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Original research paper

ANTIMICROBIAL EFFECTS OF POLYPHENOLS FROM FERMENTED AND NON-FERMENTED APPLE AND CARROT POMACE AGAINST *ESCHERICHIA COLI*

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Abstract: The pig farming industry faces significant challenges during the weaning period, often resulting in reduced growth rates and higher mortality among piglets. Traditionally, antibiotics and zinc oxide have been used to manage these issues. However, concerns about antibiotic resistance and environmental pollution have led to strict European regulations limiting or banning their use. This fact has created an urgent need for alternative solutions, with polyphenols emerging as promising candidates due to their bioactive properties, including anti-inflammatory, antioxidant, and antimicrobial effects. These properties are particularly important for preventing weaning-related problems, which are frequently caused by the *Escherichia coli* F4 (K88) bacterium. The present study focused on evaluating the antimicrobial properties of polyphenols extracted from apple pomace and carrot pomace, both in their unfermented form and after fermentation with *Saccharomyces cerevisiae*. The antimicrobial activity was assessed *in vitro* by monitoring bacterial growth through absorbance measurements at different intervals over 24 hours, at 37°C. The results indicated that polyphenols possess significant antimicrobial effects in both their unfermented and fermented states. The polyphenol extract from apple pomace inhibited *E. coli* growth at a minimum concentration of 5.6 µg (GAE)/ml, while the extract from fermented apple pomace had a higher minimum inhibitory concentration of 50 µg (GAE)/ml. For carrot pomace, only the concentration of 151 µg (GAE)/ml showed an inhibitory effect on *E. coli* growth, whereas the polyphenol extract from fermented carrot pomace significantly reduced bacterial growth at a concentration of 303 µg (GAE)/ml. The extent of bacterial inhibition was influenced by the concentration of polyphenols and the specific types of polyphenols present in the extract. Notably, despite having a lower overall concentration, the fermented polyphenol extracts achieved similar levels of bacterial growth inhibition as the unfermented extracts at higher concentrations. These findings highlight the potential of agro-industrial by-products such as apple and carrot pomace to provide bioactive compounds that could serve as effective alternatives to antibiotics and zinc oxide in livestock farming. By exploiting these natural resources, the industry might move towards more sustainable and environmentally friendly practices while still effectively managing the challenges associated with weaning piglets.

Keywords: *weaning, antimicrobial, agro-industrial by-products, polyphenols, E. coli*

INTRODUCTION

Weaning is the most challenging phase in piglet development (Zheng, Duarte, Loftus, & Kim, 2021). The shift from a milk-based diet to solid feed triggers significant morphological

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changes in the small intestine and colon of piglets, including the degradation of intestinal villi and decreased nutrient absorption (Zheng et al. 2021). These changes increase the permeability of the intestinal epithelium, leading to prolonged inflammation and making piglets susceptible to various pathogens (Zheng et al. 2021). *Escherichia coli* F4 (K88) is among the primary pathogens causing piglet mortality, inducing severe inflammatory responses and diarrhoea (Fairbrother, Nadeau & Gyles, 2005). During this period, reduced feeding may result in weight loss and often death among piglets. Consequently, the weaning crisis causes substantial economic losses in pig farming due to mortality and costly treatments. *E.coli* F4 infections are common worldwide, but the use of antibiotics is limited due to the risk of antibiotic resistance, which leads to the development of more resistant and virulent strains (Fairbrother et al., 2005).

In response to growing concerns about antibiotic resistance, the European Union took significant action in 2006 by banning the use of antibiotics as growth promoters in piglets (EC, 2005, December 22). This decision was aimed at curbing the development of antibiotic-resistant bacteria, which pose a serious threat to both animal and human health (EC, 2005, December 22). However, to address this issue, zinc oxide was introduced into piglet feed as an alternative growth promoter and health management tool (Bhatta, Chamings & Alexandersen, 2021).

Zinc oxide initially proved efficient in mitigating the weaning crisis, as it helped to control diarrhoea and improve piglet growth rates. However, its widespread use soon raised environmental and health concerns (Bhatta, et al., 2021). In 2017, a comprehensive report by the European Medicines Agency and its Committee for Medicinal Products for Veterinary Use revealed several risks associated with zinc oxide (EMA, 2017). The report highlighted that the use of zinc oxide contributed to soil pollution, as the compound accumulated in the environment through animal waste. Additionally, the report noted an increase in microbial resistance to zinc oxide, which also heightened resistance to antibiotics (EMA, 2017). The European Union reassessed the use of zinc oxide in piglet feed due to concerns about environmental health and antibiotic resistance. In 2022, the CE banned zinc oxide for managing the weaning crisis in piglets (Bhatta et al., 2021). This decision highlighted the EU's commitment to sustainable agriculture and public health, prompting the search for alternative solutions to support piglet health without harming the environment or increasing antibiotic resistance (Bhatta et al., 2021).

Agro-industrial byproducts could represent a viable alternative to the use of antibiotics and zinc oxide in piglet feed. These byproducts, often considered waste, are rich in numerous bioactive compounds, such as polyphenols, which have potential health benefits for livestock (Almaraz-Sánchez, Amaro-Reyes, Acosta-Gallegos & Mendoza-Sánchez, 2022). Using these byproducts capitalizes on their nutritional and therapeutic properties, providing a sustainable solution for managing the weaning crisis in piglets (Correddu et al. 2020; Almaraz-Sánchez et al. 2022). Incorporating polyphenols derived from agro-industrial byproducts into piglet feed could enhance the health and growth of piglets during the critical weaning period (Correddu et al. 2020; Almaraz-Sánchez et al. 2022). These compounds have been shown to possess antimicrobial, anti-inflammatory, and immunemodulatory properties, which can help mitigate the adverse effects of weaning, such as diarrhoea and growth delays (Correddu et al. 2020; Almaraz-Sánchez et al. 2022). Moreover, the use of these natural alternatives can reduce reliance on antibiotics and zinc oxide, thereby addressing concerns related to antibiotic resistance and soil pollution (Correddu et al. 2020, Almaraz-Sánchez et al. 2022).

