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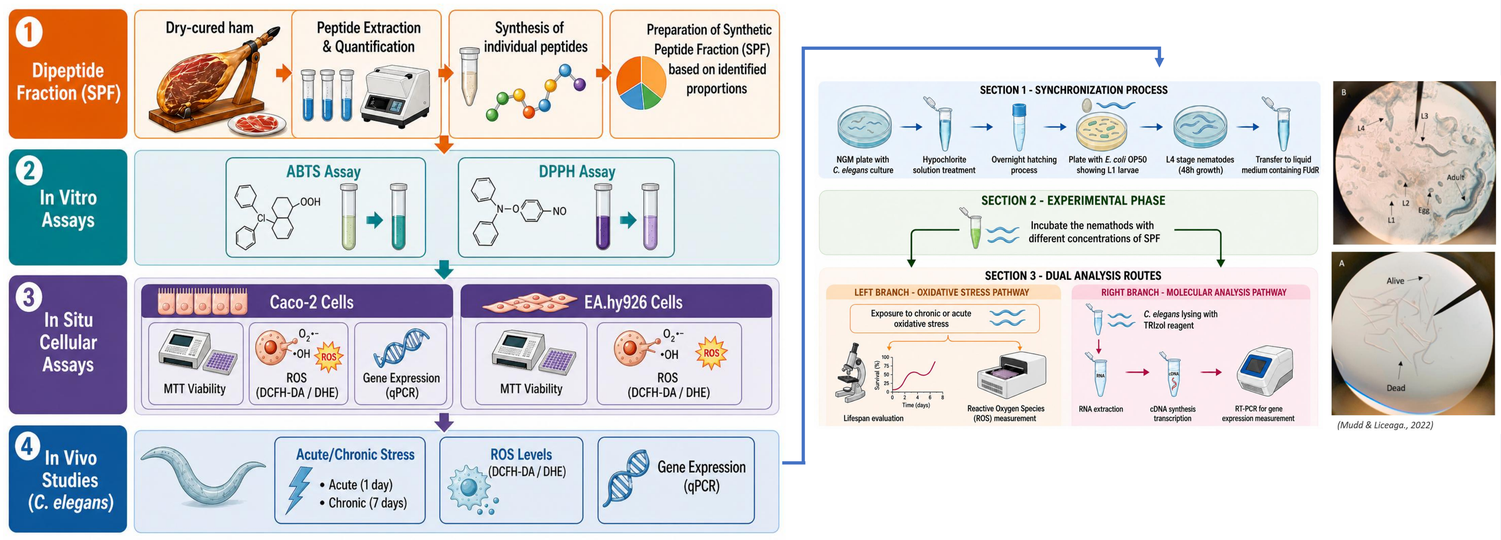
In vivo evaluation using *C. elegans*, of the bioactive potential of synthesized dipeptides previously identified in Spanish dry-cured ham.

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INTRODUCTION

Oxidative stress causes cellular damage and contributes to chronic diseases. Food-bioactive peptides (BAP) are gaining interest as potential antioxidant compounds. Our previous work identified BAP from Spanish dry-cured ham, but their specific contribution to antioxidant activity remains poorly understood. In this study, ten dipeptides previously identified in digested Spanish dry-cured ham were synthetized according to their relative abundance and evaluated for their antioxidant activity using chemical, cellular and *in vivo* methods.

METHODS



(Mudd & Liceaga., 2022)

RESULTS & CONCLUSIONS

Table 1. In vitro antioxidant activity of digested ham peptide fraction (HDP) and a reconstructed synthetic peptide fraction (SPF) determined by DPPH and ABTS radical scavenging assays, measured as IC_{50} (mg/mL) ± SEM*.

	DPPH	ABTS
Sample code[1]	IC_{50} (mg/mL)	
HDP	$0.431^{a} \pm 0.092$	$0.279^{a} \pm 0.019$
SPF	$0.722^{b} \pm 0.108$	$0.322^{a} \pm 0.082$
Control	-	-

HDP: Ham digested peptide fraction
SPF: Synthetized dry-cured ham peptide fraction
Control: Distilled water. «-»: not detected / not applicable.
**SEM: standard error of the mean.*

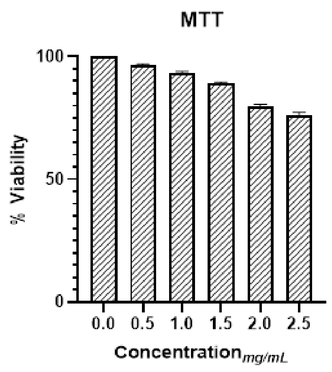


Fig. 1. Effect of ham synthetized peptide fraction (SPF) on the viability of Caco-2 cells, reported as Cell Viability % using the MTT assay.

