ANTIMICROBIAL ACTION OF NATURAL PHENOLICS AGAINST FOOD SPOILAGE YEASTS

PH.D. DISSERTATION

BERNARD GITURA KIMANI

SUPERVISORS:

DR. MIKLÓS TAKÓ

ASSISTANT PROFESSOR

DR. ERIKA BEÁTA BENCSIK-KEREKES

RESEARCH FELLOW

DOCTORAL SCHOOL OF BIOLOGY

UNIVERSITY OF SZEGED

FACULTY OF SCIENCE AND INFORMATICS

DEPARTMENT OF MICROBIOLOGY

2023

Szeged

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ANOVA: analysis of variance
BHA: butylated hydroxyanisole
BHT: butylated hydroxytoluene
BLAST: basic local alignment search tool
CAACD: computer-aided antimicrobial compounds discovery
CANDOCK: chemical atomic network docking
CB-Dock: cavity-detection guided blind docking
EGCG: epigallocatechin gallate
EPS: extra polymeric substances
FICI: fractional inhibitory concentration index
HaCaT: human epidermal keratinocytes
LDL: low-density lipoprotein
MIC: minimum inhibitory concentration
MRSA: methicillin-resistant *Staphylococcus aureus*
NCBI: national center for biotechnology information
OS: oxidative state
PDB: protein data bank
PDBQT: protein data bank, partial charge (q), and atom type (t)
PG: propyl gallate
PyMOL: molecular visualization system in bioinformatics
QSAR: quantitative structure-activity relationship
ROS: reactive oxygen species
RNS: reactive nitrogen species
SZMC: Szeged Microbiological Collection, Szeged, Hungary
TNF-α: tumor necrosis factor alpha
TPC: total phenolic content

1. INTRODUCTION

Nature has gifted mankind with a plethora of natural compounds with antimicrobial properties that can be used effectively for bacterial and fungal suppression. Natural phenolics are phytochemicals which are synthesized through the pentose, shikimic acid and phenylpropanoid pathways and can be crucial for plant-defense system. These compounds exist either in bound or in soluble form and can be divided into several sub-groups, such as flavonoids, stilbenes, phenolic acids, tannins, and lignans, based on their chemical structure.

In the food industry, there is a growing demand for consumer-friendly additives due to the concerns against synthetic compounds in foods. Natural phenolics have become popular due to their bioactive properties and have found application in food, aquaculture, cosmetic, and sport industries. In the food industry, these bioactive compounds have shown great potential as food preservatives due to their antimicrobial activity and health promoting outcomes. Spoilage yeasts such as *Debaryomyces hansenii*, *Wickerhamomyces anomalus* (formerly *Pichia anomala*), *Schizosaccharomyces pombe*, and *Saccharomyces cerevisiae*, are capable of causing food deterioration resulting in low food quality and shorter shelf-life. Natural phenolics can be used as single compounds or in combination with other antimicrobials to prolong the shelf-life of food in a consumer-friendly manner. This strategy can also mitigate food deterioration and even prevent the development of antimicrobial resistance of food spoilage microorganisms.

The main objective of this study was to determine the antimicrobial activity of natural phenolics against food-related yeasts. Our goals entailed the *in vitro* analysis of selected phenolics against the planktonic and biofilm growth, and adhesion capacity of food spoilage yeasts involved. One of our aims was to evaluate the combined effect of the most potent phenolics against the planktonic and biofilm growth, and adhesion of yeasts using the checkerboard method. We also aimed to characterize the combination of highly bioactive phenolics and food preservatives as antiplanktonic, antibiofilm and antiadhesion agents. Finally, we planned to carry out *in silico* characterization involving molecular docking of selected natural phenolics against *S. cerevisiae* receptors known to play role in modulation of cellular structure, biofilm formation and adhesion.

2. LITERATURE REVIEW

2.1. Natural phenolics

2.1.1. Chemistry and classification of natural phenolics

Phenolic compounds are diverse bioactive compounds that are widespread in nature (Cosme *et al*. 2020). These bioactive compounds boost the defense system of plants by repelling unwanted organisms by acting as toxicants, inhibitors, and pesticides of potential invaders (Bhattacharya *et al*. 2010). Some low molecular weight phenolics confer scents and pigmentation to plants that attract pollinators and animals that disperse fruits (Bhattacharya *et al*. 2010). In terms of their structure, natural phenolics have an aromatic ring with one or more hydroxyl substituents (Giada 2013). Natural phenolics differ in many aspects, e.g., on the carbon basic chain, where different groups emerge such as phenolic acids and aldehydes (C_6-C_1), coumarins and hydroxycinnamic acids (C_6-C_3), stilbenes (C_6-C_2-C_6), flavonoids (C_6-C_3-C_6), lignans (C_6-C_3), catechol (C_6), condensed tannins (C_6-C_3-C_6), simple phenols and benzoquinones (C_6). Based on the number of phenol rings and their constituent structures, these phytochemicals can broadly be classified into flavonoids and non-flavonoids (Shahidi and Ambigaipalan 2015, Cosme *et al.* 2020) (Figure 1).

2.1.2. Biosynthesis of natural phenolics

The natural phenolics are synthesized through two biosynthetic ways, namely the shikimic acid pathway that leads to the production of phenylpropanoids and the acetic acid pathway that leads to the synthesis of simple phenols (Cheynier *et al.* 2013, Lattanzio 2013, Lopez *et al.* 2022). The phenylpropanoid pathway is fed with phenylalanine from the shikimate metabolic pathway; phenylalanine undergoes condensation with acetate in the presence of phenylalanine ammonia lyase enzyme to yield trans-cinnamic acid (Cheynier *et al.* 2013, Lattanzio 2013, Lopez *et al*. 2022). Trans-cinnamic acid is converted into *p*-coumaric acid which is the precursor of *p*-coumaroyl CoA when catalyzed by 4-coumarate CoA ligase (Cheynier *et al*. 2013, Lattanzio 2013, Lopez *et al.* 2022). *p*-Coumaroyl-CoA when combined

with malonyl-CoA produces chalcones, the precursor of flavonoids. *p*-Coumaroyl-CoA produces 5-O-*p*-coumaroylquinic acid and 5-O-Caffeoylquinic acid catalyzed by hydroxycinnamoyl transferase (Cheynier *et al.* 2013, Lattanzio 2013, Lopez *et al.* 2022). *p*-Coumaroyl-CoA is also the precursor of benzoates, salicylates, coumarins, and hydrolysable tannins as well as monolignols, lignans, and lignin (Cheynier *et al.* 2013, Lattanzio, 2013, Lopez *et al*. 2022).

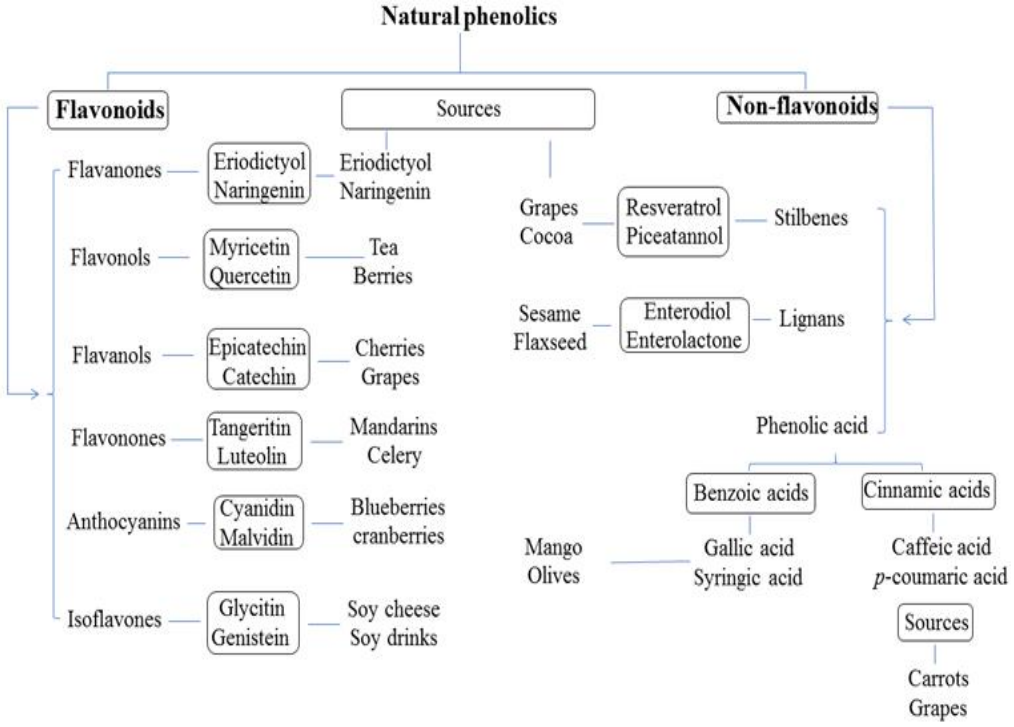


Figure 1. Classification of phenolic compounds (modified from Shahidi and Ambigaipalan 2015, Durrazzo *et al.* 2019, Diaz *et al.* 2020).

2.1.3. Main groups of natural phenolics

Flavonoids

Flavonoids are natural phenolics with low molecular weight found in different parts of plants (Dewick 2001, Panche *et al.* 2016). In most angiosperm families, most flavonoids manifest as flower pigments and aroma which is important for pollination (Dewick 2001, Panche *et al*. 2016). Apart from flower pigmentation, flavonoids have other crucial biological functions in plants. For example, they can act as signaling molecules, allopathic compounds, shielding plants from the ultraviolet light, control of enzymatic action, gene regulation, as well

as protecting the plants from biotic and abiotic stresses (Torawane *et al.* 2020). Flavonoids have become appealing compounds to researchers due to their myriad benefits to human and animal health (Torawane *et al*. 2020). Flavonoids benefits to humans have been studied by a number of authors; their applications in agriculture and neuroscience have been reviewed (Dixon and Pasinetti 2010), the usage of flavonoids in the management of Alzheimer's disease has been reviewed (Panche *et al*. 2016). Other properties of flavonoids beneficial to human health include anti-inflammatory, steroid modulation, inhibition of antibiotic resistance, diseases combating and radical scavenging activities (Torawane *et al.* 2020). The summary of flavonoids' sub-groups and their food sources is shown in Table 1.

Table 1. Flavonoids sub-groups and their natural sources (Naczk and Shahidi 2004, Kamboh *et al.* 2015, Panche *et al.* 2016).

Flavonoid sub-group	Examples of flavonoids	Food source
Flavonols	Quercetin, myricetin, morin Kaempferol, fisetin	Apple, onions, berries, citrus, tomato, red pepper, tea
Flavanols	Epicatechin, catechin, epigallocatechin-3-gallate, epicatechin gallate	Cacao, green tea, berries, grapes, lychees
Flavanones	Hesperidin, naringin, eriodictyol, naringenin, taxifolin	Lemon, orange, tomatoes, pomelo, grapefruit
Flavones	Sinensetin, nobiletin, baicalein, chrysin, tangeretin, luteolin, isosinensetin, apigenin, galangin, repoifolin	Parsley, thyme, celery, hot peppers, grape, apples
Isoflavones	Genistein, daidzein, biochanin, formononentin	Soybean and soy foods, legumes
Anthocyanins	Cyanidin, epigenidin, pelargonidin, delphinidin, malvidin	Nuts, dried fruits, fruits, vegetables
Chalcones	Phloretin, arbutin, phlioridzin chalconaringenin	Tomatoes, pears, strawberries, bearberries, wheat products

Non-flavonoids

Phenolic acids

Phenolic acids, also called phenolcarboxylic acids, are a group of natural phenolics pervasive in different parts of plants *viz.* exocarp of fruits, seeds, leaves, and plant petiole. These compounds have been divided into hydroxycinnamic acids and hydroxybenzoic acids (Kumar and Goel 2019, Cosme *et al.* 2020). Typical sources of hydroxycinnamic acid include red wine, wheat, coffee and fruits such as cherries and kiwis. Phenolic acids are rarely found in free form

and mostly will be bound in plants as esters, glycosides, and amides (Kumar and Goel 2019). Hydroxycinnamic acids have a typical phenylpropanoid C_6-C_3 structure, and include *p*-coumaric acid, ferulic acid, and caffeic acid, among others. Hydroxybenzoic acids are derived from benzoic acids and possess a typical frame of C_6-C_1, with variations among the compounds being based on methoxylations and hydroxylations of the aromatic ring. Hydroxybenzoic acids are conjugated with sugars or organic acids (Cosme *et al*. 2020). The typical examples of natural phenolics in this family include syringic acid, vanillic acid, protocatechuic acid, and *p*-hydroxybenzoic acid. Typical sources of hydroxybenzoic acids include fruits, vegetables, herbs, and spices, such as green and black tea, blackberries, blackcurrants, and raspberries (Francisco and Michael 2000). Phenolic acids have shown great potential in novel applications in diverse fields like medicine, where they have been used to manage disorders like skeletal muscle ischemia (Albuquerque *et al.* 2021). In the cosmetic industry, phenolic acids have shown great potential as multifunctional cosmeceutical ingredients (Taofiq *et al*. 2018). In the packaging industry, gallic acid and caffeic acid, have been used in chitosan-based films to inhibit the growth of food spoilage bacteria, such as *Bacillus subtilis* and *Staphylococcus aureus* (Taofiq *et al.* 2018).

Stilbenes

The name stilbene is a derivative of the Greek word “stilbos,” which means shining (Teka *et al.* 2022). Stilbenes are secondary metabolites with C_6–C_2–C_6 structure, biosynthesized through the phenylpropanoid pathway as a response to biotic and abiotic stresses (Tyunin *et al*. 2018, Sharma *et al.* 2019). Stilbenes originate from over 50 plant families such as Leguminaceae, Gnetaceae, and Vitaceae (Tyunin *et al*. 2018). This group of phenolics has attractive pharmacological properties, such as anti-cancer, anti-inflammatory, anti-atherogenic, antioxidant, estrogenic, neuroprotective, anti-aging, and anti-diabetic effects (Ziaullah and Rupasinghe 2015, Teka *et al*. 2022). Apart from plants being the main producers of stilbenes, symbiotic bacteria such as *Photorhabdus* and *Bacillus* can also biosynthesize them (Hapeshi *et al.* 2019). The main enzyme used in their biosynthesis is stilbene synthase (Teka *et al.* 2022). The 3,5,4'-trihydroxy-trans-stilbene, also known as t-resveratrol, is the most popular and extensively studied stilbene that also acts as a precursor in the biosynthesis of other stilbenes, such as oxyresveratrol and viniferins (Tyunin *et al.* 2018). Stilbenes act as phytoalexins in most

plants, crucial for defending the plants against infections (Teka *et al.* 2022). Food sources rich in stilbenes include grapes, bilberries, peanuts, purple grapes, cranberries, blueberry, acai berry, sorghum, cowberry, and lingonberry, among others (Teka *et al.* 2022).

Lignans

Lignans are naturally occurring bioactive compounds biosynthesized through the shikimic acid pathway, which may occur free or bound to sugars (Yeung *et al.* 2020, Koyama *et al*. 2022). They are synthesized in plants and in microbes inhabiting humans and animals (Yeung *et al.* 2020). Lignans are present in diverse plants, including tea, wine, coffee, wheat, rye, oat, berries, sesame, poppy, flax seeds, and cashew nuts, among others. These naturally occurring bioactive compounds have attractive health benefits such as reduced cardiovascular disease, as well as antitumor, antioxidant, and anti-inflammatory effects (Rodríguez-García *et al.* 2019). Dietary lignans include sesamin, lariciresinol, secoisolariciresinol, matairesinol, pinoresinol, syringaresinol, and medioresinol. Lignans are involved in plants as phytoalexins, biocides, and antioxidants (Durazzo *et al*. 2013).

Tannins

Tannins are a group of natural products well distributed in marine and terrestrial plants as secondary metabolites with molecular weights ranging from 500 to 20,000 Daltons (Tong *et al*. 2022, Ojo 2022). These compounds have been classified into two main groups based on their structural features, namely, hydrolysable tannins, which possess a sugar core, and condensed tannins which lack a sugar core (Tong *et al*. 2022). Tannins are available in foods such as cider, hazelnuts, cacao, strawberries, coffee, blackberries, tea, cashew nuts, peas, walnuts, grapes, and mangoes (Ojo 2022). Plant tannins have been used industrially in the leather industry for hide treatment for many centuries (Das *et al.* 2020). Tannins have also been used as animal food additives (Xiao *et al*. 2018). Traditionally, tannins were associated with anti-nutrient properties due to their ability to form complexes with food components such as proteins and carbohydrates (Ojo 2022). However, recent research has demonstrated that tannins could be beneficial for human health (Aboagye *et al*. 2019). Other applications of tannins include use as an adhesive in the timber industry, as a fungicide, insulating foam, and biocide, and they are used in the wine industry as well (Pizzi 2006, Tondi and Pizzi 2009).

2.1.4. Potential health benefits of natural phenolics

Natural phenolics as cardiovascular therapeutics

Cardiovascular diseases are conditions that affect the heart and the blood vessels (Torres-Fuentes *et al*. 2022). Such diseases include rheumatic heart disease, coronary heart disease, peripheral arterial disease, cerebrovascular disease, deep vein thrombosis, and pulmonary embolism (Torres-Fuentes *et al*. 2022). Cardiovascular diseases are one of the leading causes of deaths globally. Natural phenolics are beneficial in reducing risk factors associated with cardiovascular diseases, such as endothelial dysfunction, inflammatory signaling, and elevated high blood pressure, among others. Natural phenolics are associated with a decline in atherosclerotic plaque that leads to cardiometabolic diseases (Tangney and Rasmussen 2013). Quercetin decreases the mRNA of macrophage inflammatory protein in a murine RAW264.7 cell line (Boesch-Saadatmandi *et al*. 2011). Red wine is rich in natural phenolics such as flavonoids and has anti-inflammatory properties (Chiva-Blanch *et al.* 2012). Tea catechins reduce atheromatose lesion formation by inhibiting arterial wall smooth muscle cell proliferation (Maeda *et al.* 2003). Preclinical studies in mice have demonstrated that resveratrol improves diastolic pressure and myocardial functioning and prevents heart fibrosis (Lutz *et al.* 2019). Caffeic acid has a positive impact on the heart rate through an increase in the bioavailability of nitric oxide (Lutz *et al.* 2019).

