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Cultivar diversity in *Malus domestica* Borkh. determines bioactive potential of apple pulp extracts: evidence from *C. elegans* metabolic dysfunction model

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Abstract

The overconsumption of high-caloric diets is a primary driver of obesity and type 2 diabetes, conditions associated with oxidative stress, dyslipidaemia, and systemic metabolic dysfunction. Apples (*Malus domestica* Borkh.) are among the most widely consumed fruits globally and represent a significant dietary source of (poly)phenolic compounds with recognised health-promoting properties. However, commercial cultivar selection has substantially reduced the phytochemical richness of apple varieties available in food chains, potentially limiting their functional food value. In this study, we compared the bioactive potential of pulp extracts from an autochthonous Spanish cultivar, ‘Amarilla de Octubre’, and a commercially selected, ‘Pinova’, using a *Caenorhabditis elegans* model of glucose-induced metabolic dysfunction. Lipid accumulation, reactive oxygen species (ROS) production, bacterial intake, and health span-related markers were evaluated. ‘Amarilla de Octubre’ extract significantly reduced lipid accumulation by up to 23.24%, attenuated ROS production to levels comparable to non-stressed controls, decreased glucose-induced hyperphagia, and preserved locomotor function. In contrast, ‘Pinova’ extract showed limited and largely concentration-dependent effects across all parameters. These findings suggest that autochthonous apple cultivars with higher (poly)phenolic content like ‘Amarilla de Octubre’ may represent valuable functional food sources for the dietary management of metabolic dysfunction and highlight the nutritional relevance of preserving agrobiodiversity.

Keywords Apple cultivar diversity · (Poly)phenols · *Caenorhabditis elegans* · Metabolic dysfunction · Health span · Oxidative stress

Introduction

Non communicable diseases (NCDs) have emerged as the most important health problem worldwide. They are caused by a combination of genetic, physiological, environmental and behavioural factors, being cardiovascular diseases, cancer or diabetes the most common types¹. Overweight is a remarkable risk factor that influence the progression of NCDs and represents a real challenge for healthcare systems due to the lack of reliable drugs.

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Diabesity, defined as the co-existence of obesity and type 2 diabetes mellitus (T2DM), represents a major global health challenge characterized by metabolic dysfunction². This condition is caused because of chronic energy imbalance due to high-calorie diets and reduced physical activity, leading to excessive adiposity that promotes peripheral insulin resistance and pancreatic β -cell dysfunction³. As a T2DM consequence, chronic hyperglycaemia generates neuronal damage through the formation of reactive oxygen species and advanced glycation end products (AGEs), leading to mitochondrial dysfunction and oxidative stress. Pharmacological agents, although resulted in considerable weight loss, may lead to adverse effects⁴. In this context, dietary strategies, particularly the regular consumption of plant-based foods rich in bioactive compounds, represent accessible and sustainable approaches to reducing metabolic risk.

Apples (*Malus domestica* Borkh.) are among the most widely consumed fruits worldwide and constitute a significant component of Western and Mediterranean dietary patterns. Their regular intake has been epidemiologically associated with a reduction of cardiovascular disease, type 2 diabetes and obesity-related metabolic dysfunction^{5,6}. These benefits are attributed to their rich content of (poly)phenolic compounds, including procyanidins, catechins, quercetin glycosides, and caffeoylquinic acids, which are particularly concentrated in the pulp and exert potent antioxidant and anti-inflammatory effects^{7,8}. At the molecular level, these compounds help reduce glucotoxicity and improve insulin resistance by mitigating oxidative stress and inflammatory mediators, thus providing a multifaceted approach to metabolic regulation⁹. Apples also contribute macronutrients, dietary fibre and other non-nutrient bioactives relevant to human nutrition¹⁰.

Despite the well-documented health benefits associated with apple (poly)phenol intake, their concentration varies considerably depending on the cultivar and environmental conditions. Wild and autochthonous varieties tend to have higher (poly)phenolic content due to their protective role in plants versus biotic or abiotic stress, traits associated with bitter or astringent taste and enzymatic browning. Commercial domestication has involved extensive selection for agronomic and sensory attributes, such as increased fruit size, improved sweetness, and reduced enzymatic browning which coincided with a substantial decrease in polyphenolic compounds¹¹.

The nutritional relevance of apple cultivar diversity therefore extends beyond agronomic considerations. Traditional and locally adapted varieties, largely absent from commercial food chains, may represent underexploited dietary sources of bioactive compounds with superior health-promoting potential compared to commercially selected cultivars. Evaluating the functional properties of whole food extracts under metabolically relevant conditions is thus essential to substantiate these differences. While rodents are standard in obesity research, modern nutritional science increasingly favours more ethical, lower-order model organisms. The nematode *Caenorhabditis elegans* has emerged as a well-validated system in food and nutritional research, conserving 60–80% of human genes involved in basic energy metabolism homeostasis¹², and enabling direct assessment of how dietary compounds modulate lipid accumulation, oxidative stress, and health span markers under controlled experimental conditions.

In this context, the purpose of this work was to investigate, for the first time, whether the consumption of pulp extracts from the local Spanish apple cultivar ‘Amarilla de Octubre’ could modulate energy balance, redox homeostasis, and behavioural parameters in the *C. elegans* obese model, in comparison with the widely commercialised cultivar ‘Pinova’.

