

Growth-promoting activity of polyphenolic extracts from carrot pomace and fermented carrot pomace on *Aspergillus brasiliensis*

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ABSTRACT

This study investigated the effect of polyphenolic extracts derived from carrot pomace (CP) and fermented carrot pomace (FCP) on the growth of *Aspergillus brasiliensis*, a filamentous fungus recognized for its enzymatic activity and potential in feed fermentation. Polyphenols were recovered by microwave-assisted extraction and their total content quantified using the Folin-Ciocalteu assay. Standardized extracts were evaluated *in vitro* against *A. brasiliensis* cultures. Both preparations markedly enhanced fungal proliferation following a dose-response pattern. The strongest stimulation appeared within the intermediate to high dose range (37.8–151 µg/mL TP), where growth surpassed control values by more than 250%. At lower doses (4.72–9.45 µg/mL TP), CP preserved its growth-promoting capacity, whereas FCP progressively lost activity, indicating that the fermentation process modifies the polyphenolic profile in a manner that diminishes stimulation at reduced concentrations. The findings reveal a twofold function of carrot-derived polyphenols, promoting fungal biomass accumulation while also conferring antimicrobial activity against selected bacterial strains. Taken together, the data support the incorporation of CP and FCP into feed substrates intended for *A. brasiliensis* solid-state fermentation, thereby improving fermentation yield and nutritional attributes. The present work contributes new evidence on the interplay between polyphenols and beneficial fungi and identifies practical avenues for valorizing agroindustrial residues in sustainable livestock nutrition.

Keywords: *Aspergillus brasiliensis*, carrot pomace, fermentation, polyphenols, *S. cerevisiae*

INTRODUCTION

Farm animals play a crucial role in food security, economic stability, and environmental sustainability, supplying meat, milk, and eggs as well as dung, wool, hides, and draught power (Drucker et al., 2001; Schrobback et al., 2023; Shah et al., 2025; Van Zanten et al., 2019). Because global demand for animal-sourced food is projected to rise by 70% by 2050 (der Poel et al., 2020; Drucker et al., 2001), safe and nutritionally adequate feed remains a central requirement of animal production, with feed costs typically representing 60–70% of total farm expenditure (der Poel et al., 2020; Malenica et al., 2022; Shah et al., 2025). Conventional rations based on corn, soybean meal, and fishmeal are constrained by fluctuating prices, variable nutritional quality, and sustainability concerns linked to land and water use as well as to fertilizer-driven eutrophication (Blümmel et al., 2009; Dai et al., 2020; Gasco et al., 2020; Georganas et al., 2023; Malenica et al., 2022; Shah et al., 2025). These pressures have been further amplified by the European Commission bans on in-feed antibiotics used as growth promoters (2006) and on therapeutic zinc oxide (2023), both driven by rising antimicrobial resistance and environmental concerns.

Two complementary strategies are increasingly adopted to overcome these constraints. Microbial fermentation of feedstuffs and the reuse of agro-industrial residues. Fermentation lowers antinutritional factors, depolymerises complex carbohydrates, and enhances the bioavailability of nutrients, thereby improving feed efficiency (Dai et al., 2020; der Poel et al., 2020; Georganas et al., 2023; Iyayi, 2004; Parmar et al., 2019; Shah et al., 2025; Yafetto et al., 2023). Lactic acid bacteria and filamentous fungi, notably *Aspergillus niger*, are most frequently applied in this context because of their established safety record and their capacity to reduce trypsin inhibitors in soybean meal, raise crude protein, and hydrolyse non-starch polysaccharides into soluble sugars (Barcelos et al., 2020; Gervasi & Mandalari, 2024; Mukherjee et al., 2016; Onomu & Okuthe, 2024; Sadh et al., 2018; Schuster et al., 2002; Soares et al., 2013; Vandenberghe et al., 2020). In parallel, agro-industrial by-products, generated in large volumes and rich in proteins, fibre, oils, vitamins, minerals, and bioactive polyphenols, can be incorporated into feed formulations to reduce costs, limit waste, and support sustainable livestock productivity (Malenica et al., 2022; Shah et al., 2025).