Anti-cancer potential of natural phenolics

Natural phenolics have demonstrated immense potential in inhibiting cancer development by controlling genes and regulating the main processes leading to the initiation and progression of cancers. The application of natural products in cancer prevention is becoming more attractive due to the deleterious effects of the traditional methods of cancer management such as radiotherapy and chemotherapy (Anantharaju *et al.* 2016). Caffeic acid demonstrated anticancer properties through inhibition of tumor progression by apoptosis induction and a decrease in cell viability of the human cutaneous melanoma SK-Mel-28 cell line (Pelinson *et al.* 2019). *p*-Coumaric acid demonstrated colon cancer inhibitory effect through mitochondrial-mediated apoptosis via an increase in levels of reactive oxygen species (ROS) (Anantharaju *et al.* 2016). *p*-Coumaric acid extracted from *Rosa canina* demonstrated cytotoxicity against

human lung A549 and prostrate PC-3 cells (Kilinc *et al*. 2019). Syringic acid improved the treatment of dimethylbenz(a)anthracene-induced buccal pouch carcinogenesis in a dose-dependent manner in hamsters (Velu *et al*. 2017). Low concentrations of gallic acid had an inhibitory effect in glioblastoma multiforme T98G cell lines through the initiation of apoptotic cell death (Paolini *et al.* 2015). Vanillic acid is essential in preventing lung cancer induced by benzo(a)pyrene in Swiss albino mice (Velli *et al*. 2019). Thus, natural phenolics are becoming more attractive in cancer prevention and management due to their antioxidant, proapoptotic, and antiproliferative properties.

Antidiabetic properties of natural phenolics

Diabetes mellitus is a metabolic disorder that is characterized by high levels of blood glucose due to anomalies in insulin secretion or insulin action (Praparatana *et al*. 2022). Conventional glucose-lowering agents are synthetic in nature and can have side effects on the patient such as diarrhea, nausea, and liver disorder among others, hence, there is a need to explore more user-friendly alternatives from natural sources (Praparatana *et al*. 2022). Natural phenolics, such as phenolic acids, are capable of lowering the activity of key enzymes involved in carbohydrate metabolism like α-glucosidase and α-amylase (Hanhineva *et al.* 2010). Cinnamic acid improved glucose tolerance *in vivo,* and insulin secretion stimulation *in vitro* (Hafizur *et al.* 2015). *p*-Coumaric acid influenced the metabolism of glucose and lipids in L6 skeletal muscle cells (Yoon *et al.* 2013). Sinapic acid enhanced glucose utilization in streptozotocin-induced type1 diabetic rats (Cherng *et al.* 2013). Caffeic acid improved hepatic glucose utilization and inhibited glucose overproduction in C57BL/KsJ-db/db mice (Jung *et al.* 2006). Ferulic acid decreased blood glucose levels and remarkably increased glycogen and insulin in high fat diet-induced mice (Son *et al.* 2011). Syringic acid improved glucose homeostasis in the liver of alloxan-induced diabetic rats (Srinivasan *et al.* 2014).

Natural phenolics as potential skin therapeutics and skin protectors

Phytochemicals, such as natural phenolics, are replete with diverse skin benefits due to their antioxidant and radical scavenging activities. Natural phenolics confer direct and indirect benefits to the skin obtained through the consumption of foods rich in these compounds or through the topical application (Działo *et al.* 2016). Natural phenolics are, therefore, capable of

slowing down skin aging processes, skin protection from UV exposure and acne formation (Działo *et al*. 2016). Grape seed extracts demonstrated positive effects on human skin health (Kanna *et al*. 2003, Yamakoshi *et al.* 2003). Plant extracts from *Astragalus mongholicus* redeemed keratin expression inhibited by particulate matter exposure in human epidermal keratinocytes (HaCaT) (Nguyen *et al*. 2019). *Camellia japonica* leaf extracts demonstrated *in vitro* ROS scavenging and induced antioxidant enzymes such as catalase and superoxide dismutase in HaCaT cells (Piao *et al.* 2011). In a study involving green tea extracts, (−)-epigallocatechin gallate (EGCG) led to an increase in dermal fibroblast cell viability exposed to particulate matter (Boo 2019). Gallic acid present in *Emblica officinalis* enhanced collagen formation and proliferation in fibroblasts (Fujii *et al*. 2008). *Populus nigra* natural phenolics demonstrated amiable activity in the human dermal fibroblasts model through cell renewal and protection from oxidative stress (Dudonne *et al.* 2011). The tropical highland blackberry *Rubus adenotrichos* led to the reduction of UV-induced DNA damage as well as apoptotic damage of severely damaged skin cells (Calvo-Castro *et al.* 2013).

Natural phenolics as anti-inflammatory agents

Inflammation defends the body from harmful agents, such as pathogenic bacteria, or injurious non-microbial agents, such as toxins, through the activation of transcriptional factors and inflammatory genes (Rahman *et al.* 2022). Inflammation also occurs due to the high levels of nitric oxide (NO) produced by pro-inflammatory cytokines, such as, interleukin (IL) and tumor necrosis factor-α (TNF-α) (Rahman *et al.* 2022). Overproduction of pro-inflammatory cytokines may cause allergies, arthritis, and atherosclerosis (Chung *et al*. 2014). Natural phenolics are effective anti-inflammatory agents capable of inhibiting the production of pro-inflammatory agents or downregulation of inflammatory mediators that leads to nitrogen oxide reduction (Panzella 2020). Natural phenolics were capable of downregulating the expression of pro-inflammatory enzymes that support inflammation and modulate the production of inflammatory cytokines (Yahfoufi *et al.* 2018). Curcuminoids could lower the levels of pro-inflammatory cytokines leading to improved inflammatory markers (Peng *et al.* 2021). Natural phenolics from 15 spices had an immunomodulatory effect through NO inhibition and synthesis of TNF-α (Xu *et al*. 2020). Natural phenolics from hydroethanolic leaf extracts of *Entada africana* Guill. and Perr. demonstrated anti-inflammatory ability against TNF-α stimulated

human keratinocytes (HaCaT) with aromadendrin, ethyl gallate 3′,4′,7-trihydroxyflavone, and dihydrokaempferol-7-*O*-glucoside having a favorable release of IL-6 (Codo *et al.* 2022). Pragasam *et al*. (2013) demonstrated the anti-inflammatory ability of *p*-coumaric acid through TNF-α expression in adjuvant-induced arthritic rats. Caffeic acid and ellagic acid also demonstrated anti-inflammatory activity (Chao *et al.* 2013).

Natural phenolics as antioxidants

Phenolic compounds are known to have great potential as natural antioxidants due to their ion chelating activity, ROS and reactive nitrogen species (RNS) deactivation capacity, and free radical scavenging activity, and they can protect cell membranes from damage through inhibition of lipid peroxidation and DNA damage (Rani *et al.* 2018). Antioxidants stabilize the highly reactive species by acting as electron donors. Conventionally, synthetic antioxidants have been used as electron donors and they include propyl gallate (PG), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT). Synthetic antioxidants' usage as electron donors has been restricted by their moderate activity, low solubility, and negative impact on health. Natural phenolics are a possible alternative to synthetic antioxidants due to their natural origin and beneficial outcomes on human health. Rutin demonstrated a concentration-dependent free radical scavenging ability with an optimal free-radical scavenging ability attained at 1000 mg/day (Mutha *et al.* 2021). In addition, epigallocatechin and other tea catechins were effective *in vitro* free radical scavengers capable of reducing lipid peroxidation and protein carbonylation in animal studies (Mutha *et al.* 2021). Commonly used spices, such as cinnamon, black cumin, and turmeric, were examined for their total phenolic content (TPC), and there was a positive relationship between TPC and antioxidant efficacy (Panzella 2020). Natural phenolics inhibited xanthine oxidase, an enzyme crucial for superoxide ions synthesis, hence, capable of alleviating oxidative stress from cells (Basli *et al.* 2017). Natural phenolics supplementation in rats and athletes inhibited oxidative stress (OS) and improved the antioxidative defense system (Annunziata *et al.* 2020). Due to their broad application in pharmacology, food, medicine, and cosmetics, the potential of phenolics as natural antioxidants will continue to be an interesting area of research in the coming days.

2.2. Food-contaminating microorganisms

2.2.1. Microbial food-contamination in general

Diverse microorganisms have been utilized since ancient times as starter cultures in food production, such as beer, bread, fermented vegetables, yogurt, and sausages. However, while microorganisms have played a crucial role in food and beverage production since the dawn of human civilization, many microorganisms lead to food spoilage and some may cause food poisoning. Nowadays, there is tremendous growth in the food industry, with a variety of food products undergoing minimal processing that predisposes them to spoilage microorganisms. These spoilage microbes have led to massive economic losses and loss of lives, hence, there is a need to reduce the formation of contaminations. The range of microorganisms causing food spoilage is broad and includes bacteria, yeasts, molds, viruses, and parasites (Lorenzo *et al.* 2018).

2.2.2. Food spoilage bacteria

There are spore and non-spore-forming food spoilage bacteria. The non-spore-forming bacteria include *Carnobacterium*, *Brochothrix*, *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Weisella*, and *Streptococcus* spp. (Lorenzo *et al.* 2018). These lead to food discoloration, decreased pH through acid production, unpleasant odors, and the production of slime. *Brochothrix* spp. are Gram-positive facultative anaerobes that spoil chilled meat and meat products (Kolbeck *et al*. 2020). *Carnobacterium* spp. are Gram-positive lactic acid bacteria that produce lactic acid through carbohydrate fermentation; these bacteria cause food spoilage in chilled pork, beef, poultry, and sea foods (Kolbeck *et al.* 2020). *Lactobacillus* spp., are Gram-positive, rod-shaped bacteria that exhibit acid tolerance, they include *Lactobacillus casei*, *Lactobacillus curvatus*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum*, *Lactobacillus sakei*, and other species (Lorenzo *et al.* 2018). Most lactobacilli have probiotic capability and can exclude intestinal colonization by pathogenic strains. However, some such as *L. curvatus* and *L. sakei* lead to the formation of slime textures on meat products (Kalschne *et al.* 2015). *L. delbrueckii* subsp. *bulgaricus* is considered a beer contaminant (Lorenzo *et al.* 2018).

Pediococcus contaminates beer and wine by changing the viscosity. *Streptococcus* is associated with the spoilage of dairy products. *Lactococcus* can produce malty flavors in dairy products (Lorenzo *et al.* 2018). *Leuconostoc* spp., e.g., *Leuconostoc carnosum*, may lead to gas or slime production on preserved foods like chilled meats (Lorenzo *et al.* 2018, Kolbeck *et al.* 2020).

Spore-forming spoilage bacteria include *Bacillus* and *Clostridium* species. The non-pathogenic food spoilage *Bacillus* includes *Bacillus sporothermodurans* which is heat resistant and is capable of surviving ultra-high temperatures. These bacteria are associated with spoilage of ultraheat-treated daily products (Lorenzo *et al.* 2018). *Bacillus amyloliquefaciens* cause bread and meat spoilage (Valerio *et al.* 2015). *Bacillus coagulans* is a thermophile associated with the spoilage of canned vegetables, while clostridia lead to the spoilage of canned and refrigerated vacuum-packed foods producing a foul smell due to the production of butyric acid (Dürre 2009, André *et al.* 2017).

Pathogenic non-spore forming bacteria that may contaminate food such as unprocessed milk and poorly cooked meat include *Listeria*, *Yersinia*, *Salmonella*, *Brucella* and *Campylobacter*, among other species (Chlebicz and Śliżewska 2018). Another group of bacteria that has challenged the food industry is the pathogenic spore-forming bacteria due to their ability to survive extreme food processing conditions. These include *Bacillus cereus*, *Clostridium botulinum, Clostridium perfringens*, *Clostridium difficile*, *Clostridium tetani*, and *Clostridium sordellii* (Lorenzo *et al.* 2018, Kolbeck *et al.* 2020, Romero-Rodríguez *et al.* 2023).

2.2.3. Food spoilage yeasts and molds

Yeasts have been used since antiquity in the fermentation of food products such as alcoholic beverages, sausages, and cheese. While their significance in food production cannot be underrated, these organisms have led to massive economic losses due to food spoilage. In this regard, some of the yeasts that cause spoilage include *Saccharomyces cerevisiae*, *Dekkera bruxellensis, Pichia membranifaciens, Schizosaccharomyces pombe*, *Kloeckera apiculate, Zygosaccharomyces bailii, Issatchenkia orientalis*, *Debaryomyces hansenii*, *Zygosaccharomyces bisporus, Zygosaccharomyces rouxii, Candida holmii*. The various effects of spoilage yeasts have on foods are described in Table 2.

Table 2. Examples of food spoilage yeasts, their properties and the effects they have on foods.

Spoilage yeasts	Yeast properties	Foods affected	Effects on food	References
Saccharomyces cerevisiae	Osmotolerant, thermotolerant, ethanol tolerant	Canned foods, puree foods, soft drinks	Off-flavors, gas production, discoloration	Fleet (2011)
Debaryomyces hansenii	Osmotolerant	Salted meat products, brined vegetables, cheese, beer	Surface biofilms, off-flavor	Fleet (2011)
Wickerhamomyces anomalus (*Pichia anomala*)	Acid-tolerant, osmotolerant	Fruit products, dairy products, baked goods, silage, beer	Off-flavor in wine, biofilms	Fleet (2011)
Pichia membranifaciens	Ethanol-tolerant, oxidative tolerant	Fruits, fermented beverages	Off-flavors, biofilms	Fleet (2011)
Pichia kudriavzevii	Acid-tolerant, preservative-resistant	Dairy products, kimchi	Off-odors, biofilms, food softening	Krisch *et al*. (2016)
Zygosaccharomyces rouxii	Sugar-tolerant, osmotolerant	Sugars, dried fruits, honey	Off-flavors, off-odors	Escott *et al*. (2018)
Zygosaccharomyces bailii	Ethanol-tolerant, preservative-tolerant	Fruit and fruit juices, alcoholic beverages	Gas production, off-flavors	Krisch *et al*. (2016)
Zygosaccharomyces bisporus	Ethanol-tolerant, halotolerant, preservative-tolerant	Syrup, sauces, honey, jams	Tea, beer, gas production, off-flavors	Fleet (2011)
Rhodotorula spp.	Proteolytic, lipolytic and oxidative activity	Meat, fruit, vegetables, dairy, baked goods	Discoloration, off-flavors	Albertyn *et al.* (2014)
Yarrowia lipolytica	Proteolytic and oxidative activities, halotolerant	Fruit products, poultry, mayonnaise	Off-flavors, textural changes	Groenewald *et al.* (2014)
Hanseniaspora uvarum	Acid tolerant, osmotolerant	Fresh and processed fruits, coffee	Discoloration, flocculation	Fleet (2011)
Kluyveromyces marxianus	Proteolytic, pectinolytic activity	Dairy products like Skyr, plant products	Off-flavor	Srimahaeak *et al.*(2022)

Molds are a part of our environment and are commonly known for their hyphae development that results in the mycelium. The commonly used molds in the food industry include *Mucor*, *Aspergillus*, *Penicillium*, *Rhizopus*, *Alternaria*, and *Botrytis*. *Mucor* has been used in cheese ripening and fermentation of foods, although some species are associated with food spoilage. *Mucor circinelloides* is known to spoil yams, yogurt, and cheese (Snyder *et al.* 2016). *Mucor hiemalis* and *Mucor racemosus* cause cheese deterioration spoilage with sensory

changes (Garnier *et al*. 2017). *Aspergillus* species, e.g., *Aspergillus oryzae*, have been used for centuries in fermenting foods and beverages. *Aspergillus niger* is known to cause spoilage of apricots, grapes, yogurt, onions, nuts, and beans (Gougouli and Koutsoumanis 2017). *Penicillium* species contaminate and spoil foods such as bread, grains, fruits, and sausages, while *Rhizopus* cause the spoilage of berries, vegetables, and meat. *Alternaria* is associated with the spoilage of moist grains, vegetables, and fruits (Lee *et al.* 2015).

2.3. Antimicrobial activity of natural phenolics

2.3.1. Natural phenolics as planktonic growth inhibitors

Several studies have demonstrated that natural phenolics inhibit the planktonic growth of food spoilage and pathogenic bacteria, yeasts, and molds (Garnier *et al*. 2017, Zambrano *et al.* 2019). Due to their diversity in structure and chemical composition, natural phenolics display varied potential in their antimicrobial activity, probably due to their different inhibitory mechanisms (Takó *et al.* 2020). Table 3 shows earlier studies of pure natural phenolics and plant extracts and some of their minimum inhibitory concentration (MIC) data against the planktonic growth of bacteria, yeasts, and molds.

Table 3. Examples of activity of pure phenolic compounds and plant extracts against the planktonic microbial growth of bacteria, yeasts, and molds.