Materials and methods

Plant food material

The CITA research centre supplied two different apple pulp extracts. ‘Amarilla de Octubre’ apples were provided from mountainous areas of the Pyrenees (Spain) and recovered in collections located in Bescos de la Garcipollera (Huesca, Aragon). In contrast, ‘Pinova’ apples were obtained in the research orchards from Zaragoza, Aragon. ‘Pinova’ as commercial and ‘Amarilla de Octubre’ as autochthonous apple cultivars were collected at the ripening

period of the cultivars (17 and 23 apples respectively) from September to October 2021. The pulp tissue was frozen immediately and lyophilized. The extraction of bioactive compounds was developed by using methanol and then ultrasonication at room temperature. Extracts were stored at -80°C after elimination of solvent in a rotatory evaporator. Phenolic profiling was conducted using HPLC–MS/MS following previously described methods in a previous paper¹³. The major phenolic constituents identified in both extracts were procyanidin B2, epicatechin, and chlorogenic acid. In Amarilla de Octubre, these compounds represented 64.76%, 16.62%, and 14.43% of the total phenolic content (70,236.55, 18,029.00 and 15,653.70 μg phenolic compounds/g extract, respectively), whereas in Pinova they were 38.91%, 10.33%, and 38.60% (2871.02, 761.91 and 2848.37 μg phenolic compounds/g extract respectively). Notably, the overall phenolic mass detected was approximately 14-fold higher in the autochthonous cultivar than in the commercial Pinova sample (108,446.71 $\mu\text{g/g}$ vs. 7378.12 $\mu\text{g/g}$)¹³.

C. elegans strains and maintenance conditions.

Caenorhabditis elegans strains N2 (Bristol, wild-type) and SS104 (glp-4 (bn2)) were sourced from the Caenorhabditis Genetics Center (University of Minnesota, USA). Worms were maintained at 20°C on NGM plates seeded with *Escherichia coli* OP50. All experimental conditions were established and maintained on NGM agar plates and synchronized populations were obtained by alkaline bleaching.

Antibacterial screening assay: OP50

Escherichia coli OP50 stock cultures were grown in LB broth (Sigma) at 37°C for 24 h to reach the exponential phase. Bacteria were then distributed into 96-well plates under different conditions: a negative control (bacterial suspension only) and a positive control (5% penicillin–streptomycin in LB; Gibco). Extracts were tested at 500 and 1000 $\mu\text{g/mL}$ to evaluate antibacterial activity. Plates were incubated for 24 h at 37°C , and absorbance was measured at 600 nm using a Synergy H1 reader. Bacterial viability was normalized to the negative control. At least three independent experiments were performed for each extract concentration.

Acute toxicity assay

Synchronized L4 larvae were washed twice with M9 buffer and 7–18 worms were transferred per well into 96-well plates. Worms were exposed to extract concentrations (250, 500, or 1000 $\mu\text{g/mL}$) or M9 as a negative control, in a final volume of 200 μL per well. After 24 h at 20°C , survival was assessed and expressed as percentage survival (Eq. 1). At least 40 worms per condition were analyzed per assay.

$$\text{Survival rate} = \frac{\text{Number of alive worms} \times 100}{\text{Total number of worms}} \quad (1)$$

Chemotaxis index assay

A population of L4 *C. elegans* (N₂) were recollected and then transferred to microcentrifuge tubes. After centrifugation, they were washed with M9 solution 3 times, and the concentration of worms is adjusted so that there are between 50 and 250 per 50 μL . Plate dishes were divided in four equal quadrants and a 0.5 cm radius circle was marked in the center.

Apple pulp extracts were mixed with 0.5 M azide to anesthetize the worms, and it was applied to the “Test” quadrants. The control solution 0.5 M azide was located to the “Control” quadrants. After adding extracts and control solutions, worms were placed in the inner 0.5 cm circle, and the plates were incubated at 20°C for 90 min. Finally, the number of worms in each quadrant that have crossed the inner circle is recorded. The chemotaxis

index is calculated as shown in Eq. (2). A +1.0 score indicates maximal attraction although −1.0 is evidence of maximal repulsion.

$$\text{Chemotaxis index} = \frac{\text{Worms in both test quadrants} - \text{Worms in control quadrants}}{\text{Total scored worms}} \quad (2)$$

Fat accumulation: Nile Red O staining

An obese *C. elegans* model (N₂) was developed after exposing them to an excess of 5% glucose in the medium NGM. The conditions studied were: 5% glucose as a positive control, 5% glucose plus 250 µg/mL of apple pulp extract, 250 µg/mL of extract and as negative control, plates without adding glucose to NGM medium. Orlistat was used as reference treatment at 6 µg/mL plus 5% glucose. Total fat content was measured in nematodes by quantifying staining images according to a previously described method¹⁴. The fat reduction obtained by this drug compared to the obese worm (positive control) was considered the maximum effect.

Lipid accumulation in *C. elegans* was assessed by Nile Red staining after treatment with Pinova and Amarilla de Octubre extracts. Fluorescence intensity per area was used as a relative measure of fat content. Synchronized L1 larvae (≥300 per condition) were cultured at 20 °C for 48 h to reach the L4 stage under the specified dietary conditions previously mentioned. Nile Red fluorescence, indicative of lipid droplets, was visualized under UV illumination. For each condition, 30–40 worms were imaged using a Nikon Eclipse TS100 microscope equipped with a GFP filter (395 nm excitation/508 nm emission) at 100× magnification and 20 s exposure. Images were analysed with ImageJ to quantify fluorescence per area of each worm. The experiment was carried out at least at triplicate.

E. coli (OP50) ingestion quantification

This assay is focused on evaluating the potential feeding behavior changes related to the presence/absence of glucose and apple pulp extract in the NGM growing medium¹⁵. OP50-GFP bacteria, a strain of OP50 that contains a GFP plasmid (pFPV25.1) that is very fluorescent, were selected to feed N₂ worms for 72 h. Animals were individually transferred (until from 30 to 40 worms) with 500 µl of PBS per tube. After washing them at least twice with PBS solution, 200 µl of PBS containing 30 to 40 animals were transferred into black 96-well plates at least, per duplicate. GFP accumulation was evaluated by measuring 480 excitation and 571 nm, using the Synergy H1. The experiment was repeated at least three independent times.