Additionally, ingested polyphenols reach the colon at a rate of 90-95%, where they interact directly with the gastrointestinal microbiota (Grosu, Pistol, Taranu & Marin, 2019). The therapeutic effects of polyphenols are manifested at the site of bacterial infections through their anti-inflammatory activity, antioxidant properties, and direct antimicrobial action (Sun, Liu, Lin, Gao & Wang, 2024). The environmental benefits of using agro-industrial by-products are significant. Typically, the disposal of these materials through burning or landfilling poses considerable environmental risks, including air pollution and soil contamination (Correddu et al. 2020, Almaraz-Sánchez et al. 2022). In the last decade, by-products are used as feed additives, reducing the environmental footprint associated with their disposal. This practice aligns with circular economy principles, promoting resource efficiency and waste minimization (Correddu et al., 2020, Almaraz-Sánchez et al., 2022).

This study investigates the *in vitro* antimicrobial effect of four polyphenolic extracts derived from apple pomace (AP) and carrot pomace (CP) against *E. coli*. These plants are recognized as rich sources of polyphenols, which possess antimicrobial, antioxidant, and antiinflammatory properties (Fer-nandes et al. 2019; Cheaib, Raafat & El Darra, 2020).

In addition to testing the raw polyphenol extracts, the study also evaluates the impact of fermenting apple and carrot pomace with *Saccharomyces cerevisiae* on enhancing the antimicrobial properties of the polyphenols. Fermentation is known to alter the chemical composition of plant materials, potentially increasing the bioavailability and concentration of beneficial compounds. Therefore, tests were conducted using polyphenolic extracts from both non-fermented (AP) and fermented apple pomace (FAP), as well as non-fermented (CP) and fermented carrot pomace (FCP). Generally, fermentation led to the production of antimicrobial peptides and generated polyphenols with more potent antimicrobial effects (Rodríguez Madrera, Pando Bedriñana & Suárez Valles, 2017; Vlassa et al. 2022; Palacios-Velásquez, Quispe-Coquil, Casimiro-Soriano, Tapia-Zarate & Huamán-De la Cruz, 2023).

By exploring these natural and sustainable alternatives, the research aims to contribute to the development of new strategies for managing bacterial infections, reducing reliance on traditional antibiotics, and addressing the growing concern of antibiotic resistance.

MATERIAL AND METHODS

The main chemicals and equipment used in the experiments included Acetone HiPerSolv CHROMANORM® for HPLC, LB Broth (Luria/Miller) from Carl Roth, Folin-Ciocalteu's phenol reagent and sodium carbonate from Merck, gallic acid from Sigma, and methanol from Honeywell CHROMASOLV™ LC-MS Ultra, tested for UHPLC-MS. The devices used comprised a Centrifuge Rotina 380R from Hettich, a Microplate Reader Varioskan LUX Multimode from Thermo Fisher Scientific, an Orbital Shaker-Incubator ES-20 from Biosan, and a Rotary vacuum concentrator RVC 2-18 Cdplus from CHRIST. Additionally, a Spectrophotometer Specord 250 from Analytik Jena, Microwave-Assisted Extractor from Milestone Ethos Easy equipment (Milestone Srl, Bergamo, Italy), and syringe filter (0.45 μM, Millex Millipore) from Merck were utilized throughout the procedures.

The extraction of polyphenols from agro-industrial byproducts

Apples and carrots were sourced from a local producer. Apple pomace and carrot pomace were collected as by-products following the juice extraction process from apples and carrots. The fermentation of AP and CP was carried out using *S. cerevisiae* (commercial active dry baker's yeast produced by Pakmaya, Istanbul, Turkey), following the method described by Plaipetch and Yakupitiyage (2013). Two extraction methods were used to obtain the polyphenol extracts from the AP and CP: the conventional method for AP and the microwave-assisted extraction (MAE) method for CP, as polyphenols in CP, could not be optimally extracted using the conventional method. The conventional method involved mixing one gram of AP or FAP with 7 ml of 80% acetone, with continuous stirring for 24 hours at room temperature. The samples were then centrifuged for 10 minutes at 8000 RFC. The supernatant was subjected to acetone evaporation at 40 °C, and the extract was sterilized using 0.45 μm pore-size filters. Following the extraction of total polyphenols from carrot pomace and fermented carrot pomace with the conventional method, a low concentration of these compounds resulted, so a second extraction was made by using the microwave-assisted extraction (MAE) method. This method involved the mixing of two grams of CP or FCP with 20 ml of 80% methanol. The extraction was performed at a power of 500 W and a temperature of 70 °C for 30 minutes. The samples were then centrifuged for 10 minutes at 8000 RFC. The supernatant was subjected to methanol evaporation at 40 °C, and sterile filtered using 0.45 μm pore-size filters.

Measurement of total polyphenol content

The total polyphenol concentration was determined using the Folin-Ciocâlteu method. Initially, 10 μl of extract was diluted in 790 μl of distilled water, followed by the addition of 50 µl of Folin-Ciocâlteu reagent. After one minute, 150 µl of a 20% sodium carbonate solution was added to the mixture, which was then kept in the dark for two hours. Subsequently, a spectrophotometer was used to measure the absorbance of the homogenates at a wavelength of 750 nm. The results were compared to a standard curve obtained using gallic acid, and the final concentrations were expressed as gallic acid equivalents (GAE).

E.coli preparation

The *E.coli* F4 (K88) strain, kindly provided by Dr. Philippe Pinton (I.N.R.A.E, Toxalim, Toulouse, France), was cultured overnight in Luria-Bertani (LB) medium at 37 °C. Following the method of Roselli, Finamore, Pando Bedriñana and Suárez Valles (2003), 1 µl of culture aliquot was inoculated into 10 µl of LB medium.