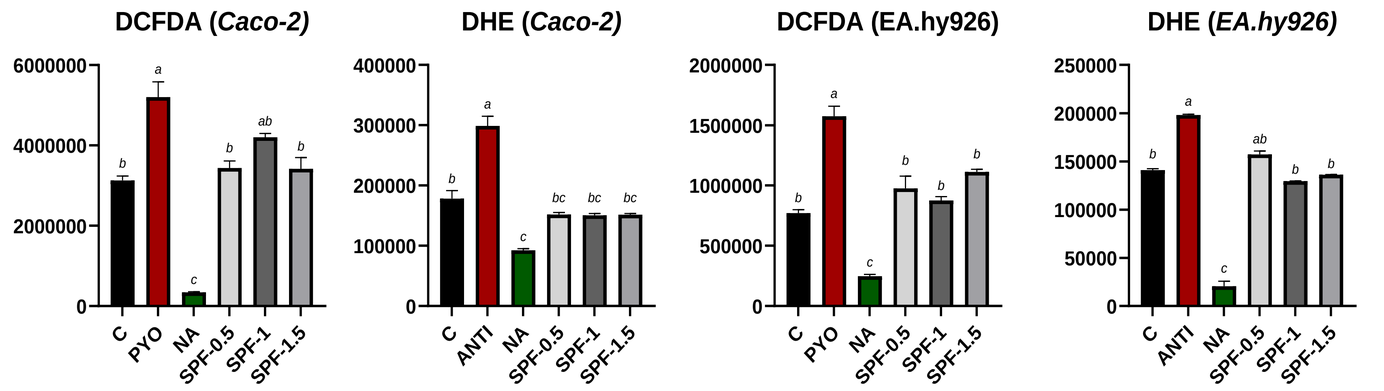


Fig. 2. Cellular antioxidant activity in Caco-2 & EA.hy926 cells, reported as ROS generation in the DCFDA & DHE assays, for synthetized Spanish dry-cured ham peptide fraction (SPF) at different concentrations (0.5, 1 and 1.5 mg/ mL). Values represent the mean of three replicates ± SEM. Trials that do not share the same letter (a, b, c) are significantly different (P<0.05).

The synthetic dry-cured ham peptide fraction (SPF) showed antioxidant activity in both DPPH and ABTS assays (**Table 1**), with activity comparable to the digested ham fraction (HDP) in ABTS. SPF showed no relevant cytotoxicity in Caco-2 cells (**Fig.1**), maintaining approximately 80% viability up to 2.0 mg/mL. In both Caco-2 and EA.hy926 cells (**Fig.2**), SPF significantly (*P<0.05*) decreased intracellular ROS generation compared with the oxidated control (PYO). No significant differences (*P>0.05*) were observed among the SPF concentrations tested (0.5–1.5 mg/mL), confirming its cellular antioxidant activity.