Carrot pomace, the solid residue obtained after juice extraction, is one such by-product whose nutritional and functional profile makes it particularly attractive for animal nutrition. It retains appreciable amounts of vitamins, minerals, carotenoids, and polyphenols, the latter two classes being well documented for their antioxidant and anti-inflammatory activity, and its dietary inclusion has produced favourable outcomes in broiler chickens, dairy cows, and pigs (Amara & El-Baky, 2023; Ikram et al., 2024; Malenica et al.,

2022; Rovai et al., 2021; Sabahi et al., 2024; Sharma et al., 2022). A promising strategy to further enhance the feed value of such residues is solid-state fermentation with *Aspergillus brasiliensis*. Like *A. niger*, this species is considered safe for animal and human applications and secretes a broad spectrum of extracellular enzymes, including tannase, xylanase, and proteases, which improve nutrient availability while lowering antinutritional factors. The metabolic proximity of *A. brasiliensis* to *A. niger* reinforces its potential for novel fermentation strategies, with the added advantage of secreting a broader variety of proteins (Ahmed et al., 2023; Chimbekujwo et al., 2020; Environment et al., 2019; Gezaf et al., 2021; Guerrero-Urrutia et al., 2021; Meijer et al., 2011; Salgado-Bautista et al., 2020; Sperandio et al., 2024; Varga et al., 2007). Despite this background, to the best of our knowledge no study has yet characterised how polyphenolic extracts from carrot pomace, in either its non-fermented or *Saccharomyces cerevisiae*-fermented form, influence the growth of *A. brasiliensis*, leaving a clear gap between the well-documented pro-fermentative and antimicrobial properties of carrot polyphenols and their possible role as a substrate for this specific fungus.

Against this background, the present work was designed to determine whether polyphenolic extracts obtained from carrot pomace, either in its native form or after fermentation with *S. cerevisiae*, can modulate the growth of *A. brasiliensis* *in vitro*. To this end, polyphenols were isolated from non-fermented (CP) and fermented (FCP) carrot pomace, quantified, and tested across a range of concentrations against *A. brasiliensis* cultures. Confirming such a growth-promoting effect would provide a rational basis for supplementing animal feeds intended for *A. brasiliensis* fermentation with carrot pomace, thereby improving fermentation yield and the overall nutritional quality of the final feed (Ikram et al., 2024; Rovai et al., 2021; Sabahi et al., 2024; Sharma & Kumar, 2017; Surbhi et al., 2018).

MATERIALS AND METHODS

The extraction of total polyphenols from agro-industrial by-products

Orange-rooted carrots (*Daucus carota* L. subsp. *sativus*, cv. *Nantes*) were obtained from a local producer located in Ilfov County, Romania. The roots were washed, peeled, and processed in a domestic juice extractor; the resulting pomace (CP) was collected as a fresh, moist, fibrous by-product and either used immediately or stored at -20°C in sealed polyethylene bags until extraction or fermentation, with a maximum storage time of two weeks to minimise oxidative loss of polyphenols. Fermentation of CP was carried out using *Saccharomyces cerevisiae* (commercial active dry baker's yeast, Pakmaya, Istanbul, Turkey) following the method outlined by Plaipetch and Yakupitiyage (2013). Polyphenol extractions (TP) were performed using the

microwave-assisted extraction (MAE) method, as conventional techniques do not allow optimal recovery of polyphenols from CP. For extraction, 2 g of CP or fermented carrot pomace (FCP) were mixed with 20 mL of 80% methanol. The treatment was carried out at 500 W and 70 °C for 30 minutes, followed by centrifugation of the samples at 8000 RCF for 10 minutes. The supernatant obtained was concentrated through methanol evaporation at 40 °C and sterilized by filtration with 0.45 µm pore-size filters.

Assessment of total polyphenol levels

The concentration of total polyphenols was determined using the Folin–Ciocâlțeu method, in which 10 µL of extract was diluted with 790 µL of distilled water and subsequently mixed with 50 µL of Folin–Ciocâlțeu reagent. After a one-minute interval, 150 µL of a 20% sodium carbonate solution was added, and the mixture was incubated in the dark for two hours. Following incubation, absorbance was measured at 750 nm using a spectrophotometer, and quantification was achieved by comparison with a gallic acid calibration curve, with results expressed as gallic acid equivalents (GAE).

