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White wine pomace mitigates hypoxia in 3D SH-SY5Y model

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INTRODUCTION & AIM

- **Hypoxia-induced reactive oxygen species (ROS) contribute to neuronal death and are a major factor in various neurodegenerative diseases. Currently, there is a need for the development of effective strategies for the control of these diseases. The application of food by-products with antioxidant properties, such as white wine pomace products (wWPP), is valuable as it not only allows their revalorization but also shows potential for disease prevention.**
- **The aim of this study was to evaluate the neuroprotective effect of the wWPP bioaccessible fractions (WPGI and WPF), obtained after in vitro gastrointestinal digestion and colonic fermentation respectively, against hypoxia in SH-SY5Y human neuroblastoma cell line. Previous research demonstrated a positive effect in 2D *in vitro* models but did not explore 3D models, so spheroids were used for a more accurate prediction of the possible protective effects of wWPP.**

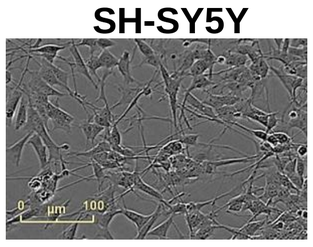
METHOD

wWPP

In vitro gastrointestinal digestión and colonic fermentation

WPGI and **WPF**

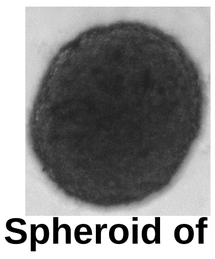
SH-SY5Y



U-well plate,
Retinoic acid 10 µM
PMA 80 nM

Hypoxia

200 µM $CoCl_2$



Spheroid of diferenciated SH-SY5Y

ROS detection
(DCF fluorescence)
Ex 480 nm
Em 530 nm

mRNA expression
(qPCR with SyBRGreen)
Nrf2, NfkB, HIF1, SOD2, Keap1

Cell viability
(PI fluorescence)
Ex 535 nm
Em 615 nm

RESULTS & DISCUSSION

- **The bioaccesible fractions did not increase cell death and ROS levels in normoxia.**
- **Hypoxia significantly increased cell death and ROS levels. The bioaccesible fractions WPGI and WPF however were able to significantly mitigate these effects.**

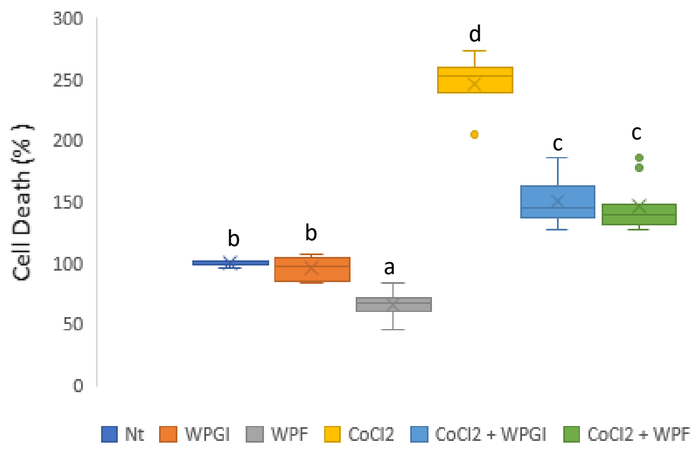


Figure 1. Cell death of the samples incubated with or without the bioaccesible fractions in normal conditions or hypoxia. Values represent mean (n ≥3) ± SD. Significant difference is indicated with Latin letters (a, b, c) (ANOVA, p<0.05). Nt: non-treated spheroids; WPGI: bioaccesible digested fraction; WPF: bioaccesible fermented fraction.

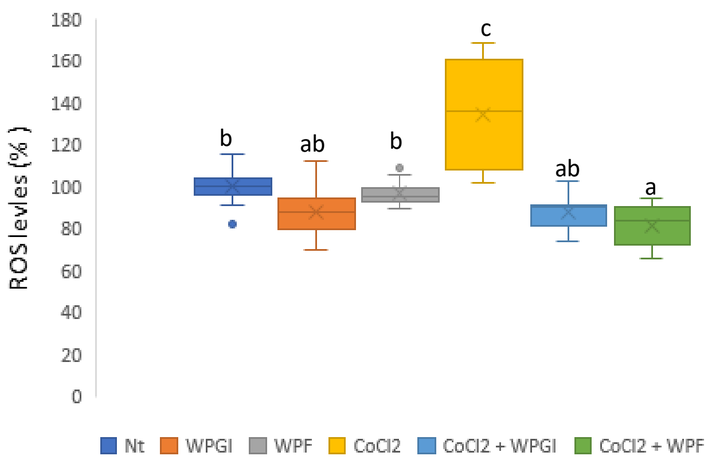


Figure 2. ROS levels of the spheroids incubated with or without the bioaccesible fractions in normal conditions or hypoxia. Values represent mean (n ≥3) ± SD. Significant difference is indicated with Latin letters (a, b, c) (ANOVA, p<0.05). Nt: non-treated spheroids; WPGI: bioaccesible digested fraction; WPF: bioaccesible fermented fraction.