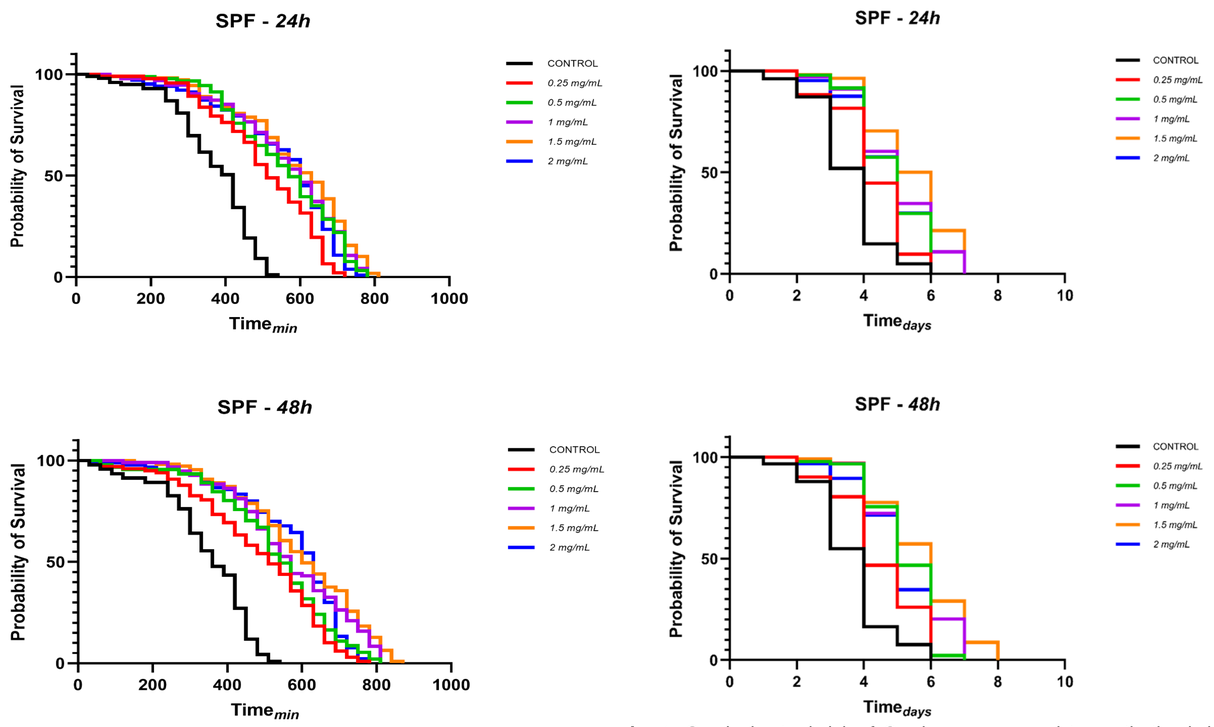


Fig. 3. Survival rate (min) of C. elegans exposed to synthetized dry-cured ham peptide fraction (SPF) at 0.25 to 2.0 mg/mL for 24 and 48 h, followed by acute oxidative stress conditions in the presence of 5mM t-BOOH.

Fig. 4. Survival rate (min) of C. elegans exposed to synthetized dry-cured ham peptide fraction (SPF) at 0.25 to 2.0 mg/mL for 24 and 48 h, followed by chronic oxidative stress conditions in the presence of 25mM paraquat.

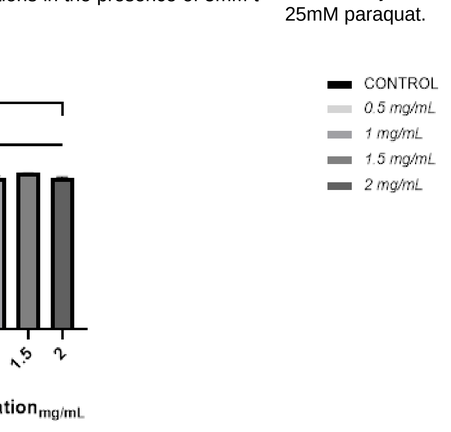


Fig. 5. Reactive oxygen species (ROS) levels in *C. elegans* pretreated with different concentrations of synthetized dry-cured ham peptide fraction (SPF) at different concentrations and then exposed to paraquat (PQ) for 48 h. Results are expressed as mean ± SEM from three independent biological experiments.

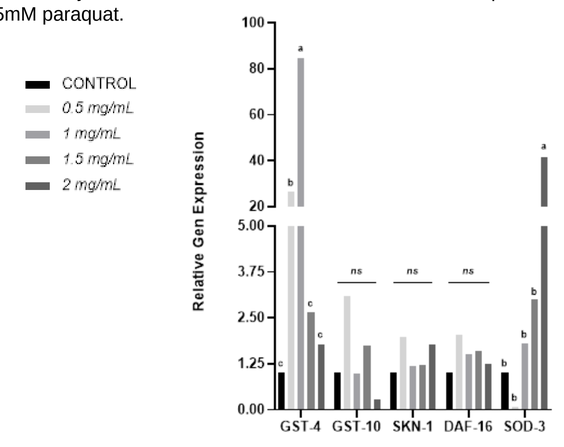


Fig. 6. Effect of synthetized dry-cured ham peptide fraction (SPF) on antioxidant gene expression in *C. elegans*. Trials for each gene that do not share the same letter (a, b, c) are significantly different (P<0.05); ns, not significant.

SPF pretreatment significantly increased *C. elegans* survival to acute oxidative stress (**Fig. 3**) conditions induced by t-BOOH (*P<0.05),* with the strongest protective effect observed at 1.0 and 1.5 mg/mL. Survival reached approximately 600–650 min compared with ~350 min in the control group. Under chronic oxidative stress (**Fig.4**) induced by paraquat, SPF also significantly increased nematode longevity *(P<0.001),* with 1.0 mg/mL increasing mean survival rate from approximately 3 to 5 days. Overall, SPF enhanced survival of *C. elegans* to both acute and chronic oxidative stress conditions.

SPF pretreatment significantly decreased intracellular ROS accumulation in *C. elegans* (**Fig. 5**) exposed to paraquat (48 h), with all tested concentrations showing lower ROS levels than the oxidant control *(P<0.05)*. ROS levels approached those of the untreated control, while no significant differences *(P>0.05)* were observed among SPF concentrations. SPF treatment modulated the expression of antioxidant defense genes (**Fig. 6**), showing an upregulation of GST-4 and SOD-3, although the response varied depending on the SPF concentration used. These findings support the antioxidant and protective effects of SPF against oxidative stress in *C. elegans*.

In **conclusion**, the synthetic dry-cured ham peptide fraction (SPF) showed consistent antioxidant activity across chemical, cellular, and *in vivo* (*C. elegans*) studies, decreasing ROS levels and enhancing resistance to acute and chronic oxidative stress. Overall, these results support the potential of the identified Spanish dry-cured ham dipeptides as bioactive antioxidant compounds.

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