Aspergillus brasiliensis preparation

Aspergillus brasiliensis was cultured overnight in Potato Dextrose (PD) medium at 30 °C. From this culture, 1 µL of aliquot was inoculated into 10 µL of fresh PD medium and subsequently diluted 100-fold in PD medium before being incubated for an additional 12 hours. Fungal growth was quantified by measuring the optical density at 600 nm using a Tecan Sunrise plate reader.

Assessment of the effect of polyphenolic carrot extracts on Aspergillus brasiliensis growth

Polyphenol extracts were prepared by serial dilution in distilled water at a 1:1 ratio. The concentration range tested for carrot pomace extract was TP 2.36–151 µg/mL, while fermented carrot pomace extract was tested at TP 4.73–303 µg/mL. Antifungal effects against *Aspergillus brasiliensis* were assessed in 96-well plates, with each well containing 277 µL of PD medium, 20 µL of extract, and 3 µL of fungal culture. The plates were incubated at 37 °C overnight, and fungal growth was tracked over 72 hours by recording absorbance at 600 nm every hour, with shaking at 120 RPM for 15 seconds prior to each measurement.

Statistical analysis

The statistical analysis of the results was performed using GraphPad Prism version 10.2.0. To evaluate differences among groups, a one-way analysis of variance (ANOVA) was applied to compare group means and determine the presence of statistically significant differences. When significance was detected, Tukey's post-hoc test was subsequently employed

to conduct pairwise comparisons, thereby identifying which specific groups differed from one another.

RESULTS AND DISCUSSION

Polyphenolic concentration in fermented and non-fermented carrot pomace

Polyphenol concentrations obtained through microwave-assisted extraction (MAE) were measured at 2.27 mg GAE/mL in carrot pomace (CP) and 4.55 mg GAE/mL in fermented carrot pomace (FCP) (Table 1); these values have been previously reported by the present authors in a complementary study focused on the antibacterial activity of the same extracts (Anghel et al., 2024).

Table 1. Polyphenolic content of the investigated by-product extracts

Meal	Total Polyphenols (mg GAE/ml)
Carrot Pomace (CP)	2.27
Fermented Carrot Pomace (FCP)	4.55

GAE= Gallic acid equivalent

The comparison of polyphenol concentration between non-fermented carrot pomace and fermented carrot pomace highlighted the fact that fermentation led to an increase in polyphenol concentration. A similar phenomenon has been observed in wine production. In this case, fermentation leads to the breakdown of cell walls, which releases higher amounts of polyphenols into the medium, especially anthocyanins and tannins (Mekoue Nguela et al., 2019). Yeast activity also contributes to polyphenol polymerization and stabilization, thereby influencing their overall composition (Mekoue Nguela et al., 2019). The increase in polyphenol concentration as a result of fermentation with *S. cerevisiae* has also been observed in fruit wines, in which this yeast was used for fermentation (Xu et al., 2022). On the other hand, in the case of rapeseed meal fermented with *S. cerevisiae*, the total concentration of polyphenols decreased, but the concentration of gallic acid and sinapic acid, polyphenols with strong antimicrobial properties, increased (Vlassa et al., 2022). Thus, the effect of *S. cerevisiae* fermentation on both the total concentration and the composition of polyphenols differs depending on the type of plant material fermented. These variations could be attributed to the molecular composition of the plant substrate as well as to the interactions among bioactive compounds during fermentation.

Fungal growth response to polyphenolic extracts

All tested polyphenol extracts consistently stimulated the proliferation of *A. brasiliensis*. The magnitude of this effect was not determined solely by the overall polyphenol concentration but was strongly influenced by the specific types of polyphenols present in the extracts. Notably, in some cases, lower concentrations produced growth responses comparable to those observed at twice the amount, indicating that fungal proliferation is more closely tied to the qualitative composition of the polyphenols than to their total quantity.