Tested phenolics/extracts	Target organism or molecule	Antimicrobial activity	Reference
	Bacteria		
11 phenolic compounds	*P. aeruginosa*, *B. subtilis*, *P. putida*, *E. coli*, MRSA, *S.* Typhimurium, *B. cereus*	The MICs ranged from 125 to ≥ 500 µg/mL	Zambrano *et al.* (2018)
2,4-dihydroxybenzoic acid, vanillic acid, syringic acid, *p*-coumaric acid	*E. coli*, MRSA, *Enterococcus faecalis*	MICs were 0.5 mg/mL for 2,4-dihydroxybenzoic acid, vanillic acid, syringic acid, and 0.1 mg/mL for *p*-coumaric acid	Alves *et al.* (2013)
Phloridzin, phloretin	*E. coli*, *S. aureus*	MICs: *E. coli* 1.50 and 0.75 mg/mL, *S. aureus* 0.50 and 0.10 mg/mL	Zhang *et al.* (2016)
p-coumaric acid	*S. pneumoniae*, *B. subtilis*, *S. aureus*, *S. dysenteriae*, *E. coli*, *S.* Typhimurium	The MIC ranged from 10 to 80 µg/mL	Lou *et al.* (2012)
Catechin, gallic acid	*E. coli*	Inhibited zone of 14 and 12 mm in the presence of 15 and 2.5 mg/well catechin and gallic acid, respectively	Diaz *et al.* (2014)
Catechin, gallic, vanillic, ferulic and protocatechuic acids	*E. coli*, *S.* Typhimurium	MICs: 15 and 20 mmol/L *E. coli* and *S.* Typhimurium, respectively	Pacheco-Ordaz *et al.* (2018)
	Yeasts		
Vanillin	*S. cerevisiae*, *Z. bailii*, *Z. rouxii*	The MICs for *S. cerevisiae*, *Z. bailii* and *Z. rouxii* were 21, 20 and 13 mM, respectively	Fitzgerald *et al.* (2003)
Tasmanian pepper leaf methanol extracts	*D. anomala*, *S. pombe*, *S. cerevisiae*	The inhibition zone (in mm): 17.6, 13.7, and 17.2 for *D. anomala*, *S. pombe*, *S. cerevisiae*, respectively	Alderees (2018)
Lemon myrtle hexane extracts	*D. anomala*, *S. pombe*, *S. cerevisiae*	The inhibition zone (in mm): 43.3, 35.7, and 34.9 for *D. anomala*, *S. pombe*, *S. cerevisiae*, respectively	Alderees (2018)
Anise myrtle hexane extracts	*D. anomala*, *S. pombe*, *S. cerevisiae*	The inhibition zone (mm) was 26.9, 14.7, and 13.2 for *D. anomala*, *S. pombe*, *S. cerevisiae*, respectively	Alderees (2018)
Cinnamic acid derivatives	*Cochliobolus lunatus*	Inhibited fungal enzyme CYP53A15	Korošec *et al.* (2014)

Table 3. Continued.

Tested phenolics/extracts	Target organism or molecule	Antimicrobial activity	Reference
	Molds or mycotoxins/molds enzymes		
Edible beans extracts	*A. oryzae* α-amylase	*Vigna angularis* (19% inhibition), *Phaseolus vulgaris* (5.4% inhibition)	Telles *et al.* (2017)
Citrus flavonoids	*Aspergillus parasiticus* aflatoxins	0.39 mM naringin, 0.24 mM neohesperidin and 0.40 mM quercetin mixture reduced aflatoxins from 85% to 100%	Pok *et al.* (2020)
5 phenolic compounds	*Fusarium graminearum* mycotoxins	TRI5 expression regulation by the tested compounds	Oufensou *et al.* (2020)
Ferulic acid	*Fusarium verticillioides*	1 mM ferulic acid 86% and 53% of fumonisin inhibition, for *F. verticillioides* INRA 63 and 64 strains, respectively	Chtioui *et al.* (2022)
Gallic acid, tannic acid	*Aspergillus flavus* aflatoxin	Gallic acid reduces aflatoxin B1 to 4% at 12 mM, and tannic acid completely inhibits production at 2 mM	Mahoney and Molyneux (2004)
Caffeic acid, chlorogenic acid, ferulic acid, *p*-coumaric acid	*A. parasiticus*	Aflatoxin production was completely inhibited by all phenolic acids at a concentration of 20 mM	Lorán *et al.* (2022)

2.3.2. Natural phenolics as antibiofilm and antiadhesion agents

Many microorganisms form multicellular aggregates called biofilms. Biofilms have unique communal properties different from the composite cells forming them which improves their survivability in hostile environments. Such communities may exhibit slower growth rates, antimicrobial resistance, increased production of secondary metabolites, and enhanced genetic exchanges. In addition, there is growing interest in these microbial structures due to their implications in the medical field, food industry and the environment. Due to the biofilms' resistance to synthetic antimicrobial agents, there is increased screening and analysis of natural inhibitory compounds such as natural phenolics which are less likely to develop antimicrobial resistance phenotypes in bacteria and fungi (Takó *et al.* 2020). Different studies have demonstrated the potential of natural phenolics to inhibit bacterial and fungal biofilms, as shown in Table 4.

Table 4. Examples of antibiofilm/antiadhesion activity of phenolic compounds/extracts against bacteria and yeasts.

Tested phenolics/extracts	Target organism	Tested activity	% biofilm/adhesion inhibition	References
Bacteria				
Gentiana asclepiadea extracts	*P. aeruginosa, S. aureus, P. mirabilis*	Antibiofilm	>50%; conc. 2.1-37 mg/mL	Stefanović *et al.* (2018)
3-*p*-trans-coumaroyl-2-hydroxyquinic acid	*S. aureus*	Antiadhesion	62% at 2.5 mg/mL	Wu *et al.* (2019)
Opuntia ficus-indica cladodes	*S. aureus*	Antibiofilm	71-85%; conc. 1-1.5 mg/mL	Blando *et al.* (2019)
Kaempferol	*S. aureus*	Antiadhesion	75%; conc. 64 μg/mL	Ming *et al.* (2017)
Isovitexin	*S. aureus*	Antiadhesion	Inhibited sortase A; IC50 of 28.98 μg/mL	Mu *et al.* (2018)
Butia odorata extract	*S. aureus*	Antibiofilm	99.9%; conc. 11.4-22.8 mg/mL	Takó *et al.* (2020)
Cranberry *Vaccinium macrocarpum*	*E. coli, E. faecalis*	Antiadhesion	>20%; conc. 500 μM	de Llano *et al.* (2020)
Yeasts				
Bauhinia holophylla extracts (EtOAc)	*Candida albicans* SC5314	Antibiofilm	82-72%; 1250-62.5 μg/mL	da Fonseca *et al.* (2022)
Curcumin	*C. albicans*	Antiadhesion	>50% inhibition at 50 μg/mL	Alalwan *et al.* (2017)

2.4. Combination of natural phenolics with other antimicrobial agents

The combination of antimicrobials in the inhibition of food spoilage microbes may become the norm in coming years, as compounds operating in consortium may have numerous benefits. The repertoire of current phytochemicals is a product of centuries of plant evolution, hence, carry with them a millennium of combating the multifactorial nature of microbial resistance. Studies have shown that antimicrobial resistance is less likely to develop in a combination of compounds unlike in single active constituents (Johnson and Perfect 2010). Apart from combating the problem of antimicrobial resistance, there are many other benefits of combining bioactive compounds, such as the reduction of toxicity, broadening of the antimicrobial spectrum in the mixture, improvement of the activity of the compounds through synergistic interactions and in case of the food grade compounds, the combination may lessen unwanted sensory attributes of individual compounds (Johnson and Perfect 2010). Some studies have demonstrated the efficacy of natural phenolics and phenolic-enriched extracts in combination with other antimicrobials. The combination of grape pomace extract with different classes of antibiotics was synergistic against *S. aureus* and *E. coli*, with FICI values ranging from 0.031 to 0.155 (Sanhueza *et al.* 2017). In another study, 13 antimicrobial drugs were combined with 8 plant extracts (carqueja-*Baccharis trimera*, guava-*Psidium guajava*, clove-*Syzygium aromaticum*, mint-*Mentha piperita*, lemongrass-*Cymbopogon citratus*, ginger-*Zingiber officinale*, guaco-*Mikania glomerata*, and garlic-*Allium sativum)*, using the Kirby and Bauer method against 15 *S. aureus* strains where the lemongrass exhibited the highest synergistic activity with antimicrobial drugs against the 15 tested strains (Betoni *et al.* 2006). Combination of *Cochlospermum regium* extracts with tannin and gallic acid against eight bacteria and five yeasts showed synergy between tannin and *C. regium* extracts against *Klebsiella pneumoniae* (FIC 0.17) and *S. aureus* (FIC 0.28), as well as against the yeasts *C. albicans* (FIC 0.46) and *Candida glabrata* (FIC 0.13) (Carvalho *et al.* 2018).

2.5. *In silico* investigation of natural phenolics

Computer-aided antimicrobial compounds discovery (CAACD) technologies are critical in identifying and developing cost-effective, novel antimicrobial candidates and aiding the

researchers in describing inhibition mechanisms of different active molecules against the target of interest (Brogi *et al.* 2020). For this to be achieved, advanced computer simulations and data analysis tools are employed to unravel the nature of the interaction between the active molecule and the protein target (Liao *et al.* 2022). *In silico* technologies can be used in optimizing phytochemical antimicrobial activity through sensible structural modifications which will solve the problems that hinder them from being used in the mainstream antimicrobial fight, such as high complexity, low specificity, poor stability, and low solubility (Yao *et al.* 2017, Rampone *et al.* 2021). The commonly used computer-aided antimicrobial discovery screening tools and associated technologies include molecular modelling methods, quantitative structure-activity relationships (QSAR) tools, *in silico* chemical absorption, distribution, metabolism, excretion, and toxicity (ADMET) tools, and pharmacophore modelling, among others (Tang *et al.* 2006, Brogi *et al.* 2020, Liao *et al*. 2022). The molecular docking method is a molecular modeling technique that predicts protein-ligand interaction and is based on the ligand positioning on the protein receptor cavity as reflected by a docking score. A good docking score, i.e., a highly negative value which reflects the change of potential energy upon complex formation between the ligand and the protein, indicates a good affinity of the ligand on a protein receptor, which implies a high antimicrobial activity of the ligand (Ferreira *et al.* 2015). The identification and validation of a viable protein target for molecular docking is the first step in designing and developing an effective antimicrobial molecule; however, identifying and validating a protein target from numerous candidate molecules is challenging (Tang *et al.* 2006). Different experimental technologies for target mining have been developed recently, such as, genomic and proteomic approaches, which are laborious and time-consuming. To complement the effort of the experimental technologies in target identification, *in silico* tools such as structure-based approaches have been employed (Tang *et al.* 2006). The target of interest could be pathogenesis-related factors and those factors which are involved in physiological processes, such as biofilm formation, depending on the antimicrobial development purpose (Agamah *et al.* 2020). In one study, molecular docking of 13 natural phenolic ligands with human aldose reductase enzyme in complex with the synthetic inhibitor showed that the phenolics binding patterns were similar to that of the co-crystallized inhibitor, the best binding affinity was that of 2-(4-hydroxy-3-methoxyphenyl) ethanoic acid (-9.8 kcal/mol) and butein (-9.8 kcal/mol) (Imran *et al*. 2022). In another study, molecular docking of derivatives from cinnamic and benzoic acids against *C.*

albicans protein targets indicated that methyl ferulate antifungal activity could be due to the inhibition of *C. albicans* multiple targets (Perez-Castillo *et al.* 2020). In *S. cerevisiae*, aminopeptidase I protein, involved in selective autophagy, showed potential inhibition by resveratrol (Kores *et al*. 2019).

2.6. Natural phenolics mechanisms of action for antimicrobial activity

The mechanisms of action of natural phenolics are yet to be fully understood. Most of the inhibitory mechanisms of natural phenolics involve their activity at the cellular membrane (De Cruz Cabral *et al.* 2013, Oulahal and Degraeve 2022). The hydroxyl group play an important role in the inhibitory mechanism of phenolics, for instance, through the modulation of the activity of target enzymes. The other possible mechanisms of inhibition of natural phenolics include their ability to modify the permeability of cells and their interaction with the membrane proteins leading to fundamental changes to membrane integrity (De Cruz Cabral *et al.* 2013, Oulahal and Degraeve 2022). Natural phenolics can also accumulate on the cell surface or in the cytoplasm altering the intracellular pH and may change the cellular functions (Oulahal and Degraeve 2022). The penetration of natural phenolics into the cytoplasm may also change vital processes such as protein synthesis and the functioning of the DNA and RNA (Oulahal and Degraeve 2022). In one study, ethanolic and water extracts of rosemary (*Rosmarinus officinalis*), roselle (*Hibiscus sabdariffa*), thyme (*Thymus vulgaris*), and clove (*Syzygium aromaticum*), led to a decline in the internal pH and cell membrane hyperpolarization of *E. coli* and *S. aureus* (Gonelimali *et al.* 2018). In another study, a polyphenolic fraction from Kombucha, a tea beverage containing catechin and isorhamnetin, led to the disruption of the *Vibrio cholerae* membrane in a time and dose-dependent manner due to the production of intracellular reactive oxygen species (ROS) (Bhattacharya *et al*. 2018). Further, solvent extracts of *Backhousia citriodora* led to the swelling and lysis of the *S. cerevisiae* cell membrane leading to cell death (Alderees *et al.* 2018). A flow cytometric analysis of *S. aureus* treated with a novel phenolic 3-*p*-trans-coumaroyl-2-hydroxyquinic acid from *Cedrus deodara* demonstrated a loss of membrane integrity and significant membrane hyperpolarization (Wu *et al.* 2016). Chlorogenic acid demonstrated antifungal activity against *C. albicans* through the disruption of the structure of the *C. albicans* cellular membrane (Sung and Lee 2010). The anti-biofilm

mechanisms of natural phenolics may entail the inhibition of global regulatory systems such as quorum sensing or the inhibition of genes crucial for biofilm formation (Payne *et al.* 2013, Silva *et al*. 2016). *S. aureus* biofilm formation was inhibited by tannic acid from black tea (*Camellia sinensis*) through a mechanism dependent on the putative transglycosylase IsaA inhibition (Payne *et al.* 2013). *Alnus japonica* extracts containing quercetin and tannic acid inhibited biofilm formation in *S. aureus* through gene regulation linked to biofilm formation (*icaA* and *icaD*) (Lee *et al.* 2013). Sub-inhibitory concentrations of tannic acid and gallic acid inhibited the biofilm formation of *Streptococcus mutans* through the inhibition of fructosyltransferase and glucosyltransferase (Sendamangalam *et al.* 2011).

2.7. Natural phenolics in food safety and shelf-life extension

Food preservation entails slowing down microbial growth or inhibiting fat oxidation, which maintains the desired level of quality. Through food preservation, food is kept safe for a prolonged period which prevents the outbreak of foodborne illnesses and reduces food wastage. This guarantees food security and increases the efficiency of food systems. Some of the approaches in food preservation include controlling the amount of moisture in food, reducing food temperature, controlling redox potential, introducing competitive microorganisms, and using preservatives (Ullah *et al.* 2022). In light of this, novel preservation techniques are emerging. These methods preserve food through their antioxidant, antimicrobial, and anti-browning activities (Ullah *et al.* 2022). Artificial food preservatives have been used in the food industry for many decades to control food spoilage due to their antimicrobial efficiency, low cost, and the fact that they are easy to obtain (Yu *et al.* 2021). Despite this, there is a growing concern of their possible detrimental effects on human health. Studies have implicated artificial food preservatives to health complications such as cancer, hypertension, cardiovascular diseases, and reduced fertility, to mention a few, when used for long above the acceptable daily intake (Yu *et al.* 2021). The use of natural compounds to control microbial proliferation and prolong the shelf life of food is becoming popular nowadays due to the desire to decrease synthetic food additives (Yu *et al*. 2021, Ullah *et al.* 2022). In this sense, natural phenolics are applied directly or indirectly to foods as bio-preservatives with beneficial health capabilities (Martillanes *et al*. 2016). The incorporation of natural phenolics into the food matrix can take

the form of phenolics-rich emitting sachets, food absorbent pads, packaging polymer, and edible food coatings/films (Martillanes *et al.* 2016). In a study investigating the development of active packaging from phenolics, tannin-cellulose films had antioxidant properties and could block UV rays (Huang *et al.* 2022). In another study, ellagic and phenolic acids made food packaging films with high antimicrobial, antioxidant, and UV light-blocking properties (Vilela *et al.* 2017). According to the study of Benbettaieb *et al.* (2018), chitosan and fish gelatin hydrocolloid films containing *p*-coumaric acid and caffeic acids could inhibit the oxidation of fatty foods. Bioactive packaging containing resveratrol could inhibit *Campylobacter* growth activities (Silva *et al.* 2016). Hamburgers containing green tea extracts had low thiobarbituric acid reactive substances values with reduced microbial deterioration and lipid oxidation (Özvural *et al.* 2016). Pork sausages fortified with green tea extracts incorporated in chitosan film could inhibit yeasts, molds, lactic acid and decrease changes in sensory characteristics, texture, and color compared to the control (Siripatrawan and Noipha 2012). Raw chicken meat containing different spice extracts of *Brassica nigra* (L.) K. Koch, *Syzygium aromaticum* (L.) Merr. and L.M. Perry, *Origanum vulgare* L., and *Cinnamomum cassia* (L.) J. Presl. had reduced lipid peroxidation and microbial growth (Özvural *et al.* 2016).

3. AIMS

For the last two decades, food hygiene and safety have emerged as significant global issues since microbial-triggered food loss and foodborne illnesses have become a menace in developing and developed countries. To this end, researchers are looking for safe broad-spectrum food preservatives that can be utilized in food fortification to inhibit microbial growth and confer other attractive properties such as antioxidative properties. Natural food additives are favored over synthetic food additives because of their endless health benefits and natural origin. These phytochemicals are being perceived as the future of food preservation when used singly or in combination with other compounds due to the health risks associated with conventional chemical food additives in food production. Natural phenolics fit the bill as innocuous compounds with broad antimicrobial activity that can inhibit planktonic growth, biofilms, quorum sensing, and microbial adhesion aspects of microbes and still have beneficial effects on human health. However, data on their action against spoilage yeast activities remain scarce.

Previously in our laboratory, natural phenolics were extracted from phenolic-rich substrates using various methods, and their antimicrobial activity was determined against pathogenic and spoilage bacteria. In the current study, our main objective was to determine the inhibitory effect of natural phenolics against the growth of common food spoilage yeasts. In this aspect, the yeast inhibition was planned to be further characterized in the presence of phenolic-phenolic and phenolic-food preservative combinations using the checkerboard assay. Our goal also included *in silico* characterization studies in which the binding potential of phenolics to protein targets was planned to be evaluated. In this study, we desire to demonstrate that natural phenolics have come of age, and it is time they become adopted in the mainstream food preservation platforms.

Our specific objectives were to:

1. determine the effects of natural phenolics on planktonic growth of food spoilage yeasts,
2. determine the effects of natural phenolics on biofilm growth of food spoilage yeasts,
3. determine the effects of natural phenolics on adhesion on a solid surface of spoilage yeasts,

4. determine the effects of phenolic-phenolic combinations on planktonic and biofilm growth, and adhesion capacity of food spoilage yeasts,
5. determine the effects of phenolic-food preservative compound combinations on planktonic and biofilm growth, and adhesion capacity of food spoilage yeasts, and
6. evaluate the interaction of phenolic compounds with yeast protein targets using the *in silico* molecular docking method.