Subsequently, the culture was diluted 100-fold in fresh LB medium and incubated for an additional 4 hours. Bacterial growth was quantified by measuring the optical density at 600 nm using a Tecan Sunrise plate reader.

Assessment of antimicrobial effects of apple and carrot polyphenolic extracts

Serial dilutions of the polyphenol extracts were prepared with distilled water using a 1:2 dilution factor. The polyphenolic concentrations tested for the apple pomace extract varied from 5.59 µg/ml to 358 µg/ml, while the fermented apple pomace extract had concentrations ranging from 3.12 µg/ml to 200 µg/ml. For the carrot pomace extract, the concentrations ranged from 2.36 µg/ml to 151 µg/ml, and for the fermented carrot pomace extract, the tested concentrations ranged from 4.73 µg/ml to 303 µg/ml. The antimicrobial activity of each resulting polyphenol concentration was tested against *E.coli* F4. The assessment was conducted in 96-well plates, with each well containing 277 µl of LB medium, 20 µl of polyphenol extract, and 3 µl of *E.coli* F4 and cultured overnight, at 37 °C. The bacterial growth curve was monitored for 27.5 hours by measuring the absorbance at a wavelength of 600 nm every 30 minutes. Prior to each measurement, the plate was shaken for 15 seconds at 120 RPM.

Statistical analysis

The results were analyzed statistically using GraphPad Prism 10.2.0 software. One-way analysis of variance (ANOVA) was utilized to compare the means of different groups and determine if there were any statistically significant differences among them. Following the ANOVA, Tukey's post-hoc test was conducted to make pairwise comparisons between group means, identifying which specific groups differed from each other.

RESULTS AND DISCUSSION

Polyphenolic concentration in fermented and non-fermented apple and carrot pomace

Following conventional extraction, the total polyphenol concentration in the unfermented carrot pomace extract was 0.6 mg GAE/ml, while the fermented carrot pomace extract contained 0.1 mg GAE/ml. In contrast, polyphenol concentrations obtained through microwave-assisted extraction (MAE) were significantly higher, with 2.27 mg GAE/ml in carrot pomace (CP) and 4.55 mg GAE/ml in fermented carrot pomace (FCP). Apple pomace (AP) displayed the highest polyphenol content at 5.37 mg GAE/ml, which dropped to 3 mg GAE/ml in fermented apple pomace (FAP) (Table 1).

While fermentation led to a decrease in polyphenol concentration in apple pomace, it resulted in an increase in polyphenol levels in carrot pomace. This can be attributed to the breakdown of cell walls during fermentation, which releases a higher concentration of polyphenols into the medium. Mekoue Nguela,

Table 1.
Concentration of total polyphenols in investigated by-products extracts

Meal	Total polyphenols (mg GAE/ml)
Apple Pomace (AP)	5.37
Fermented Apple Pomace (FAP)	3.00
Carrot Pomace (CP)	2.27
Fermented Carrot Pomace (FCP)	4.55

GAE = Gallic acid equivalent

Vernhet, Julien-Ortiz, Sieczkowski and Mouret (2019) observed a similar effect in wine production, where fermentation increased polyphenol levels by breaking down grape cell walls and releasing anthocyanins and tannins. Yeast activity also plays a role in polyphenol polymerization and stabilization, influencing their composition (Mekoue Nguela et al. 2019). Xu et al. (2022) also found that *S. cerevisiae* fermentation could boost polyphenol content in fruit wines. Similarly, Vlassa et al. (2022) showed that although fermentation of rapeseed meal with *S. cerevisiae* reduced the overall polyphenol concentration, it increased the levels of polyphenols with strong antimicrobial properties, such as gallic acid and sinapic acid. These findings suggest that the effects of fermentation on polyphenol levels vary depending on the type of pomace, likely due to differences in composition or interactions of bioactive compounds during the fermentation process.

Impact of polyphenolic extracts on bacterial growth

As a general trend, all tested polyphenol extracts significantly inhibited the proliferation of *E.coli*. Additionally, it was observed that the effectiveness in inhibiting microbial growth depended not only on the concentration of polyphenols but also on the specific types of polyphenols present in the extract. In some cases, lower concentrations of polyphenols demonstrated the same antimicrobial effect as concentrations that were twice as high. The extract with the most effective antibacterial effect was the one obtained from apple pomace.

Antibacterial properties of polyphenolic extract from non-fermented apple pomace

The results demonstrate the dose-dependent effect of polyphenols extract from apple pomace (AP) on *E.coli* growth. At the highest concentration of 358 μg GAE/ml, there is an approximate 71.28% reduction in *E.coli* growth compared to control, which is statistically significant with a p-value of <0.0001. The concentration of 179 μg GAE/ml results in a 58.73% reduction in growth (p-value <0.0001). As the concentration decreases, the inhibitory effect on *E.coli* growth becomes slightly less pronounced but remains significant compared to control. Even at the lowest tested concentration of 5.59 μg GAE/ml, there is a 48.61% reduction in *E.coli* growth, with a p-value of <0.0001. This consistent statistical significance across all concentrations indicates that polyphenols from apple pomace are highly effective in inhibiting *E.coli* growth (Fig. 1). The polyphenol content in apple pomace is approximately 5 g/kg, with 2.88 g/kg consisting of flavan-3-ols (mainly catechins and procyanidins) and 1.92 g/kg of flavonols (primarily quercetin glycosides like hyperoside, quercitrin, isoquercitrin, and rutin), 0.14 g/kg from dihydrochalcones (mainly phloridzin), and 0.03g/kg from hydroxycinnamic acids (primarily 5-caffeoylquinic acid) (Fernandes et al. 2019). However, these concentrations and the specific polyphenols in the final extract can vary depending on the solvent and its concentration used during extraction, which affects the antimicrobial activity of the extract (Zhang et al. 2016).