Additionally, the expression of GFP in OP50 bacteria was verified by making an antimicrobial test in parallel. We added a final concentration of 100 µg/ml of ampicillin to the bacteria population and compared it versus negative and positive controls per experiment as in OP50 toxicity assay (Data in supplementary materials).

ROS production

ROS levels in *C. elegans* were evaluated after exposure to the extracts and sublethal heat stress. Synchronized SS104 populations were maintained under the indicated conditions at 25 °C. Measurements were performed on days 7 and 10. Individual worms were transferred to black 96-well plates with transparent bottoms containing 75 µL PBS and exposed to 35 °C for 2 h. Then, 25 µL of 150 µM DCFH-DA (in ethanol) was added, and fluorescence was monitored for 90 min (excitation 485 nm, emission 535 nm). At least three independent experiments were conducted, analysing a minimum of 15 worms per condition in each experiment.

ROS levels were assessed on days 7 and 10 after treatment. Individual worms were transferred to black 96-well plates with transparent bottoms containing 75 µL PBS and exposed to sublethal heat stress (35 °C, 2 h). Then, 25 µL of 150 µM DCFH-DA (in ethanol) was added, and fluorescence was monitored for 90 min (485 nm excitation, 535 nm emission).

Body length

The effect of excess glucose and apple pulp extracts on *C. elegans* (N2) development was assessed by measuring L4 body length using ImageJ on the same images acquired for lipid quantification. The experiments were performed at least three independent times.

Pharynx pumping

Pharyngeal pumping was evaluated on days 7 and 10 using synchronized *C. elegans* SS104 populations under the previously described conditions. Pumping rates were manually counted for 30 s per worm using a handheld counter, with observations made at 20× magnification on a Nikon Eclipse TS100 inverted microscope. At least 30 worms per condition were analyzed across three independent replicates, and results were expressed as pumps per 30s¹⁶.

Nematode motility (*Thrashing*)

Bleached age-synchronized N2 *C. elegans* were transferred daily to fresh plates under their respective conditions until day 4 of adulthood, and every other day thereafter. Thrashing assays were performed on days 1, 4, and 7 using 15–20 worms per condition. Individual worms were placed on unseeded 60 mm NGM plates in 250 µL M9 buffer and allowed to acclimate for 1 min. Thrashing was then recorded for 50 s at 14 frames per second using the WormLab Imaging System (MBF Bioscience). Data were expressed as thrashes per 30 s.

Statistical analysis

Data are presented as mean ± SEM. Statistical analyses were carried out using GraphPad Prism (v10.5.0, GraphPad Software, USA) with one- or two-way ANOVA, followed by Tukey's post hoc test for multiple comparisons.

Results and discussion

Screening for antimicrobial activity and acute toxicity

The (poly)phenolic compounds present in apple pulp extracts are known secondary metabolites with potential antimicrobial properties, which could interfere with the viability of OP50 *E. coli*, the primary food source of *C. elegans* in laboratory conditions. Preliminary screening assays were therefore performed to rule out confounding effects on bacterial viability and nematode survival prior to the main bioactivity experiments.

Neither apple pulp extracts exhibited antimicrobial activity against OP50 bacteria at any tested conditions. Relative viability was not significantly reduced compared to untreated controls and differed significantly only from the positive control containing penicillin-streptomycin solution. Accordingly, minimum inhibitory concentrations were not determined. Acute toxicity in *C. elegans* was assessed by exposing an L4-synchronised N₂ population to 250, 500–1000 µg/mL of each extract for 24 h. Survival rates did not differ significantly from the M9 buffer control group under any tested condition, confirming the absence of acute toxicity at the concentrations selected for subsequent assays.

Chemotactic response of *C. elegans* to apple pulp extracts

As complementary screening step, the chemotactic response of *C. elegans* to the apple pulp extract was evaluated to determine whether the extracts elicited attraction or repulsion at the concentrations used in the subsequent

assays. *C. elegans* possesses a well-developed chemosensory system comprising eleven pairs of neurons that detect a wide range of olfactory and gustatory signals associated with food sources, environmental hazards, or presence of other animals¹⁷.

Chemotaxis indices were determined after 90 min exposure to each extract at 1000 and 2000 µg/mL, diluted in a sublethal concentration of sodium azide (Table 1). Neither extract elicited significant repelling response at either concentrations, indicating that the apple pulp extracts were not detected as aversive stimulus under the tested conditions, and confirming their suitability for use in the subsequent bioactivity assays.

Chemotaxis index was calculated using Eq. (2) (see [Materials and methods](#)). Values are means ± SEM from at least 3 independent experiments (≥ 150 worms per condition). A value of + 1.0 indicates complete attraction and − 1.0 indicates complete repulsion towards the tested samples. No statistically significant differences were found between conditions (one-way ANOVA).

Lipid accumulation in the *C. elegans* obese model

C. elegans constitutes a versatile and genetically tractable model for obesity research, combining conserved metabolic pathways and high-throughput compound screening capabilities. The nematode’s compact anatomy, transparency, and observable feeding behaviour offer significant experimental advantages. Its intestinal cells serve as the primary storage site for lipid droplets, enabling direct visualization and quantification of fat accumulation using lipid-specific dyes such as Nile Red¹⁸.

Nematodes were cultured on NGM, supplemented with 5% glucose to induce lipid accumulation and generate the obese model, alongside treatment with each apple pulp extract or the positive control orlistat. As expected, lipid deposition was significantly elevated in the glucose-treated population compared to the untreated controls ($p<0.0001$; Fig. 1A and B). Apple pulp extracts alone, in the absence of glucose, did not significantly affect lipid accumulation relative to the NGM control, confirming that neither extract altered baseline fat storage under non-obese conditions. In the obese model, supplementation with ‘Amarilla de Octubre’ pulp extract at both tested concentration (250 and 500 µg/mL) led to a significant reduction in *C. elegans* lipid deposition, compared to the glucose-only condition (19.40% and 23.24% reduction, respectively). In contrast, ‘Pinova’ pulp extract did not produce significant changes in lipid deposition at either concentration under glucose exposure, indicating a differential bioactive potential between the two cultivars.