4. MATERIALS AND METHODS

4.1. Phenolic compounds

Different phenolic compounds from diverse classes of natural phenolics were used for the antiplanktonic and antibiofilm growth, and antiadhesion assays (Table 5). These phenolics were (i) hydroxybenzoates, i.e., vanillic acid, gallic acid, syringic acid, protocatechuic acid, and 4-hydroxybenzoic acid; (ii) hydroxycinnamates, i.e., cinnamic acid, caffeic acid, ferulic acid, and *p*-coumaric acid; (iii) stilbenes, i.e., polydatin and resveratrol; (iv) flavonoids, i.e., quercetin and (−)-epicatechin; and (v) phenolic aldehydes, i.e., 4-hydroxybenzaldehyde and vanillin. The phenolic compounds were sourced from Sigma-Aldrich (Germany). Most compounds were selected based on a previous study (Zambrano *et al*. 2019) that had demonstrated their antimicrobial activity against food-related bacteria. The other compounds tested were also effective antimicrobial agents in studies (Fitzgerald *et al.* 2003, Alves *et al.* 2013, Shin *et al.* 2018).

Table 5. Phenolic compounds used in the study.

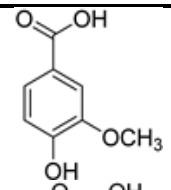
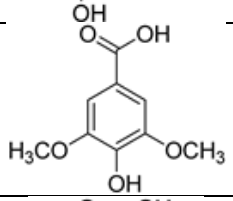
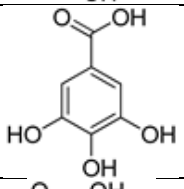
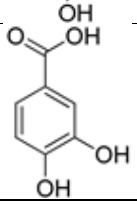
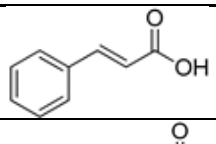
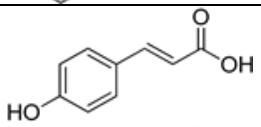
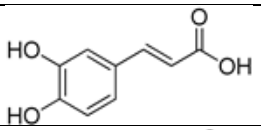
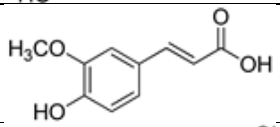
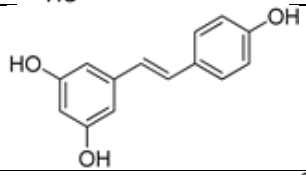
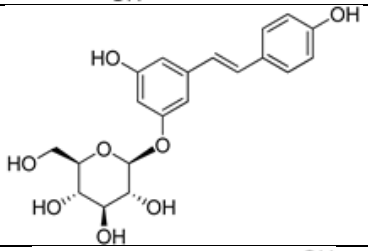
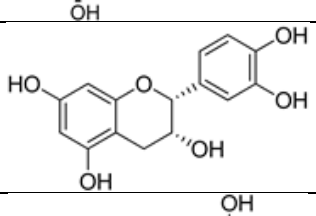
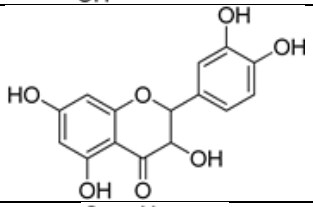
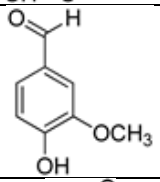
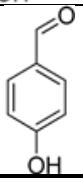
Chemical group	Compound	Chemical structure
Hydroxybenzoic acids	Vanillic acid	
	Syringic acid	
	Gallic acid	
	Protocatechuic acid	

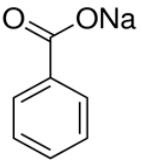
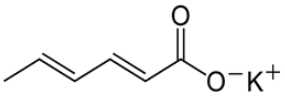
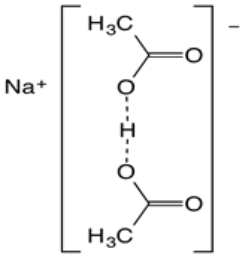
Table 5. Continued.

Chemical group	Compound	Chemical structure
Hydroxycinnamic acids	Cinnamic acid	
	p-Coumaric acid	
	Caffeic acid	
	Ferulic acid	
Stilbenes	Resveratrol	
	Polydatin	
Flavonoids	(−)-Epicatechin	
	Quercetin	
Phenolic aldehydes	Vanillin	
	4-Hydroxybenzaldehyde	

4.2. Food preservatives

Three food preservatives were used in the study for the combination work: (i) sodium benzoate; (ii) potassium sorbate; and (iii) sodium diacetate. These compounds are commonly used preservatives in foods and beverages (Piper and Piper 2017). Their properties and chemical structures are shown in Table 6.

Table 6. Food preservatives used and their properties.

Preservative	Properties	Chemical structure
Sodium benzoate	MW: 144.1 Freely soluble in water, white crystals, odorless solid	
Potassium sorbate	MW: 150.22 Soluble in water, white crystals, odorless salt	
Sodium diacetate	MW: 141.08 Soluble in water, white hygroscopic powder, acetic acid odor	

4.3. Media

Composition of the media used is listed in Table 7. Solid media were prepared by addition of 2% (*w/v*) agar-agar.

Table 7. The composition of the media used in the study.

Malt extract medium (ME)	Yeast extract peptone dextrose medium (YPD)
20% (*v/v*) malt extract*, 50 mL/L; yeast extract, 5 g/L; glucose, 5 g/L	Glucose, 20 g/L; Peptone, 20 g/L; Yeast extract, 10 g/L

*Ingredients were purchased from Merck (Hungary) or Bio Lab (Hungary).

4.4. Microorganisms

All the strains used were from the Szeged Microbiological Collection (SZMC) maintained by the Department of Microbiology, University of Szeged (Table 8).

Table 8. Yeast strains used in the study.

Yeast	Code	Growth medium	Growth temperature (°C)
Saccharomyces cerevisiae	SZMC 1279	ME*	30
Wickerhamomyces anomalus	SZMC 8061Mo	ME	30
Debaryomyces hansenii	SZMC 8045Mo	YPD	30
Schizosaccharomyces pombe	SZMC 1280	ME	30

*ME: malt extract medium; YPD: yeast peptone dextrose medium

4.5. Phenolic solutions, reagents, and solvents

Phenolic compounds for MIC determination

MICs for phenolic compounds (Table 5) against each yeast used were determined. With this regard, a stock solution with a concentration of 4 mg/mL was prepared from each phenolic compound in 10% (*v/v*) ethanol.

Solutions and reagents used in antibiofilm activity tests

- 0.1% (*w/v*) crystal violet solution (Sigma-Aldrich): 1 g of crystal violet was dissolved in 50 mL of ethanol, and the total volume adjusted to 1 L with distilled water.
- 33% (*v/v*) glacial acetic acid (Sigma-Aldrich), used for solubilizing the biofilms.
- 9% (*w/v*) physiological saline (Sigma-Aldrich) for washing the plates for planktonic cells removal.
- 0.01% (*w/v*) acridine orange (Sigma-Aldrich) used for fluorescence microscopy biofilm staining.
- 99% (*v/v*) methanol (Sigma-Aldrich) used for fixing the biofilms on the abiotic surface.

4.6. Antimicrobial activity tests for planktonic growth of yeasts

4.6.1. Determination of MIC of phenolics against the planktonic growth

The planktonic growth inhibitory effect of the phenolic compounds against the food spoilage yeasts was determined through a microplate assay (Zambrano *et al.* 2018). The stock solutions of the individual phenolic compounds were serially diluted with ethanol (10%, *v/v*) to give a range of concentration between 31.25 µg/mL to 2 mg/mL. From the diluted samples of natural phenolics and the corresponding stock solution, a volume of 100 µL was transferred to 96-well polystyrene microtiter plate wells (Sarstedt, Germany). This was followed by the addition of 100 µL of cell suspension to each microplate well prepared in a double-concentrated medium, leading to a final concentration of 15.63 µg/mL to 2 mg/mL; the final cell concentration was 10^5 CFU/mL, and was set using Bürker chamber. The positive control was made of inoculated growth medium in the absence of the phenolic compounds, while the negative control was composed of the sterile medium and the phenolic compounds. The microplates were then incubated for 24 h at 30 °C after which the absorbance was measured at 600 nm using a SPECTROstar Nano microplate reader (BMG Labtech, Germany). The MIC was defined as the concentration of the phenolic compound that caused 90% or higher growth inhibition of the food spoilage yeasts. All the measurements were done in three biological and three technical parallels.

4.6.2. Determination of MIC of food preservatives against planktonic growth

The MIC of food preservatives was determined through the microplate method as described in section 4.6.1. Serial dilution of the stock solution was done with 10% (*v/v*) ethanol to give a concentration range of 0.78 to 100 mg/mL. From the diluted samples of the food preservatives and the corresponding stock solution, a volume of 100 µL was transferred to 96-well polystyrene microtiter plates. This was followed by the addition of 100 µL of cell suspension (final concentration 10^5 CFU/mL) to each microplate well prepared in a double-concentrated medium leading to a final concentration of 0.39 to 50 mg/mL of the food preservatives. The positive control was made of inoculated growth medium in the absence of the food preservatives, while the negative control was composed of the sterile medium and the

food preservatives. The treated microplates were then incubated for 24 h at 30 °C, after which the absorbance was measured at 600 nm using a SPECTROstar Nano (BMG Labtech, Germany) microplate reader. The MIC was defined as the concentration of the food preservatives that caused 90% or higher growth inhibition of the food spoilage yeasts. All the measurements were done in three biological and three technical parallels.

4.6.3. Checkerboard assay for planktonic growth inhibition

To evaluate the antimicrobial effect of the phenolic-phenolic and the phenolic-food preservatives combinations against the planktonic growth of food spoilage yeasts, the checkerboard method was used as described by Motyl *et al.* (2005) with minor modifications. Briefly, seven serial two-fold dilutions of the food preservatives and six serial two-fold dilutions of the phenolics were prepared in 96-well polystyrene microtiter plates. Fifty µL of each dilution of the food preservatives was dispensed in each vertical row, and 50 µL of the phenolic compound dilution was dispensed in each horizontal row. The selection of the range of concentrations was based on the MICs obtained for the tested compounds against the spoilage yeasts. The final concentration of the food preservatives after microdilution ranged from 64 to 1 mg/mL, while that of the phenolic compounds ranged from 4 to 0.125 mg/mL. The final concentration of the inoculum in each well was 10^5 CFU/mL. After 24 h incubation at 30 °C under static conditions, growth in each well was quantified spectrophotometrically at 600 nm using a SPECTROstar Nano microplate reader (BMG Labtech, Germany). Wells that contained only the growth medium and the inoculum without the antimicrobial agents formed the positive control, while the negative control had the sterile medium with the antimicrobials without the inoculum. The percentage of growth in each well was calculated as previously reported (Zambrano *et al*. 2018). The MIC for each combination was defined as the concentration of compounds that reduced growth by 90% and above compared to that of the yeasts grown in the absence of the antimicrobials. All experiments were performed in triplicates in three different experiments. The fractional inhibitory concentration index was computed using the following equation: FICI = FIC-A + FIC-B = (MIC-AB / MIC-A) + (MIC-BA / MIC-B) where A and B are the antimicrobial compounds under combination. The FICI, is the fractional inhibitory concentration index, FIC-A, is the fractional inhibitory concentration of compound A, FIC-B,

is the fractional inhibitory concentration of compound B, MIC-AB is the MIC of compound A in the presence of compound B. MIC-BA, is the MIC of compound B in the presence of compound A. The interaction was interpreted as synergistic if the FICI ≤ 0.5, additive when 0.5 < FICI ≤ 1, indifferent when 1 < FICI ≤ 4, and antagonistic when FICI > 4.0.

4.7. Biofilm formation and treatment

4.7.1. Antibiofilm assay at fixed phenolic concentration

The effect of singular phenolic compounds on biofilm formation was examined by the method described by Zambrano *et al*. (2018). Wells of a 96-well polystyrene microtiter plate were filled with 200 µL of 24 h old yeast culture with approximately 10^8 CFU/mL concentration. After 4 h of cell adhesion performed at 30 °C, the planktonic cells were removed from each well and the plate was rinsed with physiological saline. The microplates were then dried in a laminar-flow box for 10 min. After drying, 200 µL of the corresponding sterile medium containing the phenolic compounds at a fixed concentration of 500 µg/mL was dispensed to each treated well. The positive control was made of 200 µL of sterile growth medium in the treated wells, while the negative controls contained the phenolic compounds in the sterile growth medium. The microplates were then incubated at 30 °C for 24 h. The biofilm cells were then detected by crystal violet staining.

4.7.2. MIC determination in antibiofilm assays

The MIC of the food preservatives and phenolic compounds on biofilm growth of yeasts was determined as follows: 200 µL of 24 h old yeast culture with approximately 10^8 CFU was pipetted in the wells of a 96-well microtiter plate and incubated for 4 h for cell adhesion at 30 °C. The non-adhered planktonic cells were removed from each well after the incubation. This was followed by the rinsing of the microplates with physiological saline after which the plates were left to dry for 10 min. After drying, 100 µL of the antimicrobial compounds were dispensed in the wells followed by the addition of another 100 µL of two-fold concentrated sterile medium to have a final concentration of 64 to 1 mg/mL for the food preservatives and 32 to 1 mg/mL

for the phenolic compounds. The positive control had the adhered cells and the sterile medium, while the negative control had the compounds and the sterile medium in the absence of the adhered cells. The plates were incubated for 24 h at 30 °C, after which biofilms were detected by crystal violet staining. The MIC was considered as the concentration of the antimicrobial compound that caused 90% or higher biofilm inhibition compared to the positive control. The experiments were performed in at least three biological parallels.

4.7.3. Fluorescence microscopy studies

The visualization of biofilms formed in the presence and absence of selected phenolic compounds was done using fluorescence microscopy. In this experiment, the surface for attachment for the yeast cells was a sterilized glass microscope slide (26 × 76 mm) placed in the center of a sterile Petri dish (diameter of 90 mm). Yeast cells were grown for 24 h at 30 °C in a medium appropriate for their growth. After a homogenization step by gentle vortexing, a volume of 6 mL from the cell suspension (approximately 10^8 CFU/mL) was supplemented with phenolic compound to be tested reaching a final concentration of 500 μg/mL. The phenolic compound contained in the suspension was then transferred to the dish covering the surface of the glass slide. A glass surface treated with phenolic-free cell suspension was used as a control. The Petri dishes were then incubated at 30 °C for 24 h, and the planktonic cells were removed using physiological saline. After 10 min fixation by methanol, the biofilms formed were stained with 20 μL of 0.01% (*w/v*) acridine orange dye (Sigma-Aldrich, Germany). The excess stain was washed out by distilled water and the dyed glass slides were dried for 10 min, after which they were visualized with AxioLab (Carl Zeiss, Germany) fluorescence microscope equipped with an Axiocam 503 mono (Carl Zeiss, Germany) camera. Excitation and emission wavelengths of 500 and 526 nm, respectively, were used to examine the acridine orange staining.

4.7.4. Checkerboard assay for biofilm formation inhibition

To assay the antibiofilm effects of phenolic-phenolic and phenolic-food preservative combinations, the compound combinations that had FICI < 1 in the planktonic growth inhibition tests were selected. Wells of a 96-well polystyrene microtiter plate were filled with 200 μL of

24 h yeast culture with approximately 10^8 CFU, except for those wells that formed the negative control. After 4 h of cell adhesion at 30 °C, the planktonic cells were removed from each well, and the plates were rinsed with physiological saline and left to dry in a laminar flow for 10 min. After drying, the compounds were then dispensed in the wells as described in section 4.6.3 for the food preservatives, and the final concentration of the phenolics ranged from 32 to 1 mg/mL. The positive control was made of the wells that contained only the sterile medium and the inoculated yeast cells without the antimicrobial agents, while the negative control wells had the sterile growth medium and the antimicrobial compounds only. The prepared plates were incubated for 24 h at 30 °C, after which biofilm formation was detected by crystal violet staining. The FIC index calculation and the definition of the interactions were as described in the section 4.6.3. All experiments were performed in triplicates in three different biological parallels.

4.8 Antimicrobial tests for adhesion inhibition

4.8.1. Adhesion inhibition assay at fixed phenolic concentration

The most active phenolics against yeast biofilm formation were tested for their antiadhesion properties on polystyrene surface using a microtiter plate-based assay (Raut *et al.* 2014). A volume of 200 µL of cell suspension (approximately 10^8 CFU/mL), which was prepared with the corresponding growth medium and contained 500 µg/mL of the phenolic compound, was transferred into the wells of a 96-well polystyrene microtiter plate. Inoculated growth medium without addition of phenolic compounds was considered as the positive control, while the negative controls contained phenolic compounds in the growth medium. After setting up the culturing environments, the plates were incubated for 4 h at 30 °C, the adhered cells were then detected by crystal violet staining. The experiments were repeated at least twice, with six parallel measurements performed after the staining.

4.8.2. MIC determinations in antiadhesion assays

In adhesion inhibition tests, the MIC for the phenolic and the food preservative antimicrobials was determined as follows: the food preservatives were dispensed into microtiter

plates to achieve a final concentration of 64 to 1 mg/mL, while the phenolics were dispensed in the wells to achieve a final concentration of 32 to 1 mg/mL, after the inoculum addition. The positive control had the sterile medium and the inoculum, while the negative control had the sterile medium and the antimicrobial compounds only. The microplates were then incubated for 4 h at 30 °C, after which the adhered cells were detected by crystal violet staining. The concentration of the compounds that caused adhesion inhibition of 90% or higher compared to the positive control was considered to be the MIC. The experiments were performed at least three times.

4.8.3. Checkerboard assay for adhesion inhibition

The combinations used in the antibiofilm assay were also evaluated for their ability to inhibit spoilage yeasts adhesion on a polystyrene surface. The compounds were dispensed as described in section 4.7.4, followed by the addition of the inoculum to achieve a final cell count of 10^8 CFU. The positive control was made of the wells that contained only the sterile medium and the inoculated yeast cells without the antimicrobial agents, while the negative control wells had the sterile growth medium and the antimicrobial compounds only. Plates were incubated for 4 h at 30 °C, after which the adhered cells were detected by crystal violet staining. The FIC index calculation and the definition of the interactions were as described in section 4.6.3. All experiments were performed in triplicates in three different biological parallels.