For instance, in a study by Zhang et al. (2016), ethyl acetate (100%) was used for polyphenol extraction, resulting in phloridzin as the most abundant polyphenol, followed by phloretin, procyanidin B2, quercetin and its derivatives (quercetin-3-O-pentoside, quercetin-3-O-rhamnoside), chlorogenic acid, gallic acid, syringin, and hyperin.

The study also assessed antimicrobial activity against *E.coli* and *S. aureus*, finding significant effects, with phloretin which showed the strongest antimicrobial activity. Also, it was demonstrated a 36% inhibition of *E. coli* (ATCC 25922) growth at a polyphenol concentration of 2500 μg/ml, with effects even at 78 μg/ml, mainly attributed to epicatechin, procyanidin, and quercetin from apple pomace extract (Zardo et al. 2020). Interestingly, Beermann, Gruschwitz, Walkowski and Annekathrin (2013) highlighted another benefit of apple pomace extract, showing not only a 15% reduction in *E.coli* growth rate with 1.5% apple pomace extract, but also an enhancement in the growth of certain *Lactobacillus* species, such as *Lactobacillus brevis*, *Lb. sanfranciscensis*, and *Lbs reuteri*, beneficial for gastrointestinal health with the same concentration (1.5%) of apple pomace extract. In contrast, films containing 4% and 8% apple pomace did not significantly inhibit *E.coli* growth, suggesting that higher concentrations may be necessary for antimicrobial effects in films (Carpes et al. 2021).

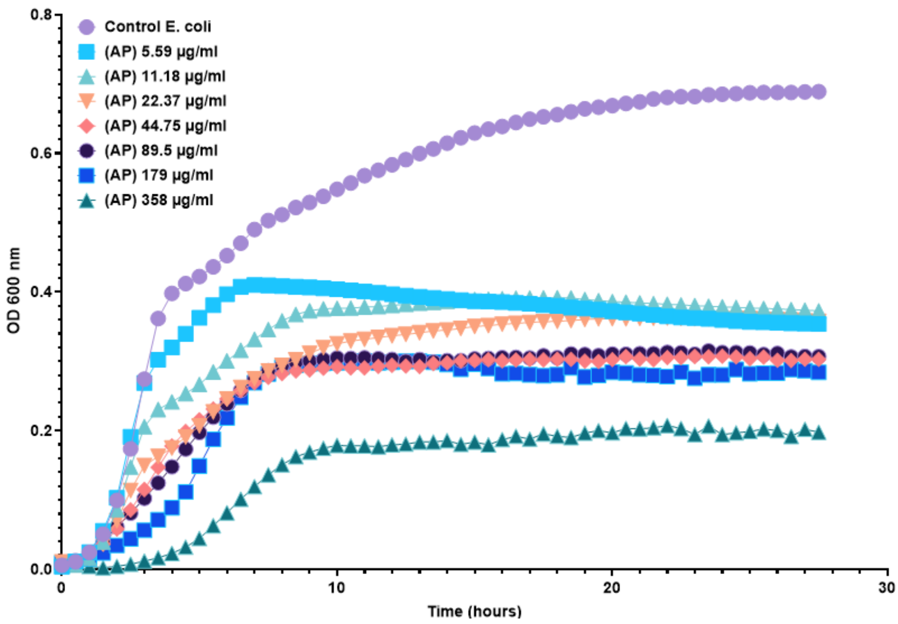

Figure 1. The impact of AP polyphenolic extract on *E.coli* F4 (K88) growth was assessed by incubating serial dilutions of the extract with varying PT concentrations for 27.5 hours. Bacterial growth inhibition was tracked by measuring the optical density at 600 nm at 30-minute intervals

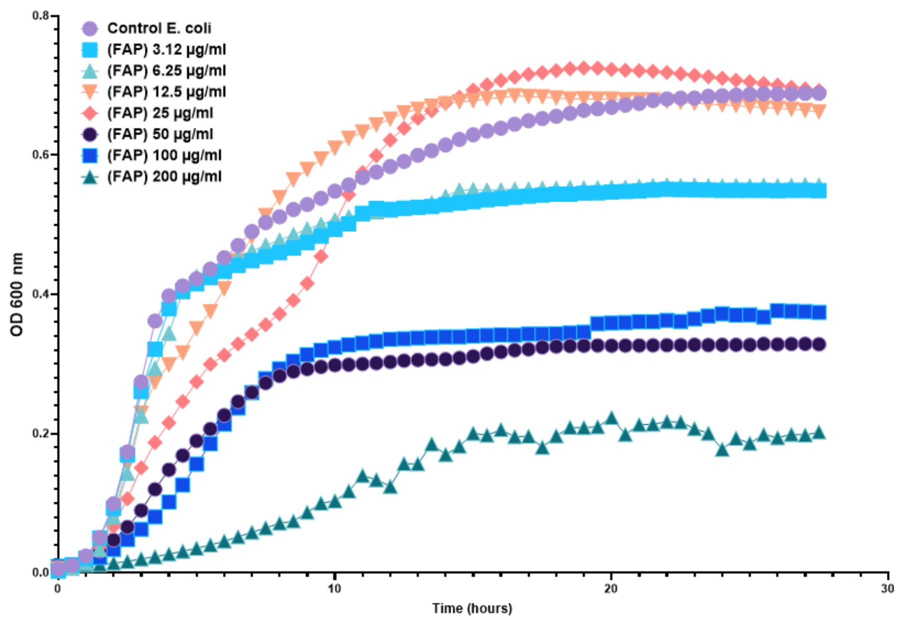

Figure 2. The impact of FAP polyphenolic extract on *E.coli* F4 (K88) growth was assessed by incubating serial dilutions of the extract with varying PT concentrations for 27.5 hours. Bacterial growth inhibition was tracked by measuring the optical density at 600 nm at 30-minute intervals

Antibacterial properties of polyphenolic extract from fermented apple pomace

The results of Fig. 2 demonstrate the inhibitory effect of polyphenols from fermented apple pomace (FAP) on *E.coli* growth comparable with the results obtained with polyphenolic extract from unfermented AP. At the highest concentration of 200 µg GAE/ml, there is an approximate 70.63% reduction in *E.coli* growth compared to the control, with a statistically significant p-value of <0.0001. The serial following concentration of 100 µg GAE

/ml led to a 45.69% reduction in bacterial growth (p-value = 0.0049), while the concentration of 50µg GAE/ml resulted in a 52.33% reduction (p-value = 0.0007). Lower concentrations, below 50µg GAE/ml, did not show significant inhibition of bacterial growth (Fig. 2).