Assessment of polyphenolic carrot extracts effect on Aspergillus brasiliensis growth

Carrot pomace polyphenols stimulate the growth of *A. brasiliensis* in a concentration-dependent manner. The most pronounced effects were observed at intermediate concentrations (37.8–75.6 $\mu\text{g/ml}$), where growth nearly quadrupled compared to control values. Importantly, these effects were highly significant ($p < 0.0001$). At lower concentrations (9.45 and 4.72 $\mu\text{g/ml}$), the stimulatory effect is present, showing growth increases of +142% and +167%, respectively, both of which remained statistically significant ($p = 0.0291$ and $p = 0.005$). However, at the lowest tested concentration (2.36 $\mu\text{g/ml}$) the results were non-significant (Figure 1).

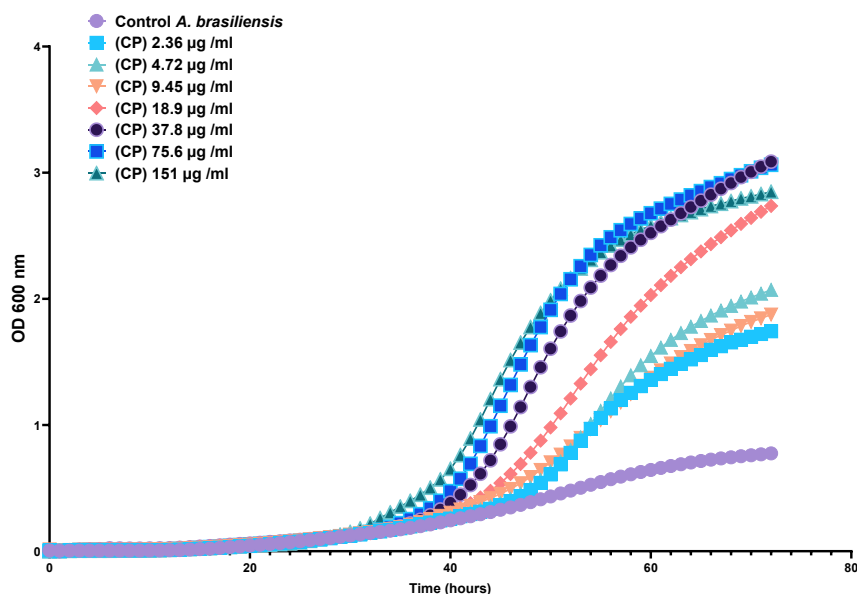


Figure 1. The effect of CP polyphenolic extract on *Aspergillus brasiliensis* growth was assessed by incubating serial dilutions of the extract at varying TP concentrations for 72 hours. Fungal proliferation was monitored by measuring optical density at 600 nm at 60-minute intervals.

In addition to promoting the growth of *A. brasiliensis*, carrot pomace also provides the advantage of exhibiting antimicrobial effects when included in animal feed. For instance, at a concentration of 20 µg/ml TP was shown to inhibit strains of Enterococci as well as Staphylococcus aureus (Cheaib et al., 2020). These antimicrobial properties are attributed to the polyphenolic compounds present in carrot pomace, such as caffeoyl quinic acid, cyanidin derivatives, and caffeoyl methyl quinic acid. However, in the case of Escherichia coli, a concentration of 20 µg/ml of TP extract did not significantly inhibit bacterial growth (Cheaib et al., 2020). Similarly, extracts from black carrot, at concentrations ranging from 0.5 to 2 g/ml in solid medium, significantly suppressed the growth of Staphylococcus aureus and Bacillus cereus, while having no notable effect on the development of E. coli or Pseudomonas. Comparable antimicrobial activity has also been reported for carrot seed extract, which significantly inhibited the growth of Staphylococcus aureus and Candida albicans (Ikram et al., 2024). Moreover, an in vivo study in pigs demonstrated that the inclusion of about 2% carrot pomace in feed significantly reduced the incidence of diarrhea, thereby supporting improved growth performance (Hagmueller et al., 2011). Taken together with the present data, these observations point to a dual functional role for carrot-derived polyphenols: on the one hand, they act as a positive growth stimulus for the beneficial fermentation fungus *A. brasiliensis*; on the other, they exert selective antimicrobial pressure on undesirable bacterial species such as *E. coli*, *Enterococcus spp.*, and *S. aureus* (Anghel et al., 2024; Cheaib et al., 2020). This combination is particularly relevant in the context of feed fermentation, as the same polyphenolic fraction can simultaneously support the development of the desired fermenting microorganism and contribute to suppressing pathogenic or spoilage bacteria in the feed matrix.