4.9. Crystal violet staining

Biofilms and adhered cells obtained in the presence or absence of phenolics were detected by the crystal violet staining method. The crystal violet staining method entailed a number of steps: after distributing the compounds in the microplates and setting the right culturing environment, the supernatant was then removed after 24 h incubation. The wells were then rinsed with physiological saline to remove the planktonic cells. The formed biofilms were then fixed on the microplate with 200 µl of methanol, after which the plates were incubated for 15 min at room temperature. After the removal and evaporation of methanol, a volume of 200 µL of 0.1% (*w/v*) crystal violet solution was added to each well and the plates were incubated

for 20 min at room temperature. The excess dye was then removed by washing the plates under slow-running tap water. The bounded crystal violet dye was solubilized by adding 200 µL of 33% (*v/v*) acetic acid after which the plates were then incubated for 10 min at room temperature. The absorbance was then measured at 590 nm (SPECTROstar Nano microplate reader, BMG Labtech, Germany), and the percentage of biofilm formation or adhered cells was calculated. The optical density of the positive control sample was considered as 100%.

4.10. Statistical analysis

Antimicrobial assays were performed in at least three independent experiments and data were expressed as means ± standard deviation. Basic statistical analysis of data, such as calculation of means and standard deviations, was conducted using Microsoft Office Excel 2016 function. Significance was calculated by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test in the GraphPad Prism 6.00 software (GraphPad Software Inc., USA). A *p*-value of < 0.05 was considered as statistically significant.

4.11. *In silico* analyses

4.11.1. Phenolics, protein targets and software selected for molecular docking

Molecular docking was performed for syringic acid, gallic acid, vanillic acid, caffeic acid, ferulic acid, cinnamic acid, *p*-coumaric acid, polydatin, resveratrol, (−)-epicatechin, quercetin and vanillin phenolics in the binding pockets of selected *S. cerevisiae* protein targets, i.e., chitin synthase III, squalene synthase and flocculation proteins Flo11 and Flo1. Analyses were carried out with the AutoDockVina (Trott and Olson 2010, Eberhardt *et al.* 2021), and the graphical user interface AutoDock Tools (Morris *et al.* 2009).

4.11.2. Ligand molecules preparation

The structures of the ligands of the phenolics were retrieved from the PubChem database in simulation description format and converted to PDB by Open Babel chemical tool box. The

ligands were then converted to protein data bank, partial charge (q), and atom type (t) (PDBQT) files using AutoDock Tools.

4.11.3. Preparation of protein molecules and binding site prediction

The crystal structure of the *S. cerevisiae* protein targets was obtained from the Protein Data Bank (PDB, https://www.rcsb.org/), and where there was no known structure, homology model was obtained from the SWISS-MODEL server for the squalene synthase (Waterhouse *et al*. 2018). The structure of the receptors was processed by removing any docked ligands, heteroatoms and water molecules from the PDB crystal structure and addition of polar hydrogen atoms. The PDBQT file of potential protein targets was generated from the PDB files using AutoDock Tools (Morris *et al.* 2009). The possible binding sites for the phenolic ligands and estimation of the druggability of the selected *S. cerevisiae* protein targets were determined using the structure-based modeling service server, ProteinsPlus DoGSiteScorer (Volkamer *et al*. 2012).

4.11.4. Preparation of configuration files

The AutoDock Tools "Grid" was used for determining the grid parameters and the data generated regarding the selected *S. cerevisiae* protein targets, natural phenolics ligands, grid size, and geometry were then saved in the form of a text file. The grid size was determined by the potential *S. cerevisiae* proteins target and designated as X, Y, Z points with a grid spacing of 0.503 Å. The exhaustiveness was set at 8 and the binding modes at 10. Blind docking and reconfirmation of configuration file parameters were done using CB-Dock (Liu *et al*. 2020), a protein-ligand docking method that automatically identifies the binding sites and determines the center and size according to the query ligand.

4.11.5. Docking

The prepared ligand molecules were docked in the binding pockets of the prepared potential targets using AutoDockVina (Trott and Olson 2010, Eberhardt *et al.* 2021) and scored

by using the scoring function. The binding free energy (ΔG, kcal/mol) for each ligand was reported in the log file. PyMOL (The PyMOL Molecular Graphics System, Version 2.0, Schrödinger, LLC) was utilized for the analysis of the binding interactions (including hydrogen bonds and hydrophobic interactions) of the ligands in the binding sites of the *S. cerevisiae* target proteins, while the LigPlot software (LigPlot+ version v.2.2.5) was used to generate 2D images of the ligand-target complex (Laskowski and Swindells 2011). The docking results were evaluated by considering the docking score, the hydrogen bonds and the different hydrophobic interactions occurring between the ligand and the receptor.

5. RESULTS AND DISCUSSION

5.1 Antimicrobial activity of singular phenolics

5.1.1. Influence of singular phenolics on planktonic growth of yeasts

Fifteen phenolic compounds, i.e., vanillic acid, gallic acid, syringic acid, protocatechuic acid, 4-hydroxybenzoic acid, cinnamic acid, caffeic acid, ferulic acid, *p*-coumaric acid, polydatin, resveratrol, quercetin, (−)-epicatechin, 4-hydroxybenzaldehyde and vanillin were screened for their potential inhibitory effect against planktonic growth of *Saccharomyces cerevisiae*, *Wickerhamomyces anomalus*, *Debaryomyces hansenii* and *Schizosaccharomyces pombe* spoilage yeasts through a broth microdilution method at 30 °C, at concentrations ranging from 0.016 to 2 mg/mL. The MIC results of the 15 phenolics against the food spoilage yeasts are summarized in Table 9. Most of the phenolic compounds had less than 90% growth inhibition against the spoilage yeasts at the highest screened concentration. A MIC of 2 mg/mL was obtained for vanillin, *p*-coumaric acid, and ferulic acid against *S. cerevisiae* and *S. pombe*. Quercetin, (−)-epicatechin, resveratrol, and 4-hydroxybenzaldehyde also had MIC at 2 mg/mL for *S. cerevisiae*, *S. pombe*, *D. hansenii* and *W. anomalus*, respectively. MIC of 1 mg/mL was identified for vanillin against *W. anomalus* and *D. hansenii*. Cinnamic acid exhibited the lowest MIC at 500 μg/mL concentration against all the screened food spoilage yeasts (Table 9). Overall, the planktonic growth of the four food spoilage yeasts was highly sensitive to cinnamic acid and vanillin, which was observed at 250 μg/mL (Figure 2), 500 μg/mL (Figure 3) and 1 mg/mL (Figure 4) phenolic concentrations.

The planktonic growth of *S. cerevisiae* was significantly inhibited by cinnamic acid even at lower concentrations than its MIC value, having a growth inhibition of more than 70% at 250 μg/mL ($p < 0.05$) (Figure 2D). Polydatin was equally effective against *S. cerevisiae* demonstrating higher planktonic growth inhibition of more than 45% at 1 mg/mL concentration ($p < 0.05$) (Figure 4D). For the rest of the phenolic compounds, a lower inhibitory effect of less than 45% was observed even at a concentration of 1 mg/mL in *S. cerevisiae*. Among these phenolics, resveratrol exhibited the lowest inhibitory activity having less than 10% growth inhibition at 1 mg/mL ($p < 0.05$) (Figure 4D). Overall, *S. cerevisiae* had remarkably higher

resistance against the planktonic growth inhibition activity of most of the tested phenolic compounds compared to the rest of the food spoilage yeasts.

Table 9. Minimum inhibitory concentration (MIC) of phenolic compounds against food contaminating yeasts.

Phenolic compounds	**MIC in mg/mL**			
	S. cerevisiae	***S. pombe***	***D. hansenii***	***W. anomalus***
Vanillic acid	>2	>2	>2	>2
Syringic acid	>2	>2	>2	>2
Gallic acid	>2	>2	>2	>2
Protocatechuic acid	>2	>2	>2	>2
4-Hydroxybenzoic acid	>2	>2	>2	>2
Cinnamic acid	0.5	0.5	0.5	0.5
p-coumaric acid	2	2	>2	>2
Caffeic acid	>2	>2	>2	>2
Ferulic acid	2	2	>2	>2
Resveratrol	>2	>2	2	>2
Polydatin	>2	>2	>2	>2
(−)-Epicatechin	>2	2	>2	>2
Vanillin	2	2	1	1
4-Hydroxybenzaldehyde	>2	>2	>2	2
Quercetin	2	>2	>2	>2

The exceptional tolerance of *S. cerevisiae* to the phenolic compounds could be attributed to several factors such as the expression of inhibitor-resistant transporters, and the conversion of phenolics into less potent antimicrobial compounds (Fitzgerald *et al*. 2003, Evensen *et al.* 2009). Currently, there is growing interest among the scientific community in unravelling the mechanisms behind phenolics tolerance in yeasts. Functional genomics tools such as chemogenomic screens will be resourceful in determining the different causes of fungal resistance to phytochemicals as well as identifying the specific genes and pathways affected by phenolics. In one study, vanillin had a MIC of 3.195 mg/mL which was close to the MIC of our study, 2 mg/mL (Fitzgerald *et al*. 2003). In that study, the authors associated the antimicrobial activity of vanillin with the presence of the aldehyde moiety in its structure. However, they have also noted that certain yeasts, including the *S. cerevisiae*, could convert vanillin to the less active vanillyl alcohol and vanillic acid derivatives at sub-MIC concentrations (Fitzgerald *et al.* 2003). The latter phenomenon could probably explain the reason why in our study vanillin had a lower inhibitory activity against *S. cerevisiae*, especially at lower concentration below the MIC (Figures 2D, 3D and 4D).

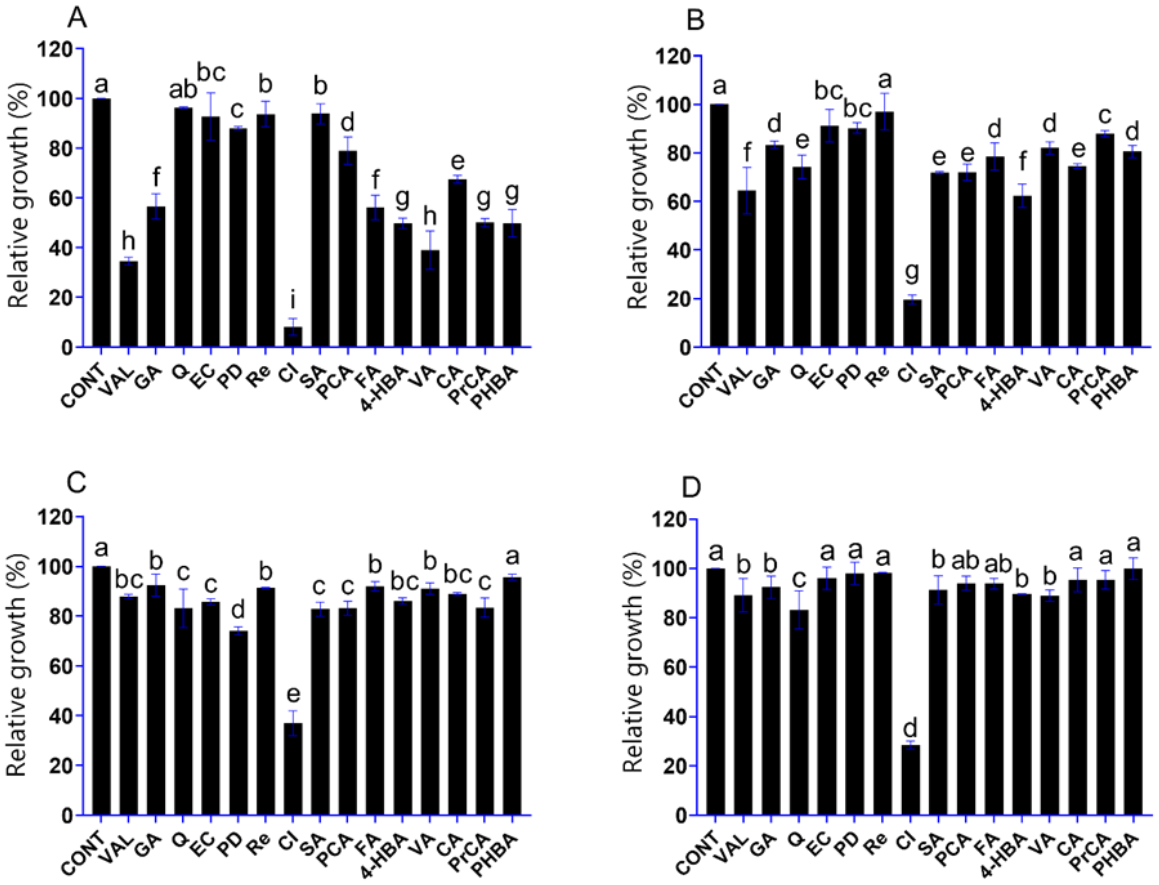


Figure 2. Effect of phenolic compounds on the growth of *D. hansenii* SZMC 8045Mo (**A**), *W. anomalus* SZMC 8061Mo (**B**), *S. pombe* SZMC 1280 (**C**) and *S. cerevisiae* SZMC 1279 (**D**) at a concentration of 250 µg/mL. Phenolic compounds: vanillin (**VAL**), gallic acid (**GA**), quercetin (**Q**), (−)-epicatechin (**EC**), polydatin (**PD**), resveratrol (**Re**), cinnamic acid (**CI**), syringic acid (**SA**), *p*-coumaric acid (**PCA**), ferulic acid (**FA**), 4-hydroxybenzaldehyde (**4-HBA**), vanillic acid (**VA**), caffeic acid (**CA**), protocatechuic acid (**PrCA**), 4-hydroxybenzoic acid (**PHBA**). The control (**CONT**) represents the growth of yeasts in the absence of phenolic compounds. Presented results are averages of three biological and three technical replicates; error bars represent standard deviations. Different letters indicate statistical differences according to one-way ANOVA followed by Tukey's multiple comparison test ($p < 0.05$).

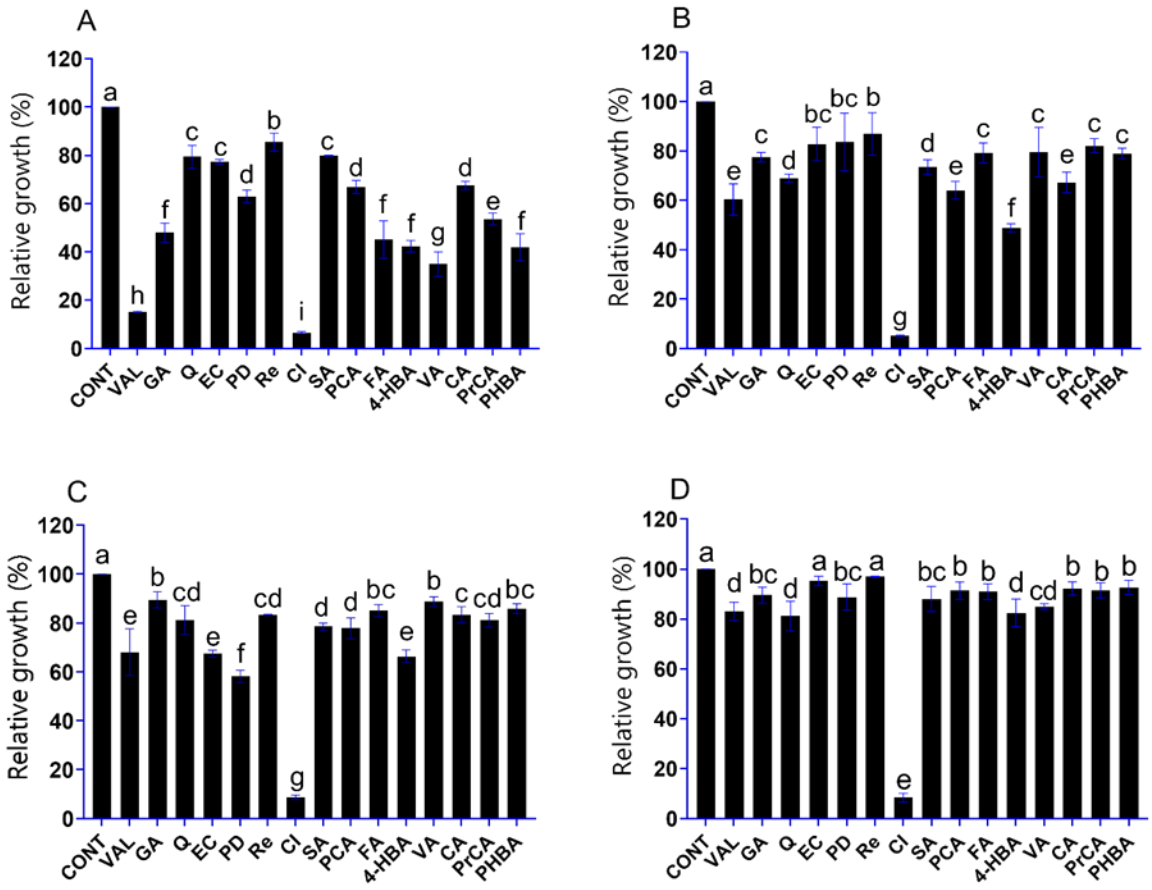


Figure 3. Effect of phenolic compounds on the growth of *D. hansenii* SZMC 8045Mo (**A**), *W. anomalus* SZMC 8061Mo (**B**), *S. pombe* SZMC 1280 (**C**) and *S. cerevisiae* SZMC 1279 (**D**) at a concentration of 500 µg/mL. Phenolic compounds: vanillin (**VAL**), gallic acid (**GA**), quercetin (**Q**), (−)-epicatechin (**EC**), polydatin (**PD**), resveratrol (**Re**), cinnamic acid (**CI**), syringic acid (**SA**), *p*-coumaric acid (**PCA**), ferulic acid (**FA**), 4-hydroxybenzaldehyde (**4-HBA**), vanillic acid (**VA**), caffeic acid (**CA**), protocatechuic acid (**PrCA**), 4-hydroxybenzoic acid (**PHBA**). The control (**CONT**) represents the growth of yeasts in the absence of phenolic compounds. Presented results are averages of three biological and three technical replicates; error bars represent standard deviations. Different letters indicate statistical differences according to one-way ANOVA followed by Tukey's multiple comparison test ($p < 0.05$).