Similar findings were also obtained from lipid droplet (LD) image analysis. A statistically significant increase in the average LD area-to-worm area ratio was observed in glucose-treated nematodes compared to the untreated controls ($p<0.001$, Fig. 1B). Supplementation with the ‘Amarilla de Octubre’ pulp extract significantly reduced this ratio at both 250 µg/mL ($p<0.05$) and 500 µg/mL ($p<0.01$) (Fig. 1B). However, the ‘Pinova’ apple extract did not produce significant changes in the LD profile at either concentration relative to the obese condition. These findings are consistent with previously reported lipid-lowering effects of (poly)phenol-rich extracts in *C. elegans*, attributed to modulation of fat synthesis, storage and consumption^{19,20}. The differential response between the two cultivars may reflect the higher (poly)phenolic content of the ‘Amarilla de Octubre’ extract, particularly its enrichment in procyanidin B2 and chlorogenic acid, which have been individually associated with lipid homeostasis modulation^{21–23}.

Table 1 Chemotaxis index of *C. elegans* exposed to apple pulp extracts at 1000 and 2000 µg/mL.

Sample	Concentration (µg/mL)	Chemotaxis index
Amarilla de Octubre	1000	0 ± 0.081
Pinova		−0.143 ± 0.045
Amarilla de Octubre	2000	0.070 ± 0.104
Pinova		−0.0786 ± 0.176