Fungal growth-promoting effects of fermented carrot pomace

Similarly, the effect of fermented carrot pomace (FCP) polyphenols on the growth of *Aspergillus brasiliensis* was concentration-dependent. At 151 µg/ml TP, FCP induced a 293% increase in fungal growth compared to the control ($p < 0.0001$), while at 75.8 µg/ml TP the effect was even stronger, with a 301% increase ($p < 0.0001$). Similarly, at 37.9 µg/ml TP, growth was enhanced by 277% relative to control ($p < 0.0001$). These results clearly demonstrate that FCP significantly promotes the growth of *A. brasiliensis* within this concentration range. At 18.9 µg/ml TP, fungal growth increased by 174%, and this effect was also statistically significant ($p = 0.0158$), although less pronounced than at higher concentrations. In contrast, at 9.47 µg/ml TP, growth was moderately enhanced (99% increase) but the effect did not reach statistical significance ($p = 0.4737$). However, at the highest tested concentration (303 µg/ml TP), the growth increase was only 107%, and the effect was not statistically significant ($p = 0.3711$). Likewise, at the lowest

concentration (4.73 $\mu\text{g}/\text{ml}$ TP) no significant difference was detected (Figure 2).

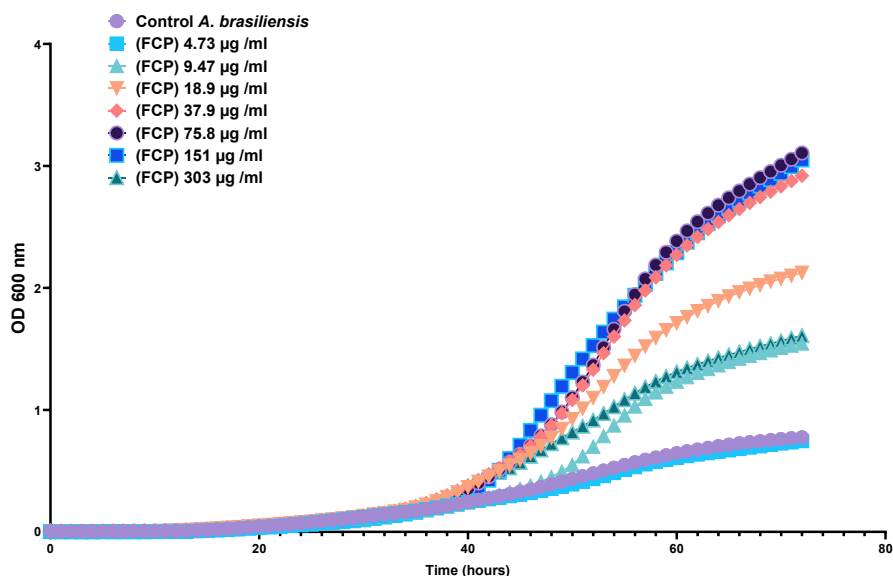


Figure 2. The effect of FCP polyphenolic extract on *Aspergillus brasiliensis* growth was assessed by incubating serial dilutions of the extract at varying TP concentrations for 72 hours. Fungal proliferation was monitored by measuring optical density at 600 nm at 60-minute intervals.

Fermentation of carrot pomace with *S. cerevisiae* produces ethanol, carbon dioxide, and various metabolites through the utilization of sugars such as glucose, fructose, and sucrose (Clementz et al., 2019; Demiray et al., 2016; Sharma & Kumar, 2017). Throughout this process, the decline in sugar concentration is accompanied by reductions in amino acids, vitamins, and minerals, while secondary products including organic acids, aldehydes, and higher alcohols are simultaneously formed (Palacios-Velásquez et al., 2023). Following fermentation, lignin and residual fibers remain as the predominant components, with sugar levels markedly reduced (Demiray et al., 2016). To date, however, no studies have directly examined the effect of fermentation on the polyphenol content of carrot pomace. Carrots are nevertheless well known for their richness in phenolic compounds and carotenoid pigments, particularly α - and β -carotenes, which are recognized for their strong antioxidant activity (Clementz et al., 2019; Demiray et al., 2016; Sharma & Kumar, 2017).