Studies have shown that the polyphenol content in apple pomace decreases after fermentation with *S. cerevisiae* (Rodríguez Madrera et al. 2017). Moreover, the levels of chlorogenic acid and protocatechuic acid remained largely unchanged.

The concentration of phloridzin, however, was influenced by the specific *S. cerevisiae* strain used, the *S. cerevisiae* C6 strain for example notably increasing phloridzin levels.

Additionally, two dihydrochalcones, labelled PG-1 and PG-2, increased during fermentation, indicating that *S. cerevisiae* fermentation might produce new phenolic derivatives.

Among flavonols, some quercetin glycosides (like hyperin and isoquercitrin) significantly decreased, while the aglycone quercetin significantly increased (Rodríguez Madrera et al. 2017).

The increase in phloridzin concentration might explain the enhanced antimicrobial effecttiveness of fermented apple pomace compared to its unfermented form, as chlorogenic acid, epicatechin, and phloridzin are known for their antimicrobial activities against bacteria like *S. aureus*, *E. coli*, and *S. enterica* (Martau, Teleky, Ranga, Pop & Vodnar, 2021).

Furthermore, the addition of apple pomace during cider fermentation helped maintain polyphenol levels and increased antioxidant activity, particularly in polyphenols like phloridzin, caffeic acid, p-coumaric acid, and flavonols (Bortolini et al. 2020).

By contrast, Munekata et al. (2021) highlighted that after fermenting apple pomace with *S. cerevisiae*, there was a slight decrease in polyphenol content.

In some instances, an initial increase in polyphenol levels was observed due to the release of bound polyphenols by microbial enzymes, although prolonged fermentation might lead to a decrease due to polyphenol degradation (Munekata et al. 2021).

Comparison between the inhibitory effect of polyphenolic extract from AP and FAP

The results indicate that although the polyphenolic concentration in unfermented apple pomace is nearly double that in fermented apple pomace, their effectiveness in inhibiting *E. coli* growth is almost equivalent, with no statistically significant differences observed between corresponding concentrations of serial dilutions from AP and FAP.

This suggests that the fermentation process does not significantly alter the antimicrobial efficacy of polyphenols from both sources of apple pomace, even when their concentration is reduced. There is a possibility that fermentation enhances the activity of the polyphenols, compensating for the lower concentration in FAP.

For instance, the highest polyphenolic concentration from AP of 358 µg GAE/ml achieves an approximate 71.28% reduction in *E.coli* growth compared to the control (p-value <0.0001), while correspondingly, the highest concentration in FAP of only 200 µg GAE/ml results in a similar 70.63% reduction (p-value <0.0001).

The comparison between these two concentrations (AP 358µg GAE /ml vs. FAP 200µg GAE /ml) reveals no significant difference in the inhibition of bacterial growth.

Similarly, the second serial dilution concentration of 179µg GAE/ml in AP resulted in a 58.73% reduction in *E. coli* growth compared to the control (p-value <0.0001), whereas the corresponding serial dilution concentration of 100µg GAE /ml in FAP leads to a comparable 45.69% reduction compared to control (p-value <0.0001).

The comparison between these concentrations (AP 179 µg GAE/ml vs. FAP 100 µg/ml) was also non-significant. Similarly, 89.5 µg GAE /ml in AP showed a 55.42% reduction in *E.coli* growth compared to control (p-value <0.0001), while the corresponding concentration of 50µg GAE /ml in FAP resulted in a 52.33% reduction (p-value <0.0001).

Again, the comparison between these two groups (AP 89.5 µg GAE/ml vs. FAP 50 µg GAE/ml) shows no significant difference. Despite the lower polyphenolic concentration in FAP, its ability to inhibit *E.coli* growth is comparable to that of AP, underscoring the potential of FAP as an effective antimicrobial agent (Fig. 3).

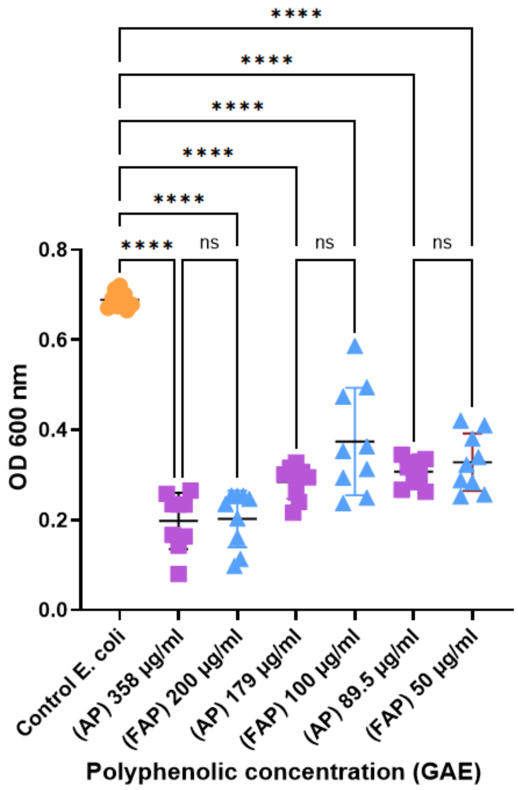

Figure 3. Comparison between similar polyphenolic concentrations extracted from AP and FAP against *E.coli* F4 (K88). Serial dilutions of AP and FAP extracts with varying total polyphenol (TP) concentrations were prepared, and the bacterial growth inhibition was monitored over 27.5 hours. The inhibition was tracked by measuring the optical density at 600 nm at 30-minute intervals.