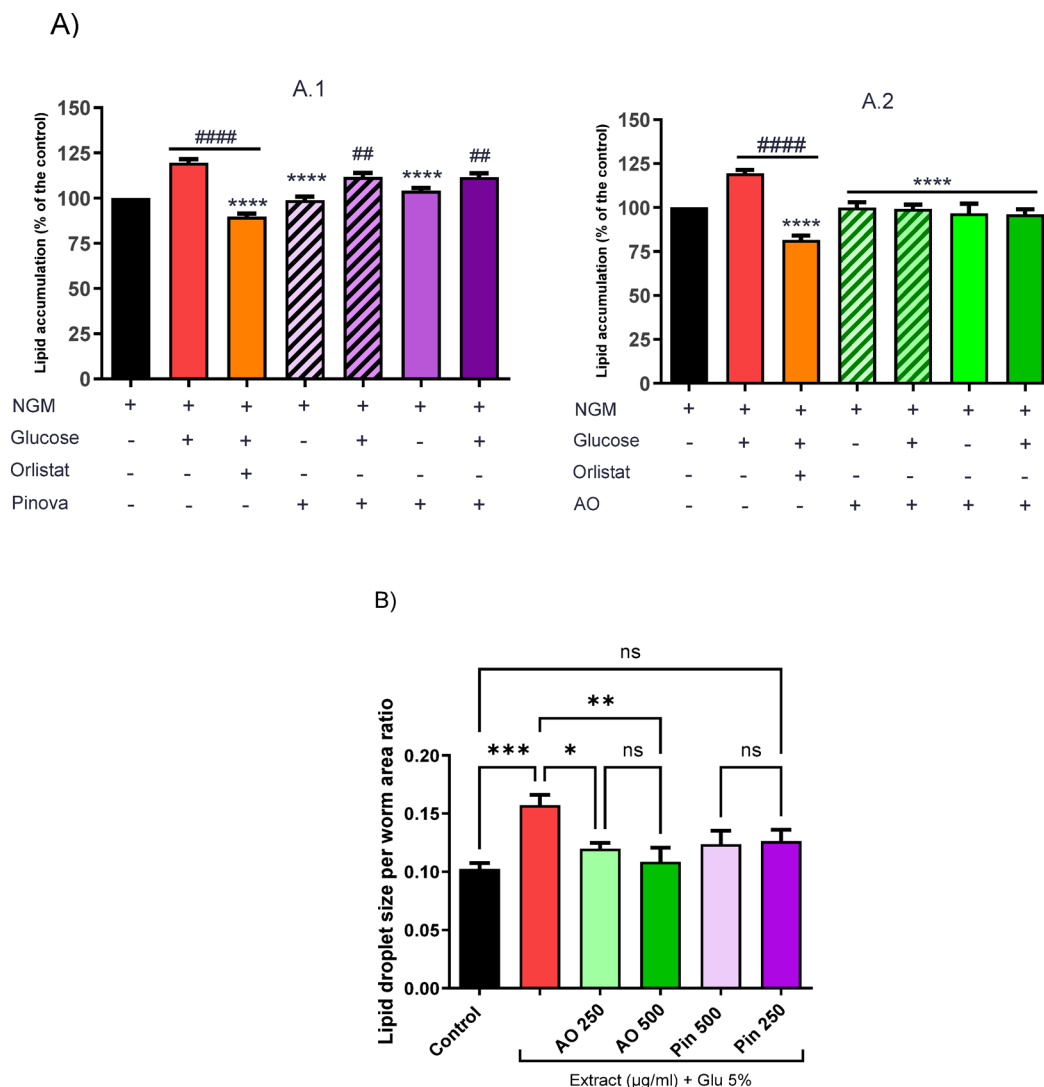


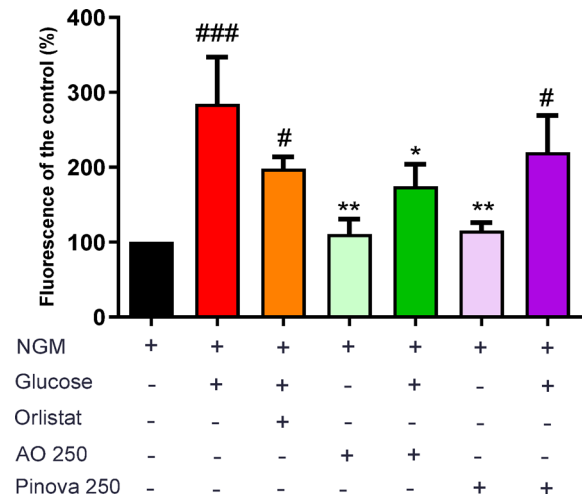
Fig. 1 Effect of apple pulp extracts on lipid accumulation in the *C. elegans* obese model. **(A)** Relative lipid content in nematodes exposed to ‘Pinova’ (A.1) or ‘Amarilla de Octubre’ (A.2) extracts at 250 μg/mL (stripped bars) and 500 μg/mL (solid bars); data from ≥ 40 worms per condition. **(B)** Average lipid droplet area-to-worm area ratio under all tested conditions. Results are expressed as mean $\pm$ SEM; **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ versus Glucose; ##### $p < 0.0001$, ## 0.01 versus NGM.

Bacterial intake quantification

Bacterial intake was determined by measuring the fluorescence accumulated in the gut of nematodes fed GFP-expressing OP50 *E. coli* under all tested conditions (Fig. 2). Under basal conditions, neither apple pulp extracts significantly affected bacterial consumption relative to untreated NGM controls, suggesting no influence on feeding behaviour in non-obese worms.

In the obese model, glucose supplementation led to a general increase in bacterial accumulation, consistent with previously reported feeding alterations under high-glucose conditions²⁰. Orlistat similarly reduced bacterial accumulation as previously described²⁰. Notably, only the ‘Amarilla de Octubre’ extract significantly reduced bacterial intake in glucose-supplemented nematodes compared to the obese control ($p < 0.05$), while the ‘Pinova’ extract had no significant effect. This cultivar specific attenuation of glucose-induced hyperphagia, without affecting baseline feeding behaviour, suggest that the bioactive compounds present in ‘Amarilla de Octubre’ may exert

Fig. 2 Effect of apple extracts on bacterial intake in *C. elegans* under basal and high-glucose conditions. Fluorescence intensity of GFP-expressing OP50 *E. coli* accumulated in the gut of L3 N2 worms after 48 h exposure to different growing conditions. Data are normalized to the NGM control group and represent means $\pm$ SEM from ≥ 3 independent replicates (≥ 120 worms per condition). * $p < 0.005$, ** $p < 0.01$ vs. glucose; ### $p < 0.001$, # $p < 0.05$ vs. NGM.



a satiety-related effect under conditions of metabolic overload, consistent with findings reported for other apple (poly)phenol extracts in murine models¹⁹.

ROS production under sublethal thermal stress

Increased carbohydrate-lipid intake has been demonstrated that promotes deleterious effects in *C. elegans* and glucose could act as a toxic compound affecting the normal development and physiological state of the worms²⁴. The abnormal functioning of mitochondria and the dysregulation of ROS production play a crucial role in lifespan, longevity or fat accumulation in nematodes. Sub-lethal treatments such as thermal stress enhances ROS production and activates antioxidant defences to counteract the pro-oxidant situation.

Sublethal thermal stress was applied to 10-day old nematodes to assess the capacity of the apple pulp extract to modulate oxidative stress under conditions that promote ROS overproduction. Our results show that the ‘Amarilla de Octubre’ pulp extract significantly reduced ROS production at both tested concentrations (250 and 500 $\mu\text{g}/\text{mL}$) in the obese model, with the combined ‘Amarilla de Octubre’ + glucose treatment showing substantially lower ROS values compared to the glucose-only condition (Fig. 3A). Moreover, ‘Amarilla de Octubre’ also attenuated ROS production in non-obese worms at both concentrations, and at 500 $\mu\text{g}/\text{mL}$ stabilized ROS levels at 80 and 90 min to values not significantly different from those of the non-stressed population. The ‘Pinova’ extract showed a significant reduction in ROS levels only at 500 $\mu\text{g}/\text{mL}$, while no significant effects were observed at 250 $\mu\text{g}/\text{mL}$ relative to the obese condition (Fig. 3B), indicating a more limited and concentration-dependent antioxidant capacity compared to the autochthonous cultivar.

These cultivar-dependent differences in antioxidant capacity may be attributed to the higher-total (poly)phenolic concentration in ‘Amarilla de Octubre’ relative to ‘Pinova’, particularly its enrichment in procyanidin B2 and chlorogenic acid, which remained notably even at 250 $\mu\text{g}/\text{mL}$ ¹³. Although the two cultivars share a broadly similar (poly)phenolic profile, quantitative differences in these key compounds likely underlie the observed difference in bioactivity. Flavonoids and phenolic acids have outstanding antioxidant properties, and when present together, as in many fruits like apples, they may act synergistically to counteract abnormal ROS accumulation by promoting enzyme action or through their direct scavenging potential as demonstrated in previous in vitro assays^{13,25}. Particularly, procyanidin B2 has been associated with lower ROS production in mice via factor-erythroid 2-related factor 2 (Nrf2)²⁶, while chlorogenic acid has been shown to protect *C. elegans* against thermal stress via hypoxia-inducible factor (HIF-1), heat-shock transcription factor (HSF-1), and autophagy-dependent mechanisms²⁷. Together, these findings suggest that the superior antioxidant response observed for ‘Amarilla de Octubre’ is likely driven by the combined and potentially synergistic action of these compounds at physiologically relevant concentrations.