Comparison of fungal growth-promoting activity of polyphenolic extracts from CP and FCP

The comparison between *A. brasiliensis* control and treatments with carrot pomace (CP) or fermented carrot pomace (FCP) demonstrated a clear growth-promoting effect, particularly at higher concentrations (Figure 3).

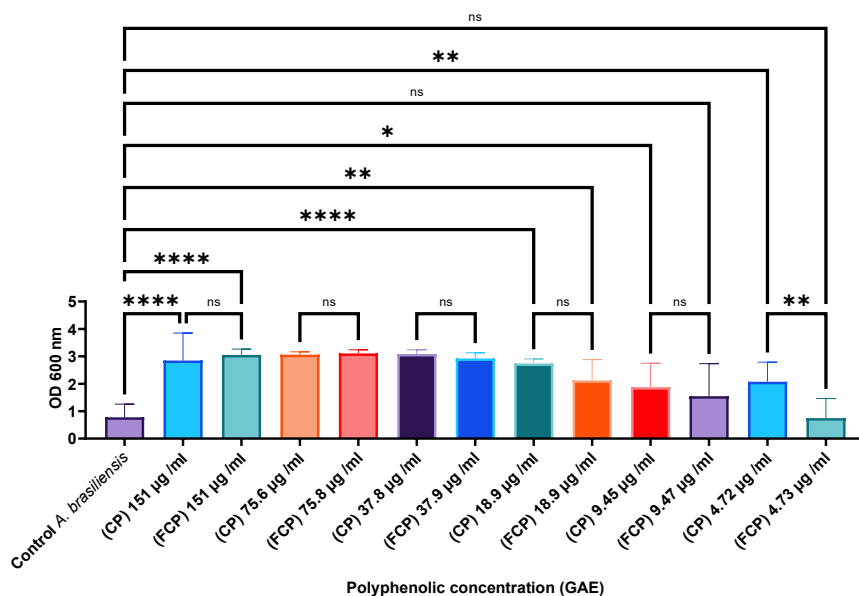


Figure 3. Comparison between CP and FCP extracts at equivalent polyphenolic concentrations to evaluate their effects on *A. brasiliensis*. Serial dilutions of the extracts with varying total polyphenol (TP) levels are prepared, and fungal growth is monitored over a 72-hour period. Growth inhibition is assessed by measuring optical density at 600 nm at 60-minute intervals.

At the highest tested dose (151 µg/ml TP), both CP and FCP significantly enhanced fungal growth compared to the control. Specifically, CP resulted in a 267% increase ($p < 0.0001$), while FCP produced an even greater increase of 293% ($p < 0.0001$), suggesting that both substrates provide substantial nutritional support for fungal proliferation, with a slightly stronger effect observed for FCP. At the intermediate concentration of 18.9 µg/ml TP, CP promoted a 253% increase relative to control ($p < 0.0001$), whereas FCP showed a lower, though still statistically significant, increase of 174% ($p = 0.0011$), but lower than the same concentration from CP. This indicates that at this level, CP was more effective than FCP in stimulating growth. At lower concentrations, differences between CP and FCP became more apparent. At 9.45–9.47 µg/ml TP, CP produced a 142% increase compared to control ($p = 0.0198$), whereas FCP resulted in a 99% increase that did not reach statistical significance ($p = 0.3309$), highlighting that only CP retained a consistent