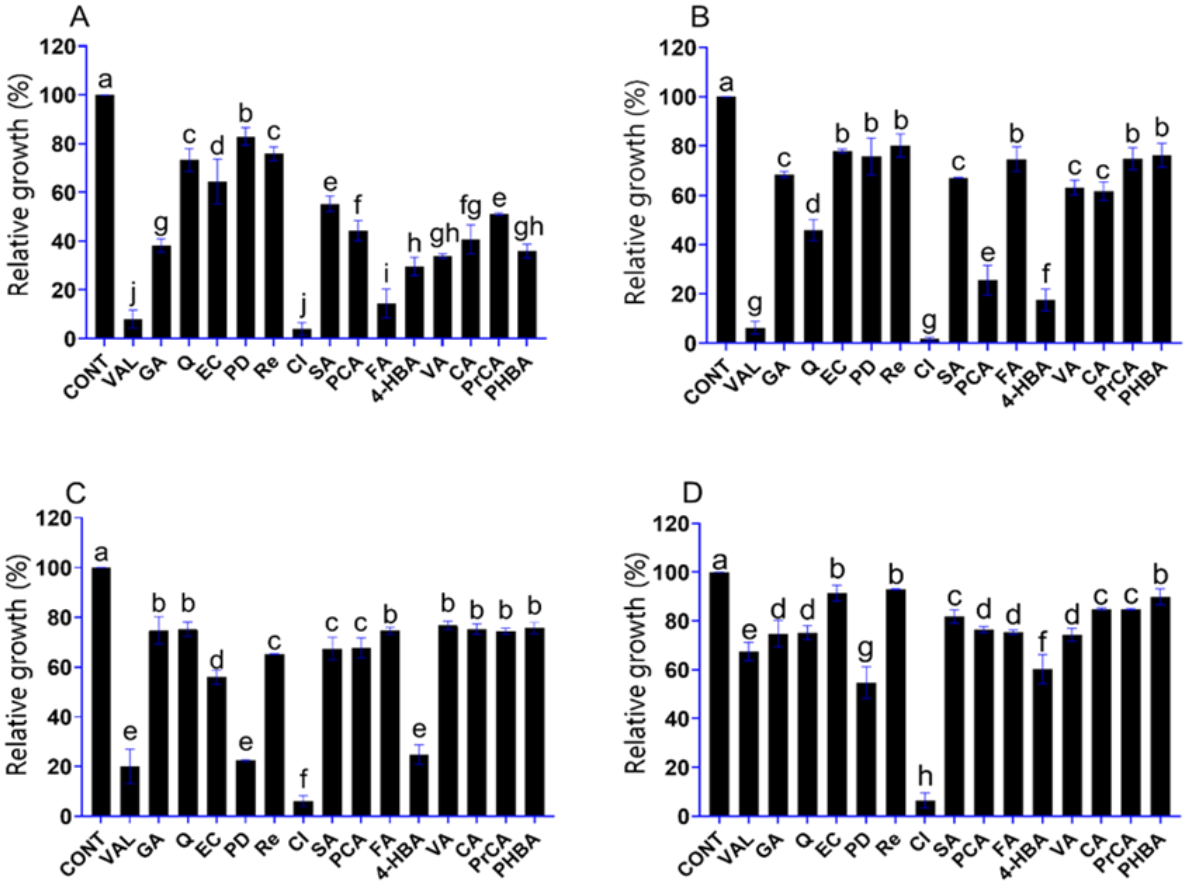


Figure 4. Effect of phenolic compounds on the growth of *D. hansenii* SZMC 8045Mo (**A**), *W. anomalus* SZMC 8061Mo (**B**), *S. pombe* SZMC 1280 (**C**) and *S. cerevisiae* SZMC 1279 (**D**) at a concentration of 1 mg/mL. Phenolic compounds: vanillin (**VAL**), gallic acid (**GA**), quercetin (**Q**), (−)-epicatechin (**EC**), polydatin (**PD**), resveratrol (**Re**), cinnamic acid (**CI**), syringic acid (**SA**), *p*-coumaric acid (**PCA**), ferulic acid (**FA**), 4-hydroxybenzaldehyde (**4-HBA**), vanillic acid (**VA**), caffeic acid (**CA**), protocatechuic acid (**PrCA**), 4-hydroxybenzoic acid (**PHBA**). The control (**CONT**) represents the growth of yeasts in the absence of phenolic compounds. Presented results are averages of three biological and three technical replicates; error bars represent standard deviations. Different letters indicate statistical differences according to one-way ANOVA followed by Tukey's multiple comparison test ($p < 0.05$).

The *S. pombe* planktonic growth was significantly inhibited by cinnamic acid having more than 60% growth inhibition at a lower concentration of 250 µg/mL ($p < 0.05$) (Figure 2C). Cinnamic acid and its derivatives have been shown to inhibit microbial enzymes. In one study, cinnamic acid could competitively inhibit benzoate 4-hydroxylase, an important enzyme in aromatic compounds degradation in fungi (Korošec *et al.* 2014). In addition, cinnamates can inhibit the enzyme β-ketoacyl-[acyl carrier protein] reductase, a highly conserved enzyme that

is pervasive in prokaryotic organisms and important in the fatty acid biosynthesis (Kristan *et al.* 2009). Vanillin was equally highly active against *S. pombe* at 1 mg/mL with a planktonic growth inhibition of 80% ($p < 0.05$) (Figure 4C), this planktonic growth was reduced to 32% (Figure 3C) and 13% (Figure 2C) at concentrations of 500 µg/mL and 250 µg/mL, respectively. Polydatin and 4-hydroxybenzaldehyde demonstrated higher than 70% growth inhibition at 1 mg/mL ($p < 0.05$) (Figure 4C). In addition, a 26% inhibition of *S. pombe* growth was recorded for polydatin at a concentration of 250 µg/mL (Figure 2C).

The planktonic growth of *D. hansenii* was significantly inhibited by vanillin, cinnamic acid, and 4-hydroxybenzaldehyde. At a low concentration of 250 µg/mL, vanillin, cinnamic acid, and 4-hydroxybenzaldehyde exhibited inhibitory effects of 66%, 92% and 50%, respectively ($p < 0.05$) (Figure 2A). At a concentration of 500 µg/mL, all the phenolic compounds had significant planktonic growth inhibition against *D. hansenii* (Figure 3A). At 1 mg/mL, most of the phenolic compounds had moderate to high growth inhibition with the least planktonic growth been recorded in polydatin of 17% (Figure 4A). In one study, vanillin could inhibit *D. hansenii* and other food spoilage yeasts when used at a concentration of 1.978 mg/mL for 40 days in culture medium and apple puree (Fitzgerald *et al*. 2003).

W. anomalus was vulnerable to cinnamic acid with 80% planktonic growth inhibition at a low concentration of 250 µg/mL (Figure 2B). Vanillin was also equally potent against *W. anomalus* having about 40% planktonic growth inhibition at concentrations of 250 and 500 µg/mL (Figures 2B and 3B). In addition, *p*-coumaric acid had >70% while 4-hydroxybenzaldehyde had >80% planktonic growth inhibition against *W. anomalus* at 1 mg/mL (Figure 4B).

5.1.2. Influence of singular phenolics on biofilm formation

In this study, the antibiofilm formation inhibitory activity of the 15 phenolic compounds against the studied spoilage yeasts was investigated at a fixed concentration of 500 µg/mL. Vanillin was highly potent as an antibiofilm agent against *D. hansenii* with a 97% biofilm inhibitory effect (Figure 5A). Vanillin was also highly potent against the biofilm formation of *W. anomalus* and *S. pombe*, with a 77% and 82% biofilm inhibitory effect, respectively (Figures

5B and 5C). There was a significantly lower activity of vanillin against biofilm formation in *S. cerevisiae* which was below 50% (Figure 5D).

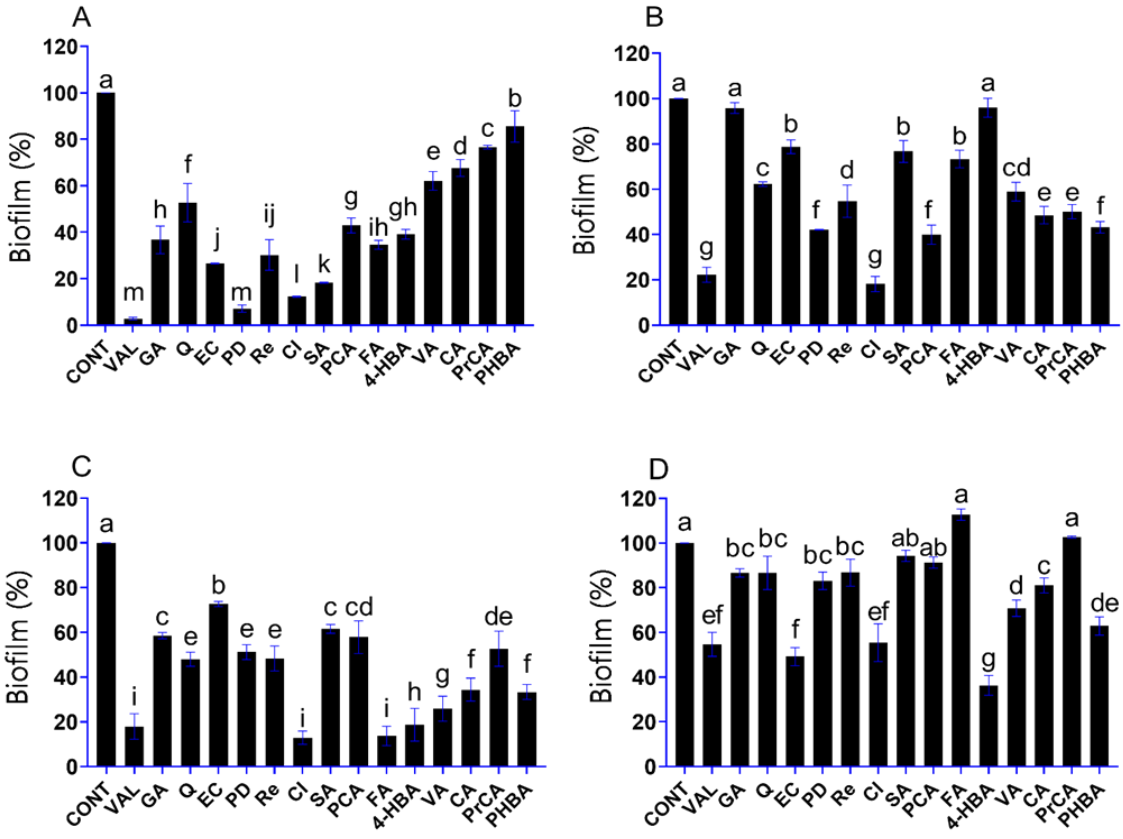


Figure 5. Effect of phenolic compounds (in 500 µg/mL) on the biofilm formation of *D. hansenii* SZMC 8045Mo (**A**), *W. anomalus* SZMC 8061Mo (**B**), *S. pombe* SZMC 1280 (**C**) and *S. cerevisiae* SZMC 1279 (**D**). Phenolic compounds: vanillin, **VAL**; gallic acid, **GA**; quercetin, **Q**; (–)-epicatechin, **EC**; polydatin, **PD**; resveratrol, **Re**; cinnamic acid, **CI**; syringic acid, **SA**; *p*-coumaric acid, **PCA**; ferulic acid, **FA**; 4-hydroxybenzaldehyde, **4-HBA**; vanillic acid, **VA**; caffeic acid, **CA**; protocatechuic acid, **PrCA**; 4-hydroxybenzoic acid, **PHBA**. The control (**CONT**) represents the biofilm formation in the absence of phenolic compounds. The results shown are the mean percent biofilm formed relative to control biofilm; error bars represent standard deviation. The different letters above the columns indicate significant differences according to one-way ANOVA followed by Tukey's multiple comparison test ($p < 0.05$).

Results showed that the biofilm inhibitory activity of the phenolic compounds differed depending on the studied food spoilage yeast. In pathogenic fungi *Candida albicans*, vanillin inhibited biofilm formation at a concentration of 500 µg/mL, through a potential mechanism of inhibition of fungal ergosterol biosynthesis (Jayant *et al.* 2013). Cinnamic acid was highly active

as an antibiofilm agent against *D. hansenii*, *W. anomalus*, and *S. pombe* with a biofilm formation inhibition of more than 80% at the studied concentration (500 µg/mL) ($p < 0.05$) (Figure 5A-C). In *S. cerevisiae*, the most active phenolic compound was 4-hydroxybenzaldehyde, which had a 64% biofilm formation inhibition ($p < 0.05$) (Figure 5D). Most of the phenolic compounds had low activity against the biofilm of *S. cerevisiae*. It is worthwhile to note the moderate inhibitory activity of quercetin against all the assayed food spoilage yeasts, which was less than 50% in most strains (Figure 5). Based on this finding, it is noteworthy to mention that quercetin can have a protective ability against yeast stress factors, which it does through modulation of signaling pathways like those involved in the biogenesis of the cell wall and carbohydrate metabolism (Vilaça *et al.* 2012). The tolerance of yeasts to antifungals has been associated with a number of mechanisms one being the production of a resilient extracellular matrix (ECM) that contains beta-1,3 glucan and extracellular DNA (Bojsen *et al.* 2014). Among the tested phenolics, only (−)-epicatechin and 4-hydroxybenzaldehyde had more than 50% biofilm formation inhibition in *S. cerevisiae* ($p < 0.05$). It is worth mentioning that ferulic acid and protocatechuic acid had a slight invigorative effect on *S. cerevisiae* biofilm formation (Figure 5D). Nevertheless, ten of the assayed phenolics, i.e., gallic acid, (−)-epicatechin, vanillin, polydatin, resveratrol, syringic acid, cinnamic acid, *p*-coumaric acid, 4-hydroxybenzaldehyde, and ferulic acid, had high antibiofilm activity against *D. hansenii*, an osmotolerant and halotolerant spoilage yeast, with more than 50% inhibitory effect (Figure 5A). In one study, gallic acid and quercetin had a moderate antibiofilm effect on fungal biofilms at 1.25 mg/mL (Alves *et al*. 2014). In another study, cinnamic acid derivatives had more than 50% biofilm inhibition against fungal biofilms (De Vita *et al*. 2016).

The biofilm inhibitory ability of the most bioactive compounds, i.e., vanillin, cinnamic acid and (−)-epicatechin, against the studied spoilage yeasts was also investigated on the glass surface of a microscope slide. The images from fluorescence microscopy showed fragmented biofilms for the samples containing the phenolic compounds and intact mature biofilms for the control samples (Figure 6), meaning that the analyzed phenolic compounds could block the formation of all yeast biofilms on glass surface at 500 µg/mL. From these results, further studies were considered necessary to analyze whether the compounds could inhibit only the biofilm formation, or they also could affect the cell adhesion during incubations.

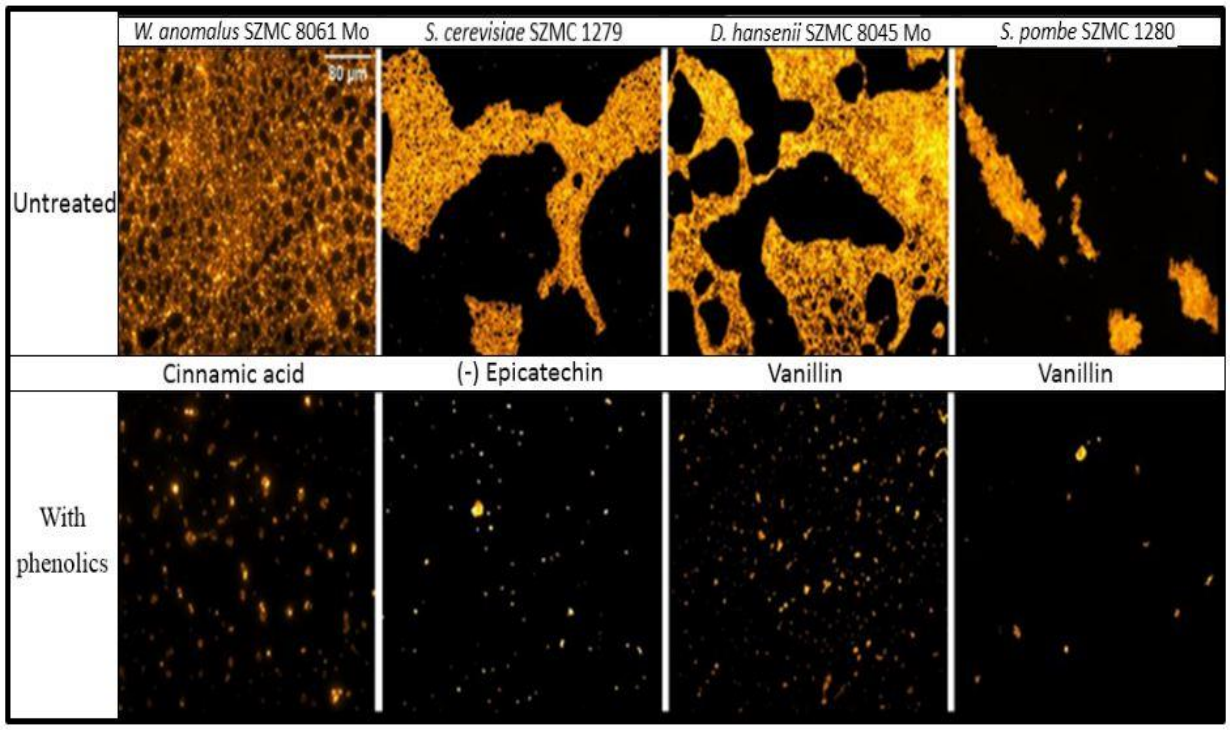


Figure 6. Biofilm formation of yeasts on a glass surface in the presence (sample) or absence (control) of selected phenolic compounds. Phenolic compounds were used in the samples at a concentration of 500 µg/mL. After incubation at 30 °C for 24 h biofilms were stained with acridine orange and examined by fluorescence microscope. Magnification 20x.