Fig. 3 ROS production in *C. elegans* under sublethal thermal stress following treatment with ‘Amarilla de Octubre’ (A) and ‘Pinova’ (B) apple pulp extracts. Fluorescence intensity data are normalized to the non-stress control and expressed as mean values±SEM (*n*=3). Statistical significance between groups at each time point was determined by two-way ANOVA and is showed in the table below each figure (** *p*<0.01, *** *p*<0.001 **** *p*<0.0001 vs. obese condition).

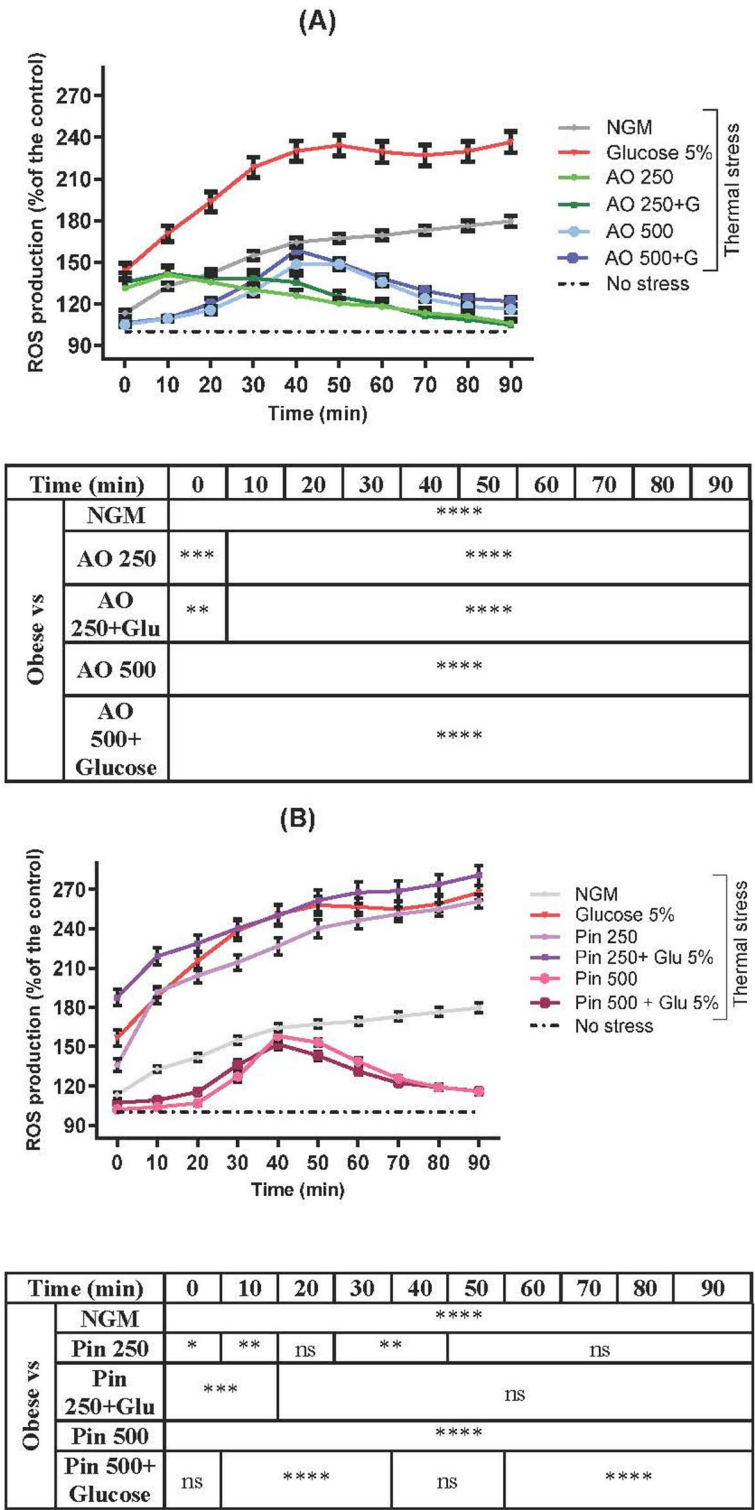
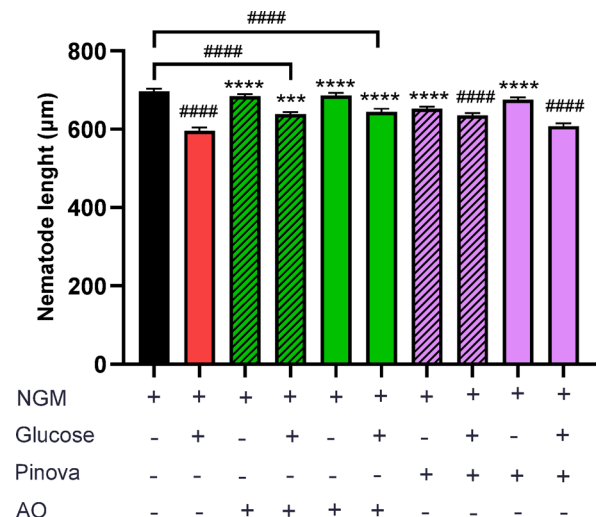


Fig. 4 Effect of apple pulp extracts on body length of *C. elegans* under basal and high-glucose conditions. Striped bars represent the 250 $\mu\text{g/mL}$ treatment; solid bars represent the 500 $\mu\text{g/mL}$ treatment. Results are expressed as mean $\pm$ SEM ($n=3$; ≥ 70 worms per condition). Statistical differences were determined by one-way ANOVA; **** $p<0.0001$, *** $p<0.001$, ** $p<0.01$, * $p<0.05$ versus glucose; ##### $p<0.0001$ vs. NGM.



Health span-related parameters

To assess whether the apple pulp extracts could attenuate the broader physiological deterioration associated with chronic glucose exposure, three health span-related markers were evaluated in the presence of 5% glucose: body length, pharyngeal pumping rate, and thrashing rate. Enhanced stress resistance and improved locomotion in *C. elegans* serve as indicators of an extended health span and healthier aging. Interestingly, chlorogenic acid, one of the major compounds in our extracts, also have been linked with delayed age-related reduction in pumping rates as well as improved stress resistance in *C. elegans*²⁸.

