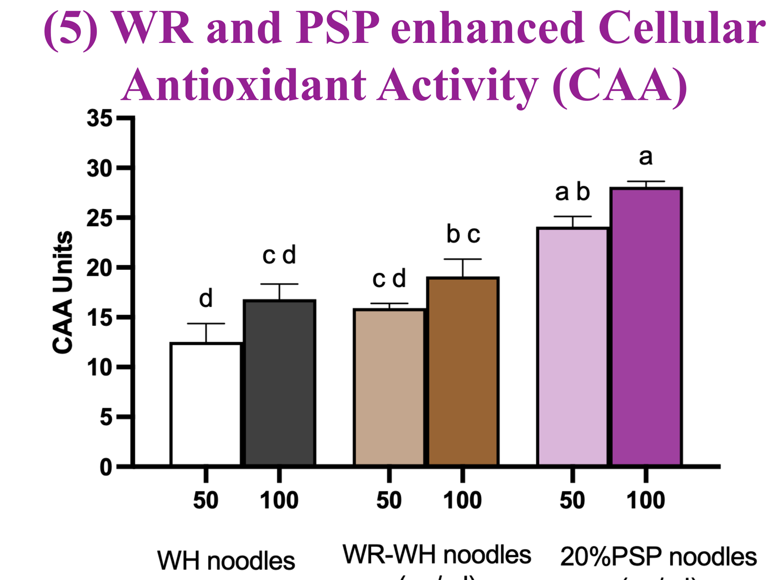


Fig. 7 CAA of the purified noodle extracts

Caco-2 cells were co-treated with DCFH-DA Probe and 50 or 100 µg/ml purified extracts from WH, WR-WH, and 20%PSP noodles for 1h, and then exposed to AAPH (200µM) to collect the fluorescence intensity. Results are expressed as CAA units and each bar represents mean ± SD (n=3).

Conclusion

- The incorporation of WR flour and PSP powder at 20% significantly enhanced the physicochemical and antioxidant properties of wheat noodles, improved Caco-2 cells viability and mitigated AAPH-induced oxidative damage.
- Fortifying wheat-based products with phenolic-rich ingredients, such as WR and PSP, could be a promising strategy for developing functional foods with added health benefits.

Acknowledgments

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