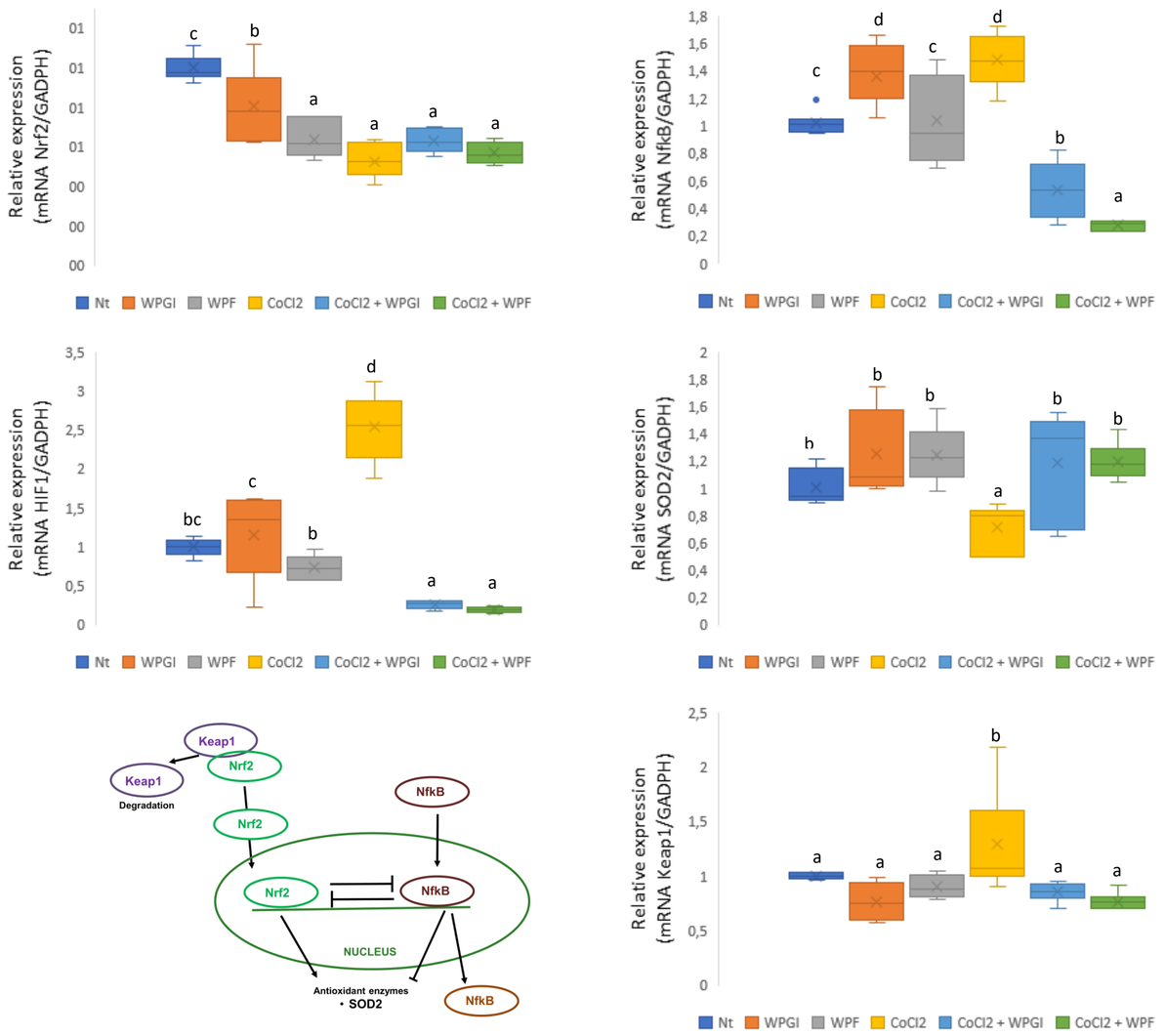


Figure 3. mRNA expression of genes involved in hypoxia and antioxidant response of the spheroids incubated with or without the bioaccesible fractions in normal conditions or hypoxia. Values represent mean (n ≥3) ± SD. Significant difference is indicated with Latin letters (a, b, c) (ANOVA, p<0.05). Nt: non-treated spheroids; WPGI: bioaccesible digested fraction; WPF: bioaccesible fermented fraction.

- **Hypoxia significantly increased the hypoxia-inducible factor 1 (HIF1) and altered the expression of Nrf2, NfkB and Keap1, involved in the regulation and response to oxidative stress. This resulted in a significant decrease of the antioxidant enzyme SOD2. The bioaccesible fractions were able to reverse these changes, downregulating NfkB, HIF1, Keap1 and increasing SOD2 to control levels.**

CONCLUSION

- **In conclusion, bioaccessible wWPP showed significant potential in mitigating hypoxia effects in 3D SH-SY5Y model. These results suggest a potential neuroprotective effect of wine pomace and highlight the relevance of using natural products from the food industry in disease prevention. However, in vivo studies are necessary to better understand the potential use of these food by-products as functional foods.**

REFERENCES

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