Body length

The cumulative metabolic disruptions due to high-glucose diets (HGD) in *C. elegans* compromise their normal growth and body size²⁰. Apple pulp extracts alone did not significantly affect body length relative to the NGM control, indicating no intrinsic effects on growth under basal conditions. However, supplementation with ‘Amarilla de Octubre’ extract at 250 and 500 $\mu\text{g/mL}$ partially rescued this glucose-induced growth reduction, yielding worms 5.47% and 6.36% longer than the obese group, respectively. In contrast, the ‘Pinova’ extract showed no significant differences among any of the glucose-treated conditions.

Pharyngeal pumping rate

Nematodes adapt to variations in metabolic and nutritional status through behavioural responses, such as alterations in food intake and hunting, while their pharyngeal pumping behaviour is regulated by both internal and external neuromodulation²⁹. In young adult worms, pumping rates are at their peak; however, they decline rapidly with age from day 1 to day 7 of adulthood, and by day 10 to day 15, pumping becomes insignificant. This decline, which correlates with reductions in other locomotory and bending markers, underscores the association between pharyngeal function and the progressive loss of physiological integrity.

Regarding previous effective treatment lowest concentration, we assessed the extracts at 250 $\mu\text{g/mL}$. Pharyngeal pumping rates declined with age in all groups, with the untreated control population showing a 41.36% reduction from day 7 to day 10 of adulthood, consistent with normal age-related physiological decline³⁰. Apple pulp extracts alone did not significantly affect pumping rates at either time point compared to the NGM control, indicating no intrinsic effects on pharyngeal function under basal conditions. Glucose supplementation

significantly reduced pumping rate compared to untreated controls, with reductions of 22.78% and 39.98%, recorded at days 7 and 10, respectively.

Significant attenuation of the glucose-induced pumping decline was observed in nematodes co-treated with ‘Amarilla de Octubre’ extract and glucose, with pumping rates reduced by only 6.34% at day 7 and 2.57% at day 10 compared to the NGM control, substantially less than the reductions observed in the obese group. Notably, pumping rate in the ‘Amarilla de Octubre’ plus glucose group were not significantly different from those of nematodes treated with ‘Amarilla de Octubre’ extract alone (Fig. 5). In addition, nematodes treated with ‘Amarilla de Octubre’ extract alone showed a 13.48% higher pumping rate than untreated controls at day 10, suggesting a positive effect on pharyngeal function independent of metabolic stress. The ‘Pinova’ extract did not produce significant effects on pumping rates under any tested conditions, either in presence or absence of glucose.

These results are consistent with a protective role of ‘Amarilla de Octubre’ against glucose-induced deterioration of pharyngeal function during ageing. The attenuated decline in pharyngeal pumping observed upon treatment with these autochthonous extracts aligns with previous reports in which polyphenol-rich extracts preserved pumping rates in aged *C. elegans*, further supporting the association between (poly)phenolic content and health span maintenance in this model³¹.

Nematode locomotion: thrashing rate

Thrashing rate was assessed as an additional locomotion-based health span marker over the first seven days of adulthood. Glucose supplementation significantly impaired locomotion from day 1 of adulthood, with the obese population showing 41.22% fewer thrashes per 30 s as compared to untreated controls. This is consistent with previously reported glucotoxicity-induced motility impairment in *C. elegans*³². Supplementation with both apple pulp extracts at 250 µg/mL improved thrashing rates compared to the obese groups at days 1 and 4 of adulthood. However, only the ‘Amarilla de Octubre’ extracts sustained this improvement through day 7, with thrashing rates 16.87%, 15.24% and 24.96% higher than the glucose-supplemented group at days 1, 4 and 7 of adulthood, respectively (Fig. 6). The ‘Pinova’ extract did not maintain significant differences beyond day 4. These findings

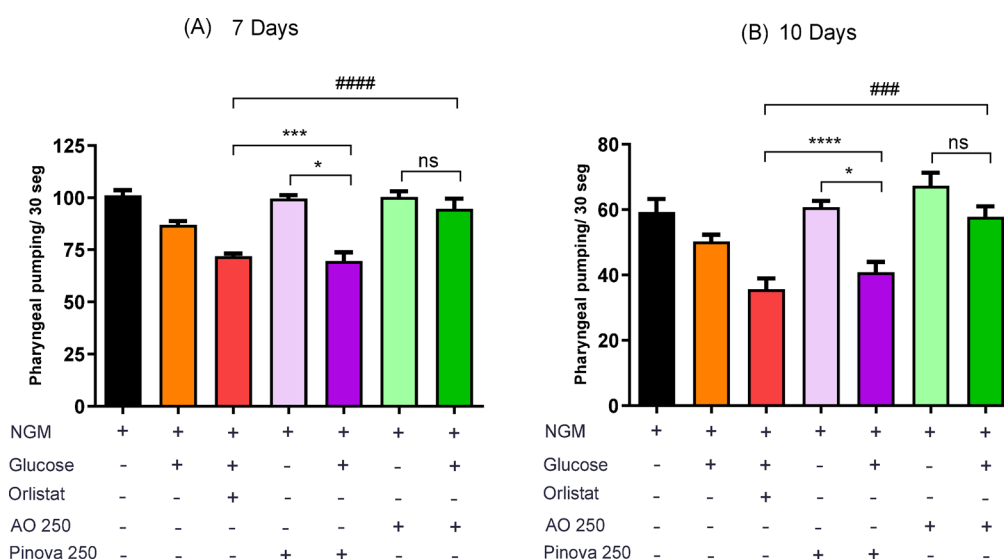
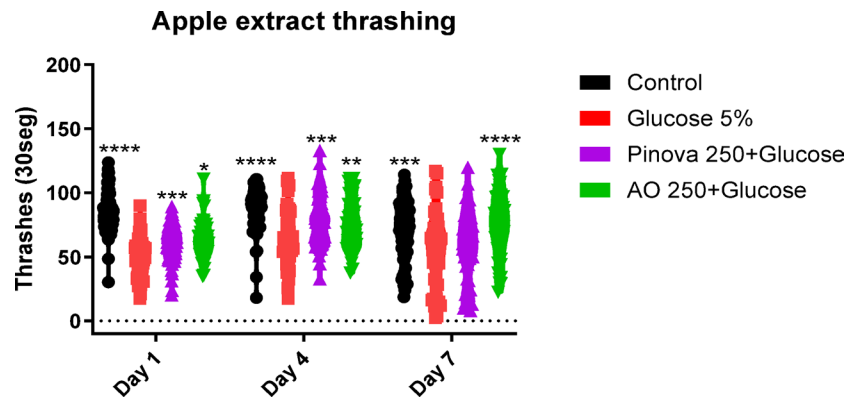