Antibacterial properties of carrot pomace

The results indicate that carrot pomace polyphenols (CP) at a concentration of 151µg GAE /ml significantly inhibit *E. coli* growth, leading to an approximate 17.63% reduction compared to the control, with a p-value of 0.0294. However, at concentrations of 75.6 µg GAE /ml and lower, the inhibition of *E. coli* growth was not statistically significant. This suggests that a higher concentration of carrot pomace polyphenols is necessary to achieve a meaningful reduction in bacterial growth (Fig. 4).

Cheaib et al. (2020) highlighted that carrot pomace exhibits antimicrobial activity against several strains of *S. aureus* and Enterococci at a concentration of 20 µg/ml. The most abundant polyphenols in carrot pomace, such as caffeoyl quinic acid, cyanidin derivatives, and caffeoyl methyl quinic acid, contribute to this antimicrobial effect. However, the same concentration of 20 µg extract/ml was found insufficient to inhibit *E.coli* growth (Cheaib et al. 2020). It was shown that carrot extract between 0.5 and 2 g/ml was not able to inhibit *E.coli*, but also *Staphylococcus aureus*, and *Bacillus cereus* (Saleem et al., 2018). Ikram et al. (2024) reported significant inhibition against *Staphylococcus aureus* and *Candida albicans* of carrot seed extract. Similarly, Merino, Bellassi, Morelli and Athanassiou (2024) found a significant antimicrobial effect against *E. coli* when testing films containing carrot pomace combined with 3% and 5% eugenol concentrations.

To illustrate furthermore the efficacy of carrot pomace, Hagmueller et al. (2011) reported that a mixture containing 40% carrot pomace added to piglet feed showed a significant reduction in the incidence of diarrhea although there was no change in *E.coli* strains among the experimental groups.

Antibacterial properties of fermented carrot pomace

The results indicate that fermented carrot pomace polyphenols (FCP) at a concentration of 303 µg GAE /ml significantly inhibit *E.coli* growth, resulting in an approximate 84.76% reduction compared to the control, with a p-value of <0.0001. However, at concentrations of 151 µg GAE/ml and lower, the inhibition was not statistically significant (Fig. 5).

During the fermentation of carrot pomace with *S. cerevisiae*, fermentable sugars such as glucose, fructose, and sucrose are converted into ethanol, carbon dioxide, and various byproducts (Sharma&Kumar, 2017; Clementz et al., 2019; Demiray, Karatay, Dönmez & Dönmez et al., 2016). Along with the reduction in sugars, fermentation also results in the depletion of amino acids, vitamins, and minerals. Additionally, fermentation generates byproducts like organic acids, aldehydes, and higher alcohols (Palacios-Velásquez et al., 2023).

Post-fermentation, the remaining carrot pomace has reduced sugar content and is primarily composed of non-fermentable materials, including lignin and residual fibres (Demiray et al., 2016). To date, no studies have specifically investigated changes in polyphenol content in carrot pomace during fermentation. However, carrots are known for their high phenolic content, particularly carotenoids such as α- and β-carotenes, which possess strong antioxidant properties.

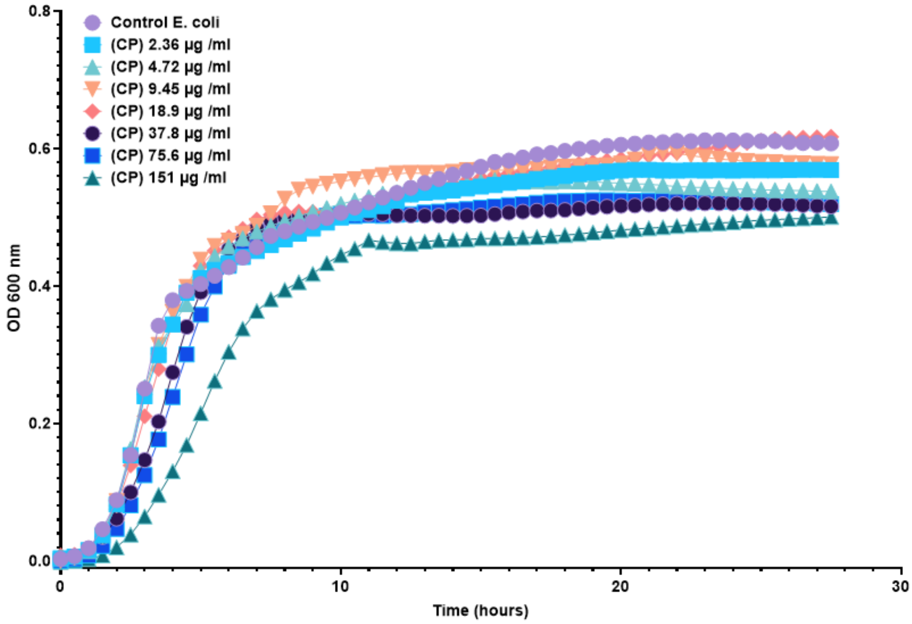

Figure 4. The impact of CP polyphenolic extract on *E.coli* F4 (K88) growth was assessed by incubating serial dilutions of the extract with varying PT concentrations for 27.5 hours. Bacterial growth inhibition was tracked by measuring the optical density at 600 nm at 30-minute intervals