stimulatory effect at this range. At the lowest tested concentration (4.72–4.73 µg/ml), CP still promoted fungal growth, yielding a 167% increase relative to control ($p = 0.0021$). In contrast, FCP failed to promote growth and in fact showed a 4% decrease compared to control ($p > 0.9999$), suggesting that fermentation diminished the growth-promoting potential of carrot pomace at this low level. Direct comparison between CP and FCP at 4.72 µg/ml TP confirmed this divergence, with CP being significantly more effective ($p = 0.0014$). When directly comparing CP and FCP across all concentrations, no significant differences were observed at higher and intermediate levels (151, 75.6, 37.8, 18.9, and 9.45 µg/ml TP; all $p > 0.69$), indicating that fermentation did not substantially modify growth-promoting effects at these concentrations. However, at the lowest concentration (4.72 µg/ml TP), CP clearly outperformed FCP ($p = 0.0014$), underscoring a dose-dependent influence of fermentation on fungal growth. Overall, these findings demonstrate that both CP and FCP act as strong growth substrates for *A. brasiliensis*, particularly at higher inclusion levels, with FCP exerting a slightly stronger effect only at the maximum tested dose. However, at lower concentrations, non-fermented carrot pomace consistently stimulated greater growth, while fermentation reduced or even abolished this stimulatory activity.

These results suggest that the fermentation of carrot pomace may enhance fungal growth potential at high concentration of total polyphenols but it can also attenuate it at lower concentrations, probably due to the modifications in bioactive compounds during the fermentation process. Such a dose-dependent behaviour is consistent with the notion that *S. cerevisiae* fermentation does not only alter the overall polyphenol content but also modifies its qualitative profile. At intermediate-to-high TP concentrations (37.8–151 µg/mL), FCP probably delivers a sufficient amount of fungal-utilisable phenolic carbon and stimulatory co-metabolites to sustain, and even slightly exceed, the stimulation obtained with CP, explaining the 277–301% increases in fungal growth observed in this range. At low TP concentrations (4.72–9.45 µg/mL), by contrast, stimulatory fractions are no longer present in sufficient amounts to sustain growth, while the relative contribution of fermentation-derived inhibitory metabolites, such as organic acids, higher alcohols, and specific antimicrobial polyphenols (e.g., gallic and sinapic acid derivatives reported in yeast-fermented substrates; Vlassa et al., 2022), becomes proportionally greater and masks the residual stimulatory effect. CP, which lacks these additional inhibitory species, retains its growth-promoting activity even at the lowest tested doses. The net effect is therefore biphasic: fermentation is beneficial when high inclusion levels of polyphenols are foreseen in the feed, but becomes neutral or slightly detrimental at very low

inclusion levels, an aspect that must be considered when designing practical feed formulations.

CONCLUSION

The present work shows that polyphenolic extracts from carrot pomace, used either in non-fermented (CP) or *S. cerevisiae*-fermented (FCP) form, act as positive growth modulators for *Aspergillus brasiliensis*, with a clear dose-response profile. The strongest and most reproducible stimulation was recorded in the intermediate-to-high range of total polyphenols (37.8–151 µg/mL TP), where fungal growth was enhanced by more than 250% over the control for both preparations, identifying this interval as the practical working window for feed applications. At low TP levels (4.72–9.45 µg/mL), a clear divergence between the two preparations became apparent: CP maintained a statistically significant stimulatory effect, whereas FCP lost its capacity to promote fungal growth, suggesting that the fermentation-induced reorganisation of the polyphenolic profile is advantageous when high inclusion rates are intended, but counterproductive when the feed would only deliver trace amounts of polyphenols. From a practical standpoint, these findings support the use of carrot pomace as a functional additive in feed formulations intended for solid-state or liquid fermentation with *A. brasiliensis*, with non-fermented CP being preferable when only small inclusion levels are feasible and FCP being a valid alternative when higher polyphenol doses can be incorporated. The simultaneous capacity of the extracts to inhibit undesirable bacteria reported elsewhere further strengthens their value as a dual-function ingredient, supporting the beneficial fungus while exerting selective pressure against spoilage and pathogenic species. Several limitations should nevertheless be acknowledged. The experiments were conducted in vitro, under standardised liquid medium conditions, and therefore do not fully reproduce the physicochemical complexity of a real feed matrix undergoing solid-state fermentation. Only total polyphenol content was quantified, without resolving the contribution of individual phenolic compounds or of non-phenolic fermentation metabolites, and a single strain of *A. brasiliensis* and one yeast starter were tested. Future studies should therefore address the phenolic profile by HPLC-MS, extend the evaluation to pilot-scale feed fermentation trials, and assess possible in vivo effects on target animal species in order to confirm the translational potential of CP- and FCP-supplemented diets.

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