5.1.3. Influence of phenolic compounds on surface adhesion

The most effective phenolic compounds as antibiofilm agents were assayed as antiadhesion agents at 500 µg/mL. Apart from vanillin and cinnamic acid, other compounds assayed against the spoilage yeasts were polydatin and syringic acid in *D. hansenii*, *p*-coumaric acid and 4-hydroxybenzoic acid in *W. anomalus*, ferulic acid and 4-hydroxybenzaldehyde in *S. pombe*, and (−)-epicatechin and 4-hydroxybenzaldehyde in *S. cerevisiae* (Figure 7). From the antiadhesion study, all the assayed phenolic compounds had significant adhesion inhibition against the 4-h adhesion of spoilage yeast cells ($p < 0.05$) (Figure 7). This is exemplified in *D. hansenii*, where vanillin and cinnamic acid had more than 50% adhesion inhibition on the abiotic surface ($p < 0.05$) (Figure 7A). In *W. anomalus*, cinnamic acid, vanillin, and *p*-coumaric acid had higher antiadhesion activity than 4-hydroxybenzoic acid, with the latter exhibiting less than 50% antiadhesion capacity against *W. anomalus* when compared to the control (Figure 7B). Cinnamic acid and vanillin were potent antiadhesive agents against *S. pombe* with adhesion

inhibitions of 91% and 81%, respectively ($p < 0.05$) (Figure 7C). This high potency of these two compounds is also attributable to their antiplanktonic activity on *S. pombe* cells, most of which could have been decimated before attaching to the abiotic surface. It was interesting to note the resistant nature of *S. cerevisiae* against the adhesion inhibition activity of the assayed phenolics compared to the other yeast strains. Cinnamic acid exhibited the highest antiadhesive potency against *S. cerevisiae*, with an adhesion inhibition of 51%. The rest of the phenolic compounds had less than 30% antiadhesion activity against the resistant *S. cerevisiae* (Figure 7D).

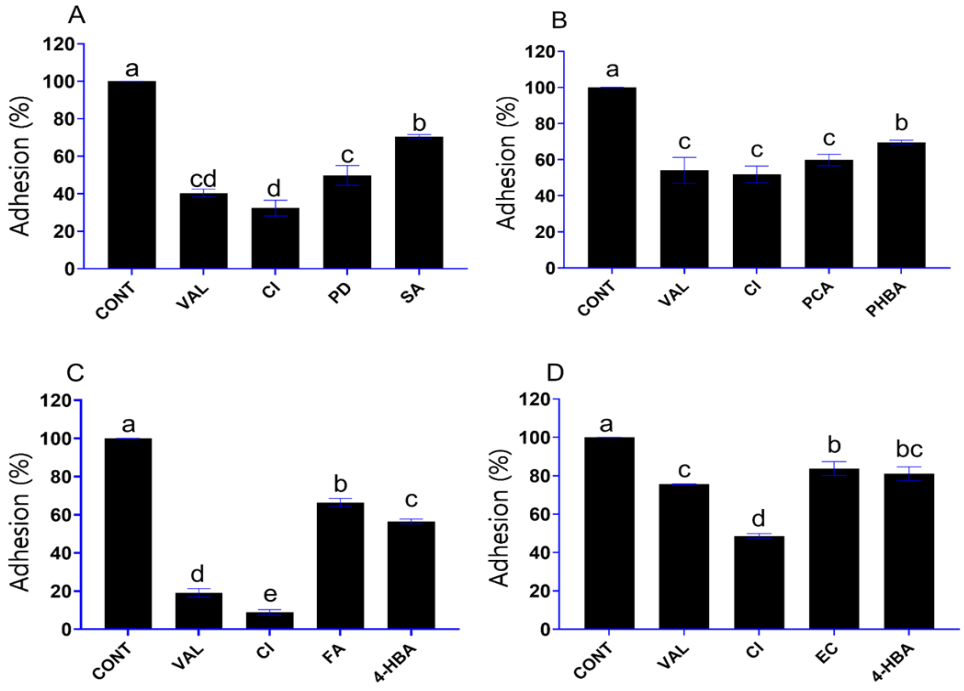


Figure 7. Effect of selected phenolic compounds (in 500 µg/mL concentration) on the adhesion of *D. hansenii* SZMC 8045Mo (**A**), *W. anomalus* SZMC 8061Mo (**B**), *S. pombe* SZMC 1280 (**C**) and *S. cerevisiae* SZMC 1279 (**D**) yeasts to polystyrene surface. Phenolic compounds: vanillin, **VAL**; cinnamic acid, **CI**; polydatin, **PD**; syringic acid, SA; *p*-coumaric acid, **PCA**; 4-hydroxybenzoic acid, **PHBA**; ferulic acid, **FA**; 4-hydroxybenzaldehyde, **4-HBA**; (–)-epicatechin, **EC**. Adhesion of cells in the absence of phenolic compounds was taken as 100%. Results are presented as mean of replicates; error bars represent standard deviation. The different letters above the columns indicate significant differences according to one-way ANOVA followed by Tukey's multiple comparison test ($p < 0.05$).

Several mechanisms explain the possible routes of adhesion inhibition of phenolic compounds against yeasts, including interference with localization of glycosylphosphatidylinositol anchored proteins, inhibition of biosynthesis of cell wall components, and downregulation of genes that encode adhesins (Martin *et al.* 2021). Previous

studies have also demonstrated the antiadhesive ability of natural phenolics against fungal strains. In one study, crude methanolic extracts from the mangrove *Laguncularia racemosa* were assayed as antiadhesive agents against *C. albicans* and *Candida glabrata*, with a substantial reduction of adhesion against *C. albicans* (Glasenapp *et al.* 2019). In other studies, magnolol, curcumin, and honokiol downregulated the genes encoding adhesins in *C. albicans* impeding considerably the surface adhesion abilities of the yeast (Martins *et al*. 2009, Shahzad *et al.* 2014).

5.2. Phenolics and food preservatives in combination against growth activities of yeasts

Having established that cinnamic acid and vanillin were the most potent singular phenolics against the studied food-related yeasts, the antiplanktonic, antibiofilm, antiadhesion activity of cinnamic-vanillin, cinnamic-food preservatives, and vanillin-food preservatives combinations were examined using a checkerboard approach. The three food preservatives used were sodium benzoate, sodium diacetate, and potassium sorbate all of which are common food preservatives against food spoilage yeasts. The checkerboard tests were performed after establishing the MICs of phenolic and preservative compounds to be combined (see Tables 10-16).

5.2.1. Influence of phenolic compound combinations on planktonic growth

The two phenolic compounds selected for the combination assay, vanillin and cinnamic acid, were combined to create a bipartite solution, as previously described in section 4.6.3, which was screened for antiplanktonic growth activity against the food spoilage yeasts. The MICs of the two phenolic compounds were reconfirmed during checkerboard assays, and for comparison they were included in Table 10 as well. As for the checkerboard assay, synergistic interaction was present in all the assayed spoilage yeasts (Table 10). In *D. hansenii* and *W. anomalus*, the FICI was ≤0.31 ($p < 0.05$), while in *S. pombe* and *S. cerevisiae*, the FICI was ≤0.28 ($p < 0.05$) (Table 10). There was a reduction of ≥32-fold in the MIC of vanillin in *S. pombe* and *S. cerevisiae* and ≥16-fold in *W. anomalus* and *D. hansenii* (Table 10). Cinnamic acid also recorded a ≥4-fold reduction in the MIC in all the strains (Table 10). Checkerboard

layouts depicting the activity of vanillin-cinnamic acid combinations against the planktonic growth of the spoilage yeasts tested is shown in Appendix 1. Vanillin is a known inhibitor of planktonic growth in yeasts through translational repression (Iwaki *et al.* 2013). In one study, vanillin caused the disintegration of the fungal cell surface and mitochondrial functioning (Saibabu *et al.* 2021). In our opinion, the high activity of vanillin-cinnamic acid combination against the planktonic growth of tested yeasts could have been contributed by the multitarget effects of the combination.

Table 10. Effect of combination of vanillin and cinnamic acid on planktonic growth of food spoilage yeasts**.**

Yeasts	Phenolic compounds	MIC (mg/mL)		MIC reduction (fold)	FIC index	Outcome
		Single	Combined			
D. hansenii	Cinnamic acid + Vanillin	0.5 1	0.125 0.0625	≥4 ≥16	≤0.31	Synergy
W. anomalus	Cinnamic acid + Vanillin	0.5 1	0.125 0.0625	≥4 ≥16	≤0.31	Synergy
S. pombe	Cinnamic acid + Vanillin	0.5 2	0.125 0.0625	≥4 ≥32	≤0.28	Synergy
S. cerevisiae	Cinnamic acid + Vanillin	0.5 2	0.125 0.0625	≥4 ≥32	≤0.28	Synergy

5.2.2. Phenolics-food preservative combinations against planktonic growth

Vanillin and cinnamic acid were combined separately with the three studied food preservatives to create binary antifungal solutions that were screened for growth inhibitory potential. For the vanillin-food preservatives' growth inhibitory activity, synergistic interaction was present in all combinations in *S. pombe*, with a FICI range of 0.41-0.46 (Table 11). In *S. cerevisiae*, synergistic interaction was present in vanillin-sodium benzoate and vanillin-potassium sorbate with a FICI of 0.34 (Table 11). In *W. anomalus*, synergistic interaction was present in vanillin-sodium benzoate with a FICI of 0.33 (Table 11). In *D. hansenii*, all vanillin-food preservatives combinations were indifferent with a FICI range of 2.02-2.64. (Table 11). Checkerboard layouts depicting the action of some effective phenolic-food preservatives combinations against the planktonic growth of spoilage yeasts is shown in Appendix 2. A synergistic interaction of antimicrobial compounds has been ascribed to a number of factors such as the formation of complexes that are more stable than the individual compounds in

combination, such complexes may be in form of dimers or adducts with higher anti-yeast activity than the singular compounds (Olszowy-Tomczyk 2020, Corrêa *et al.* 2021). The new compounds formed due to combination may also target multiple metabolic pathways, modulate cellular transport and permeation, and inhibit the development of disease resistance mechanisms (Olszowy-Tomczyk 2020, Corrêa *et al.* 2021).

Table 11. Effect of combination of vanillin and food preservatives on planktonic growth of food spoilage yeasts.

Yeasts	Phenolic agent + food preservative	MIC (mg/mL)		MIC reduction (fold)	FIC index	Outcome
		Single	Combined			
D. hansenii	Vanillin +	1	2	0.5	2.02	Indifferent
	Sodium benzoate	50	1	50		
	Vanillin +	1	2	0.5	2.64	Indifferent
	Potassium sorbate	50	32	1.56		
	Vanillin +	1	2	0.5	2.32	Indifferent
	Sodium diacetate	50	16	3.13		
W. anomalus	Vanillin +	1	0.25	4	0.33	Synergy
	Sodium benzoate	12.5	1	12.5		
	Vanillin +	1	0.25	4	0.58	Additive
	Potassium sorbate	3	1	3		
	Vanillin +	1	4	0.25	4.67	Antagonism
	Sodium diacetate	6	4	1.5		
S. pombe	Vanillin +	2	0.5	4	0.41	Synergy
	Sodium benzoate	6.25	1	6.25		
	Vanillin +	2	0.25	8	0.46	Synergy
	Potassium sorbate	3	1	3		
	Vanillin +	2	0.5	4	0.41	Synergy
	Sodium diacetate	12.5	2	6.25		
S. cerevisiae	Vanillin +	2	0.5	4	0.34	Synergy
	Sodium benzoate	25	2.34	10.68		
	Vanillin +	2	0.5	4	0.34	Synergy
	Potassium sorbate	25	2.34	10.68		
	Vanillin +	2	1	2	0.66	Additive
	Sodium diacetate	50	8	6.25		

When equal concentrations of vanillin-food preservative combinations were compared with singular food preservatives for their activity against planktonic growth of the studied yeasts, it was observed that vanillin-food preservative combinations had a higher activity than the singular food preservatives (Figure 8). This demonstrated that the presence of vanillin had a

promoting effect on the food preservatives' anti-yeast activity, even at low concentrations (Figure 8).

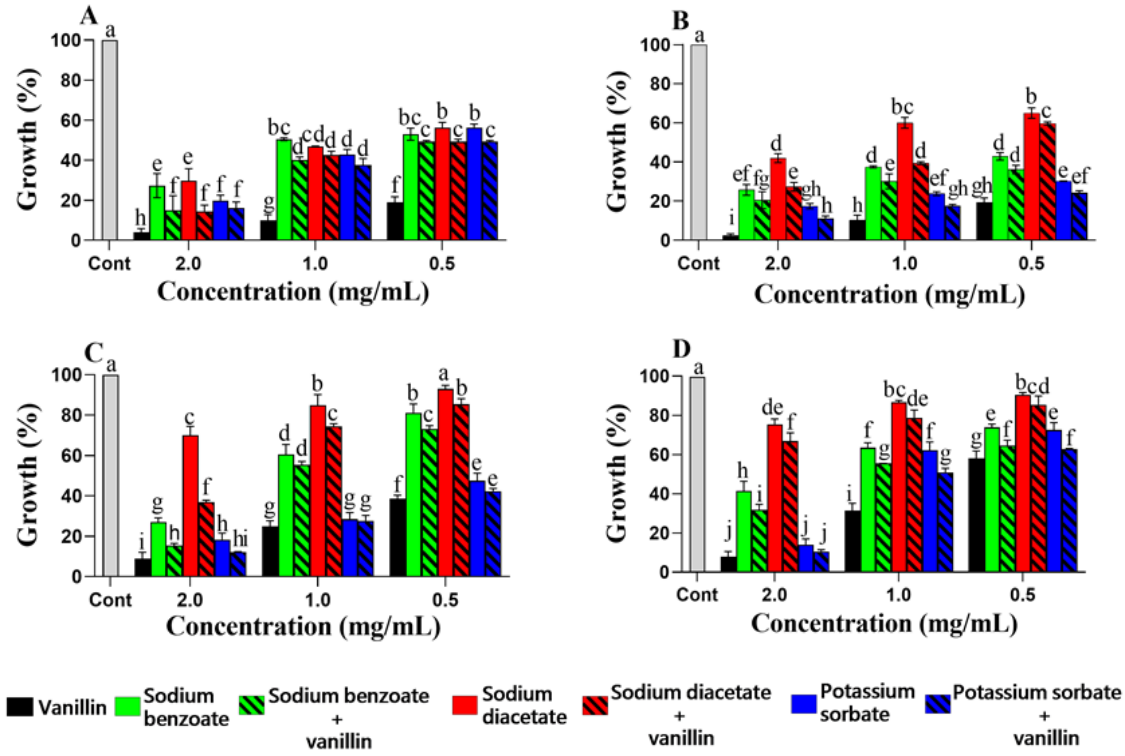


Figure 8. Effect of the antimicrobial agents on planktonic growth of *D. hansenii* SZMC 8045Mo (**A**), *W. anomalus* SZMC 8061Mo (**B**), *S. pombe* SZMC 1280 (**C**), and *S. cerevisiae* SZMC 1279 (**D**) when the vanillin and food preservatives were used alone or in combination in equal proportions. The control (Cont.) represents growth in the absence of the compounds. The results are the mean percent growth relative to the control; error bars represent standard deviation. The different letters above the columns indicate statistically significant differences ($p < 0.05$).

There was less activity against the planktonic growth of studied yeasts for the cinnamic acid-food preservative combinations, with synergistic interaction (FICI of ≤0.34) only occurring in the cinnamic acid-sodium diacetate combination against *S. pombe* (Table 12). Synergistic compound combinations have been known to be highly efficacious against their targets than singular compounds (Yin *et al.* 2014). The cinnamic acid-sodium benzoate and cinnamic acid-potassium sorbate combinations in *S. pombe* were additive in activity with a FIC index of 0.67 and 0.83, respectively (Table 12). In *W. anomalus*, cinnamic acid-sodium benzoate and cinnamic acid-potassium sorbate combinations were additive in activity with a FIC index of 0.58, while cinnamic acid-sodium diacetate was antagonistic in activity, with a FIC index of

4.16 (Table 12). In *S. cerevisiae*, cinnamic acid-sodium diacetate was additive in activity, with a FICI of 0.9, while cinnamic acid-sodium benzoate and cinnamic acid-potassium sorbate were indifferent, with each having a FICI of 2.82 (Table 12). In *D. hansenii*, higher resistance against the cinnamic acid-food preservative combinations was quite evident, with cinnamic acid-sodium benzoate (FICI >9.28) and cinnamic acid-sodium diacetate (FICI 5.28) being antagonistic. Antimicrobial compounds antagonism is often undesirable but could be helpful in curbing the selection for antimicrobial resistance (Chait *et al.* 2007). The cinnamic acid-potassium sorbate combination in *D. hansenii* was indifferent, with a FICI of 1.78 (Table 12). The relative fortitude of *D. hansenii* against the combinations could have been caused by its halotolerant nature attributed to its plasma membrane adaptability to environmental salinity (Turk *et al*. 2007).

Table 12. Effect of combination of cinnamic acid and food preservatives on planktonic growth of food spoilage yeasts.

Yeasts	Phenolic agent + food preservative	MIC (mg/mL)		MIC reduction (fold)	FIC index	Outcome
		Single	Combined			
D. hansenii	Cinnamic acid +	0.5	>4	<0.13	>9.28	Antagonism
	Sodium benzoate	50	>64	<0.78		
	Cinnamic acid +	0.5	0.25	2	1.78	Indifferent
	Potassium sorbate	50	64	0.78		
	Cinnamic acid +	0.5	2	0.25	5.28	Antagonism
	Sodium diacetate	50	64	0.78		
W. anomalus	Cinnamic acid +	0.5	0.25	2	0.58	Additive
	Sodium benzoate	12.5	1	12.5		
	Cinnamic acid +	0.5	0.13	3.85	0.58	Additive
	Potassium sorbate	3.13	1	3.13		
	Cinnamic acid +	0.5	2	0.25	4.16	Antagonism
	Sodium diacetate	6.25	1	6.25		
S. pombe	Cinnamic acid +	0.5	0.25	2	0.67	Additive
	Sodium benzoate	6	1	6		
	Cinnamic acid +	0.5	0.25	2	0.83	Additive
	Potassium sorbate	3	1	3		
	Cinnamic acid +	0.5	≤0.13	≥3.85	≤0.34	Synergy
	Sodium diacetate	12.5	≤1	≥12.5		
S. cerevisiae	Cinnamic acid +	0.5	0.13	3.85	2.82	Indifferent
	Sodium benzoate	25	64	0.39		
	Cinnamic acid +	0.5	0.13	3.85	2.82	Indifferent
	Potassium sorbate	25	64	0.39		
	Cinnamic acid +	0.5	0.13	3.85	0.9	Additive
	Sodium diacetate	50	32	1.56		

When the antimicrobial activity of equal concentrations of cinnamic acid-food preservative combinations were compared with that of singular synthetic preservatives against the planktonic growth of the studied yeasts, it emerged that cinnamic acid-food preservatives combinations were more active even at low concentrations, compared to singular food preservatives (Figure 9). This demonstrated that the presence of cinnamic acid had a boosting effect on the anti-yeast activity of the food preservatives.