Fig. 5 Pharyngeal pumping rates in *C. elegans* at day 7 (A) and day 10 (B) of adulthood under basal and high-glucose conditions. Results are expressed as mean ± SEM ($n=3$, ≥ 30 worms per conditions). Statistical differences were determined by one-way ANOVA **** $p < 0.0001$, *** $p < 0.001$, * $p < 0.05$ versus glucose: ##### $p < 0.0001$, ### $p < 0.001$ versus NGM. Significant differences were determined by using ANOVA.

Fig. 6 Thrashing rates of *C. elegans* N2 at days 1, 4 and 6 of adulthood under basal and high-glucose conditions. Results are expressed as mean $\pm$ SEM ($n=3$, ≥ 45 worms per condition). Statistical differences were determined by one-way ANOVA; * $p<0.05$; ** $p<0.01$, *** $p<0.001$ **** $p<0.0001$ vs. glucose group)



suggest that the higher (poly)phenolic content of ‘Amarilla de Octubre’ may confer a sustained protective effect on locomotor function during ageing under metabolic stress conditions.

The anti-ageing and locomotion-promoting effects observed for ‘Amarilla de Octubre’ may be attributed to its rich (poly)phenolic composition, particularly the abundance of procyanidin B2 and chlorogenic acid. Health span enhancement by polyphenol-rich extracts has been previously reported in *C. elegans* treated with compounds derived from other plant foods, including olives³² and *Camellia sinensis*³⁴. In *C. elegans*, such effects are thought to be mediated at least in part through modulation of conserved metabolic signalling pathways, whereby (poly)phenolic compounds activate the insulin/IGF-1 signalling (IIS) pathway and stimulate the downstream transcription factor DAF-16/FOXO, leading to upregulation of stress defence genes encoding antioxidant enzymes such as superoxide dismutase (SOD)³⁵. The consistent improvement in pharyngeal pumping, thrashing rate, and body length observed for ‘Amarilla de Octubre’ but not ‘Pinova’, suggests that a threshold (poly)phenolic concentration may be required to engage these protective pathways under conditions of metabolic overload.

Conclusions

This study demonstrated that pulp extracts from the autochthonous Spanish apple cultivar ‘Amarilla de Octubre’ exert a broad spectrum of protective effects in a *C. elegans* model of glucose-induced metabolic dysfunction, outperforming the commercially selected cultivar ‘Pinova’ across all evaluated parameters. Specifically, ‘Amarilla de Octubre’ significantly reduced lipid accumulation, attenuated ROS overproduction under thermal stress, decreased glucose-induced hyperphagia, and preserved health span-related markers at concentrations as low as 250 $\mu\text{g/mL}$. The ‘Pinova’ extract showed limited and largely concentration-dependent effects, highlighting that commercial cultivar selection, which prioritises agronomic and sensory traits over phytochemical richness, may result in a substantial reduction in the health-promoting potential of apple as a functional food.

This comparison highlights differences related to the geographic and agricultural origin of the apple varieties. Commercial cultivars such as ‘Pinova’ have been selectively bred to meet consumer and market demands, whereas local varieties such as ‘Amarilla de Octubre’ have undergone adaptation to ambient and regional growing conditions. These differences may influence the phenolic composition of the fruit because of distinct selective pressures and environmental adaptation. Consequently, such variability provides a relevant opportunity to investigate potential implications for human health.

From a translational nutrition perspective, these findings suggest that the regular dietary consumption of (poly)phenol-rich apple varieties, such as traditional autochthonous cultivars currently underrepresented in commercial food chains, may contribute to the dietary management of metabolic risk factors associated with obesity and type 2 diabetes. While the concentrations used in this *C. elegans* model require further contextualisation against

realistic dietary intake levels in humans, the results provide a compelling preclinical basis for evaluating the functional food potential of heritage apple cultivars in human nutritional intervention studies.

More broadly, this work underscores the nutritional and public health value of preserving agrobiodiversity. Autochthonous cultivars such as ‘Amarilla de Octubre’ represent underexploited dietary sources of bioactive compounds whose health-promoting potential may be substantially superior to that of commercial dominant varieties. Their integration into functional food development strategies and dietary recommendations warrants further investigation, including assessment of (poly)phenol bio accessibility and bioavailability following consumption and gastrointestinal processing¹.

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Author contributions A.M. wrote the first draft of manuscript and performed experiments. S.N. set up experimental protocols. P.E. provided plant food material and apple extracts. M.E. supervised the research stay of A.M. at Kent University. C.G. and V. L. supervised the work and provided financial support for the project.

Data availability Data is available upon request to the corresponding or first author of the manuscript.

Declarations

Competing interests The authors declare no competing interests.

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