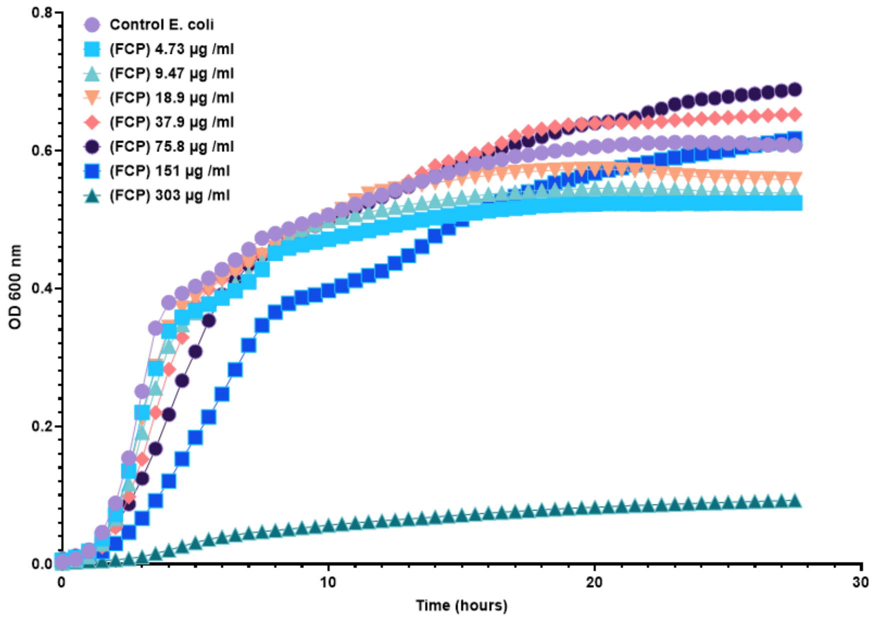

Figure 5. The impact of FCP polyphenolic extract on *E.coli* F4 (K88) growth was assessed by incubating serial dilutions of the extract with varying PT concentrations for 27.5 hours. Bacterial growth inhibition was tracked by measuring the optical density at 600 nm at 30-minute intervals

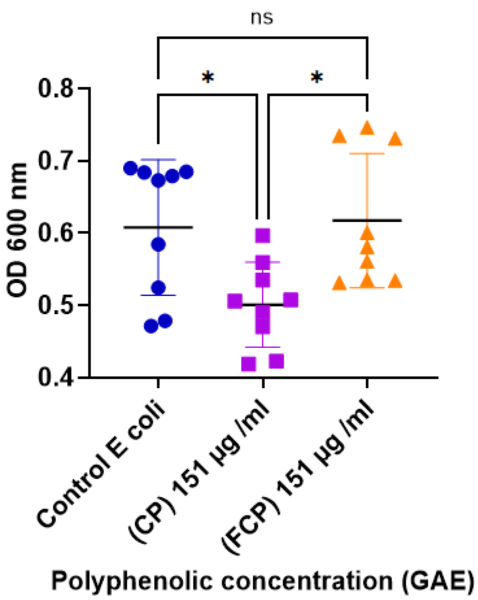

Figure 6. Comparison between similar polyphenolic concentrations extracted from AP and FAP against *E.coli* F4 (K88). Serial dilutions of CP and FCP extracts with varying total polyphenol (TP) concentrations were prepared, and the bacterial growth inhibition was monitored over 27.5 hours. The inhibition was tracked by measuring the optical density at 600 nm at 30-minute intervals

Comparison between the inhibitory effect of polyohenolic extract from CP and FCP

The CP extract at 151 µg GAE /mL significantly inhibited *E. coli* growth, reducing it by 17.6% compared to the control group ($p = 0.0312$).

In contrast, the FCP extract at the same concentration did not show a significant inhibitory effect on *E.coli* growth. When comparing the CP and FCP extracts directly, the FCP extract resulted in significantly higher *E. coli* growth, with an increase of 23.3% ($p = 0.018$). These findings highlight the potential of CP extract as a natural antibacterial agent and suggest that fermentation may not be a suitable process if the goal is to maintain its antibacterial properties (Fig. 6).

Comparison between AP, CP, FAP and FCP

A comparison of the inhibition efficiency of various polyphenol extracts reveals that fermented carrot pomace (FCP) at the highest concentration of 303 µg GAE /ml provides the highest inhibition, reducing *E. coli* growth by 85.71% compared to the control (p-value <0.0001) and showing 16.91% greater effectiveness than FAP at the highest concentration of 200 µg GAE /ml (p-value 0.0176). Furthermore, FCP at 303 µg GAE /ml exceeded AP at 358 µg GAE /ml by 16.24% (p-value 0.0249), indicating that antimicrobial activity is influenced not only by the concentration of polyphenols but also by the specific polyphenolic compounds present in the extract.

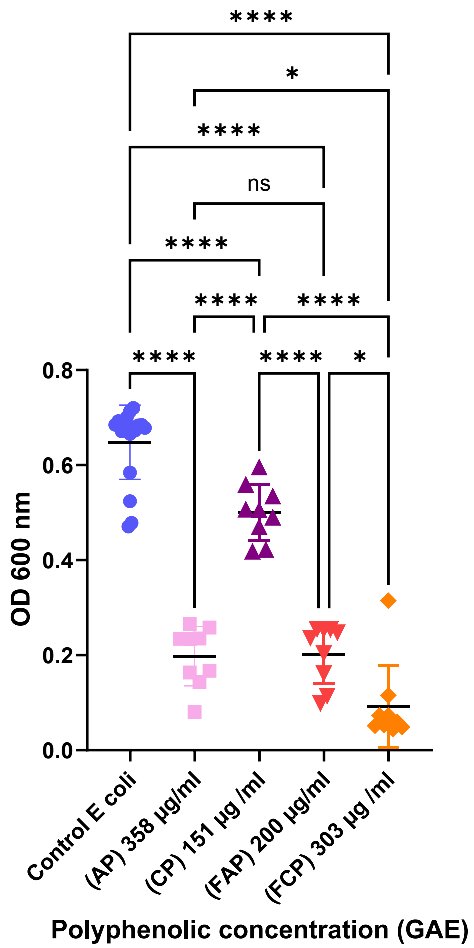

Figure 7. Comparison of polyphenolic extracts obtained from FCP, FAP, AP, and CP against *E.coli* F4 (K88). Total polyphenolic concentrations were incubated with *E.coli* for 27.5 hours. Bacterial growth inhibition was tracked by measuring the optical density at 600 nm at 30-minute intervals

Polyphenolic extract from FAP at 200 µg GAE /ml and from AP at 358 µg GAE /ml exhibited comparable inhibition capacities, reducing *E.coli* growth by 68.79% and 69.47%, respectively, both of which being highly significant compared to the control group (p-values <0.0001). On the other hand, polyphenolic extract from CP at 151µg GAE /ml shows a significantly lower inhibition capacity, with only a 22.79% reduction compared to the control (p-value <0.0001).

Additionally, CP at 151 μg GAE /ml is 46.01% and 46.98% less effective than FAP at 200 μg GAE /ml and AP at 358 μg GAE/ml, respectively (p-values <0.0001). These results suggest that FCP is the most potent extract for inhibiting *E. coli* at the concentrations tested, while CP is the least effective. The fermentation process seems to enhance the anti-microbial activity of the extracts, particularly in the case of carrot pomace. Future research could aim to optimize fermentation conditions to enhance the efficacy of these extracts in antimicrobial applications (Fig. 7).

CONCLUSIONS

Most polyphenolic extracts derived from apple pomace, carrot pomace, fermented apple pomace, and carrot pomace fermented with *S. cerevisiae* (commercial active dry baker's yeast) demonstrated antimicrobial activity against *E.coli* F4 (K88). Notably, extracts from fermented apple pomace and fermented carrot pomace generally exhibited stronger antimicrobial activity compared to those from non-fermented by-products.

Among the polyphenol extracts, those obtained from fermented carrot pomace showed the most potent antimicrobial effects, followed by fermented apple pomace, unfermented apple pomace, and unfermented carrot pomace, with the last showing no antimicrobial activity.

This study underscores the potential of polyphenols extracted from agro-industrial by-products as effective substitutes for antibiotics and zinc oxide, offering a promising solution for addressing the weaning crisis. However, further *in vivo* experiments are needed to confirm these findings.

AUTHOR CONTRIBUTIONS

Conceptualization, A.C.A., I.T., A.O., N.E.B.; Methodology, A.C.A.; Investigation, formal analysis, validation, writing-original draft preparation, A.C.A.; Writing-review and editing, A.C.A., I.T., A.O., N.E.B.; Supervision, I.T., A.O., N.E.B.

DATA AVAILABILITY

Data contained within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ANTIMIKROBNI EFEKTI POLIFENOLA IZ FERMENTISANOG I NEFERMENTISANOG TROPA JABUKE I MRKVE PROTIV *ESCHERICHIA COLI*

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Sažetak: Industrija uzgoja svinja suočava se sa značajnim izazovima tokom perioda odlučivanja prasadi od sisanja, što često rezultira smanjenim stopama rasta i većom smrtnošću među prasadima. Tradicionalno, antibiotici i cink oksid se koriste za rešavanje problema u ovom periodu. Međutim, zabrinutost zbog rezistencije na antibiotike i zagađenja životne sredine dovela je do strogih evropskih propisa koji ograničavaju ili zabranjuju njihovu upotrebu. Ovo je dovelo do hitne potrebe za alternativnim rešenjima, a polifenoli se pojavljuju kao opcija koja obećava zahvaljujući njihovim izraženim bioaktivnim svojstvima, uključujući antiinflamatorna, antioksidaciona i antimikrobna dejstva. Bioaktivna svojstva polifenola su veoma važna u prevenciji problema u periodu odlučivanja prasadi a koja su najčešće izazvana bakterijom *Escherichia coli* F4 (K88). Sadašnja studija se bavi određivanjem antimikrobnih svojstava polifenola ekstrahovanih iz tropa jabuke i mrkve, pre i posle njihove fermentacije sa kvascem *Saccharomyces cerevisiae*. Antimikrobna aktivnost je određena *in vitro* praćenjem bakterijskog rasta merenjem apsorbancije u različitim intervalima tokom 24 h na 37 °C. Rezultati su pokazali da polifenoli pokazuju značajan antimikrobni efekat, bez obzira da li je trop fermentisan ili ne. Polifenolni ekstrakt iz tropa jabuke je inhibirao rast *E. coli* pri minimalnoj koncentraciji od 5.6 µg (GAE)/ml dok su polifenoli iz fermentisanog tropa jabuke pokazali veću minimalnu inhibitorsku koncentraciju od 50 µg (GAE)/ml. U slučaju tropa mrkve, samo je doza od 151 µg (GAE)/ml polifenola iz nefermentisanog tropa šargarepe pokazala značajno inhibitorno delovanje na rast E. coli dok su polifenoli ekstrahovani iz fermentisanog tropa mrkve inhibirali bakterijski rast pri većoj dozi (303 µg (GAE)/ml). Efekat bakterijske inhibicije je zavisio of koncentracije i vrste polifenola u ekstraktu. Fermentisani polifenolni ekstrakti su pokazali slično inhibitorno delovanje sa nefermentisanim ekstraktima ali pri većim koncentracijama. Rezultati ukazuju na potencijal agro-industrijskih sporednih proizvoda kao što su trop jabuke i mrkve, kao izvora bioaktivnih jedinjenja sa potencijalom da budu efikasne zamene za antibiotike i cink oksid u stočarstvu. Eksploatacijom prirodnih resursa, industrija može da se preorijentiše na praksu koja je održiva i prihvatljiva sa stanovišta zaštite okoline a istovremeno efikasna u pogledu rešavanja izazova koji prate period odlučivanja prasadi.

Ključne reči: *period odlučivanja prasadi, antimirobno delovanje, sporedni agroindustrijski proizvodi, polifenoli, E. coli*

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