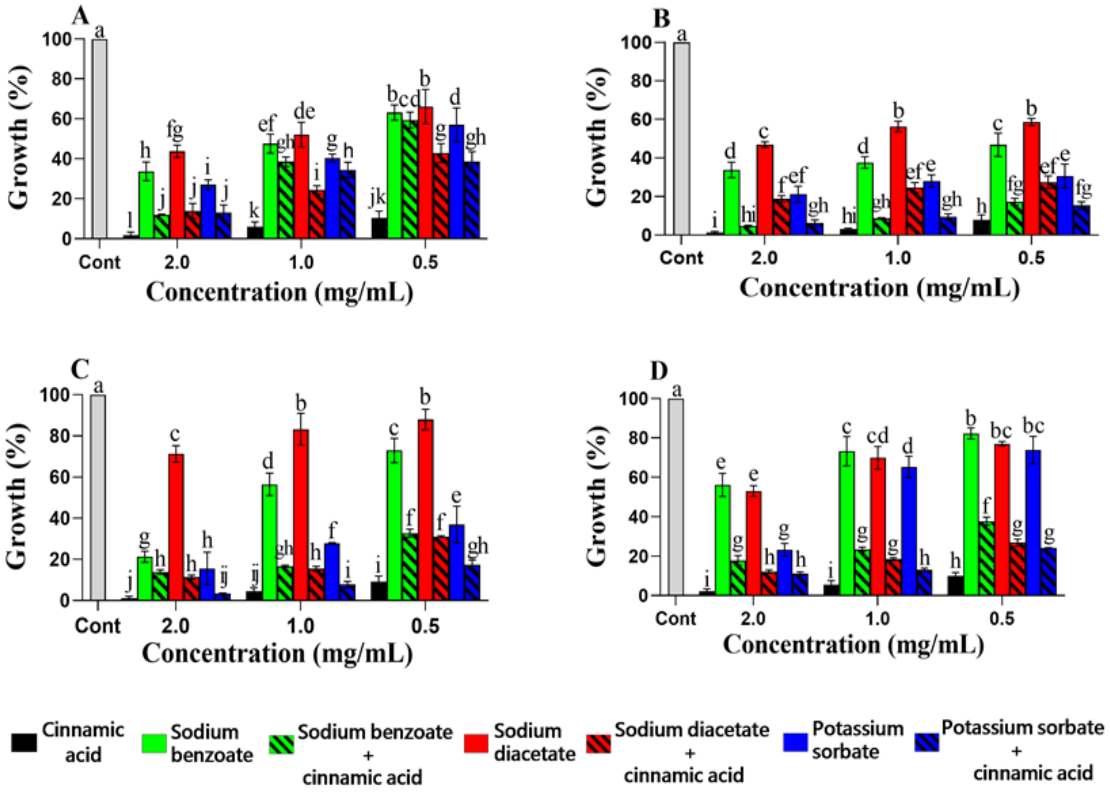


Figure 9. Effect of the antimicrobial agents on the planktonic growth of *D. hansenii* SZMC 8045Mo (**A**), *W. anomalus* SZMC 8061Mo (**B**), *S. pombe* SZMC 1280 (**C**), and *S. cerevisiae* SZMC 1279 (**D**) when the cinnamic acid and food preservatives were used alone or in combination in equal proportions. The control (**Cont.**) represents growth in the absence of the compounds. The results are the mean percent growth relative to the control; error bars represent standard deviation. The different letters above the columns indicate statistically significant differences ($p < 0.05$).

5.2.3. Influence of phenolic compound combinations on biofilm formation

The vanillin-cinnamic acid combination was also assayed against the biofilm formation of the spoilage yeasts. The combination had synergistic interaction in all the studied yeasts with a FIC index of ≤0.5 ($p < 0.05$) (Table 13). The combination also reduced the MIC of the two

phenolic compounds by a range of 4 to ≥16 (Table 13). The checkerboard layout depicting the effect of vanillin-cinnamic acid combinations against biofilm formation of spoilage yeasts is shown in Appendix 3. The fungal biofilm cells are distinct from planktonic cells since they have an extraordinary tolerance to conventional antifungal treatment (Nett and Andes 2015). As such, the discovery of novel antifungal compounds and innovative strategies against fungal biofilms is of great interest. Vanillin-cinnamic acid combination seems to provide a feasible solution to the fungal recalcitrant biofilms based on the results of our study.

Table 13. Effect of combination of vanillin and cinnamic acid on biofilm formation of food spoilage yeasts.

Yeasts	Phenolic compounds	MIC (mg/mL)		MIC reduction (fold)	FIC index	Outcome
		Single	Combined			
D. hansenii	Cinnamic acid +	8	2	4	0.5	Synergy
	Vanillin	8	2	4		
W. anomalus	Cinnamic acid +	8	≤1	≥8	≤0.19	Synergy
	Vanillin	8	≤0.5	≥16		
S. pombe	Cinnamic acid +	8	1	8	0.25	Synergy
	Vanillin	8	1	8		
S. cerevisiae	Cinnamic acid +	8	2	4	0.31	Synergy
	Vanillin	8	0.5	16		

5.2.4. Influence of phenolics-food preservative combinations on biofilm cells

The cinnamic acid/vanillin-food preservative combinations with a FICI less than 1 in the antiplanktonic growth assay were tested for their antibiofilm activity against the studied yeasts. According to the results of this antibiofilm assay, most of the phenolic-food preservative combinations were indifferent to the biofilm formation. The only exception was the vanillin-sodium diacetate combination in *S. pombe* that was additive in activity, with a FICI of 0.75 (Table 14). However, it was interesting to note the high reduction in the MIC of most food preservatives in the presence of phenolic compounds (Table 14). In *W. anomalus*, for instance, potassium sorbate and sodium benzoate had a 16-fold MIC reduction when combined with vanillin (Table 14). In *S. pombe*, sodium benzoate had a 64-fold reduction in MIC with cinnamic acid and a 32-fold reduction when combined with vanillin (Table 14). In *S. cerevisiae*, a 32-fold MIC reduction was recorded for sodium benzoate and potassium sorbate when combined with vanillin (Table 14). The checkerboard layout depicting the effect of some phenolic-food

preservative combinations against the biofilm formation of spoilage yeasts is shown in Appendix 4. Although the phenolic-food preservative combinations were not so strongly active against the biofilm cells as to result in a synergistic association, combination of the compounds against fungal biofilms is advisable as it may prevent or slow down the selection of antifungal resistance (Nett and Andes 2015).

Table 14. Effect of combination of phenolic compounds and food preservatives on biofilm formation of food spoilage yeasts.

Yeasts	Phenolic agent + food preservative	MIC (mg/mL)		MIC reduction (fold)	FIC index	Outcome
		Single	Combined			
W. anomalus	Vanillin +	8	16	0.5	2.06	Indifferent
	Sodium benzoate	32	2	16		
	Vanillin +	8	16	0.5	2.06	Indifferent
	Potassium sorbate	16	1	16		
	Cinnamic acid +	8	2	4	1.25	Indifferent
	Sodium benzoate	32	32	1		
	Cinnamic acid +	8	4	2	2.5	Indifferent
	Potassium sorbate	16	32	0.5		
S. pombe	Vanillin +	8	16	0.5	2.06	Indifferent
	Potassium sorbate	16	1	16		
	Vanillin +	8	8	1	1.03	Indifferent
	Sodium benzoate	64	2	32		
	Vanillin +	8	4	2	0.75	Additive
	Sodium diacetate	32	8	4		
	Cinnamic acid +	8	4	2	2.5	Indifferent
	Sodium diacetate	32	64	0.5		
	Cinnamic acid +	8	8	1	1.02	Indifferent
	Sodium benzoate	64	1	64		
	Cinnamic acid +	8	8	1	1.06	Indifferent
	Potassium sorbate	16	1	16		
S. cerevisiae	Vanillin +	8	16	0.5	2.03	Indifferent
	Potassium sorbate	32	1	32		
	Vanillin +	8	16	0.5	2.03	Indifferent
	Sodium benzoate	32	1	32		
	Vanillin +	8	4	2	1.00	Indifferent
	Sodium diacetate	64	32	2		
	Cinnamic acid +	8	2	4	1.25	Indifferent
	Sodium diacetate	64	64	1		

5.2.5. Influence of phenolic compound combinations on adhesion capacity

The vanillin-cinnamic acid combination was evaluated for its ability to inhibit the adhesion of the spoilage yeasts on a polystyrene surface. The combination was synergistic in interaction in all the spoilage yeasts tested, except in *D. hansenii*, where additivity effect was observed (FICI 0.63) (Table 15). In addition, the combination of vanillin and cinnamic acid had a reducing effect with 2- to 16-fold MIC reduction on the effective antiadhesion concentration of the compounds ($p < 0.05$) (Table 15). Adhesion is the first stage of microbial biofilm formation; therefore, it is a crucial step to intervene in biofilm management. The inhibitory effect of natural products on the adhesion activity of yeasts is less studied, even though it is important to develop synthetic preservative-free mechanisms for preventing the establishment of biofilms.

Table 15. Effect of combination of vanillin and cinnamic acid on adhesion on polystyrene surface of food spoilage yeasts.

Yeasts	Phenolic compounds	MIC (mg/mL)		MIC reduction (fold)	FIC index	Outcome
		Single	Combined			
D. hansenii	Cinnamic acid +	8	4	2	0.63	Additive
	Vanillin	8	1	8		
W. anomalus	Cinnamic acid +	8	2	4	0.31	Synergy
	Vanillin	8	0.5	16		
S. pombe	Cinnamic acid +	8	1	8	0.38	Synergy
	Vanillin	8	2	4		
S. cerevisiae	Cinnamic acid +	8	2	4	0.5	Synergy
	Vanillin	8	2	4		

5.2.6. Influence of phenolics-food preservative combinations on adhesion capacity

The cinnamic acid/vanillin-food preservative combinations were evaluated for their ability to inhibit adhesion on a polystyrene surface. The vanillin-potassium sorbate combination had synergistic interaction in the case of *W. anomalus* and *S. pombe*, with FIC index values of 0.25 and 0.38, respectively ($p < 0.05$) (Table 16). Most of the phenolic-food preservative combinations in *S. pombe* were additive in activity with a FICI range of 0.53-0.75 ($p < 0.05$), while in *S. cerevisiae*, all the phenolic-food preservative combinations were indifferent, with a FICI range of 1.5-2.5 ($p < 0.05$) (Table 16). In *W. anomalus*, the phenolic-food preservative

combinations were indifferent to adhesion inhibition, except the vanillin-potassium sorbate combination which showed synergistic interaction (Table 16). The presence of phenolic compounds led to 1 to 32-fold reduction in the MICs of food preservatives (Table 16).

Table 16. Effect of combination of phenolic compounds and food preservatives on polystyrene surface adhesion of food spoilage yeasts.

Yeasts	Phenolic agent + food preservative	MIC (mg/mL)		MIC reduction (fold)	FIC index	Outcome
		Single	Combined			
W. anomalus	Vanillin +	8	16	0.5	2.03	Indifferent
	Sodium benzoate	32	1	32		
	Vanillin +	8	1	8	0.25	Synergy
	Potassium sorbate	16	2	8		
	Cinnamic acid +	8	4	2	1.5	Indifferent
	Sodium benzoate	32	32	1		
	Cinnamic acid +	8	8	1	1.5	Indifferent
	Potassium sorbate	16	8	2		
S. pombe	Vanillin +	8	2	4	0.38	Synergy
	Potassium sorbate	8	1	8		
	Vanillin +	8	4	2	0.63	Additive
	Sodium benzoate	16	2	8		
	Vanillin +	8	4	2	0.53	Additive
	Sodium diacetate	32	1	32		
	Cinnamic acid +	8	4	2	0.75	Additive
	Sodium diacetate	32	8	4		
	Cinnamic acid +	8	4	2	0.56	Additive
	Sodium benzoate	16	1	16		
	Cinnamic acid +	8	4	2	0.63	Additive
	Potassium sorbate	8	1	8		
S. cerevisiae	Vanillin +	8	16	0.5	2.06	Indifferent
	Potassium sorbate	16	1	16		
	Vanillin +	8	16	0.5	2.5	Indifferent
	Sodium benzoate	16	8	2		
	Vanillin +	8	8	1	1.5	Indifferent
	Sodium diacetate	32	16	2		
	Cinnamic acid +	8	16	0.5	2.5	Indifferent
	Sodium diacetate	32	16	2		

In conclusion, most of the phenolic-food preservative combinations were indifferent against yeast biofilms and in the adhesion tests. However, the vanillin-cinnamic acid combination seems to be an effective strategy. The vanillin-cinnamic acid combination was also effective against yeast adherence on the abiotic surface and might influence the initial stage of biofilm formation. Vanillin is an aromatic aldehyde with an odor threshold of 0.008 and 100

μg/L in air and water, respectively (de-la-Fuente-Blanco and Ferreira 2020), and is lauded for its appetite-enhancing effects when present in food as an additive (Ogawa *et al.* 2018). According to the United States Food and Drug Administration (FDA), the maximum permitted level of vanillin is 70 mg/kg of body weight (Zhao *et al.* 2018). Cinnamic acid is known for its rapid absorption and elimination from the body and has no safety concerns when used as a flavoring agent (Adams *et al.* 2004). According to the joint FAO/WHO expert committee on food and additives (JECFA), the acceptable daily intake (ADI) levels of sodium diacetate, potassium sorbate, and sodium benzoate is 0-15, 0-25, and 0-5 mg/kg, per body weight, respectively (Mpountoukas *et al.* 2008, Mohammadzadeh-Aghdash *et al.* 2018). Based on our study, all the combinations with synergistic interactions were within the permitted concentrations of food additives (see Tables 10-16). All the compounds used in the study are generally recognized as safe (GRAS), therefore, quite appealing for use in food realms.

5.3. *In silico* investigation of phenolics against *S. cerevisiae* protein targets

5.3.1. Protein targets

Twelve phenolic compounds belonging to different classes were selected for *in silico* investigation through molecular docking studies, having demonstrated significant anti-yeast properties in previous *in vitro* experiments. The phenolics were syringic acid, gallic acid, vanillic acid, caffeic acid, ferulic acid, cinnamic acid, *p*-coumaric acid, polydatin, resveratrol, (−)-epicatechin, quercetin, and vanillin. The protein targets selected for the docking studies were from *S. cerevisiae*, a well-characterized yeast; these proteins were chitin synthase III (PDB ID: 4WJW), squalene synthase (SWISS-MODEL), Flo11 (PDB ID:4UYR) and Flo1 (PDB ID: 4LHL) (Appendix 5). Based on the literature, the protein targets selected are important for yeasts' flocculation, structural integrity, adhesion, and biofilm formation (Table 17). A protein basic local alignment sequence similarity search, as well as other information in the public databases of proteins, would help reveal the similarity between the selected protein targets and other food-related yeasts proteins.

Table 17. Physiological functions of *S. cerevisiae* protein targets used in the *in silico* analysis against the natural phenolics.

***S. cerevisiae* protein target**	**Physiological function(s)**	**UniProtKB**	**Reference**
Chitin synthase III	Cell wall chitin synthesis	P29465	Bulik *et al*. (2003)
Squalene synthase	Biosynthesis of ergosterol for cell membrane formation. Important in yeasts cell growth	P29704	Jennings *et al.* (1991), Jordá and Puig (2020)
Flocculation protein Flo11	Cell-cell interactions during flocculation, cell-substrate adhesion, biofilm development, haploid invasive growth	P08640	Reynolds and Fink (2001), Goossens *et al.* (2011)
Flocculation protein Flo1	Cell wall protein important in cell-cell interactions during yeast flocculation	P32768	Soares (2011), Goossens *et al.* (2011)

5.3.2. Binding potential of phenolics to protein targets

Most of the phenolic ligands had promising binding potential on the *S. cerevisiae* protein targets when their hydrogen and hydrophobic bond interactions as well as free energy of binding were analyzed (Table 18).

Table 18. Molecular docking output of the phenolics ligands binding to *S. cerevisiae* protein target.

Natural phenolics	**Protein targets**	**Binding energy (kcal/mol)**	**Hydrogen bond interactions**
Polydatin	Chitin synthase III	-10.4	Tyr688, Asp685, Lys197, Lys681
	Squalene synthase	-8.7	Arg76, Asn222, Phe295, Tyr72, Gln219
	Flo11	-8.3	Asn147, Thr102, Asn180, Trp144
	Flo1	-7.2	Asn84, Thr124, Ser123, Asp125
Quercetin	Chitin synthase III	-10.0	Lys681, Asn191, Lys197, Gly200, Tyr688, Leu201
	Squalene synthase	-7.8	Gln219, Phe295, Val182, Asn222
	Flo11	-8.0	Thr135, Asn147, Met145, Trp144
	Flo1	-7.0	Tyr46, Gln45, Thr132, Trp122, Asn135, Tyr130
(−)-Epicatechin	Chitin synthase III	-9.0	Lys681, Lys197, Asn690 Gly200, Leu201
	Squalene synthase	-8.1	Arg76, Tyr72
	Flo11	-7.6	Asn147, Thr135, Tyr133
	Flo1	-7.1	Gln45, Thr132, Asn135, Thr131, Tyr130, Tyr46
Resveratrol	Chitin synthase III	-7.6	Leu201, Gly200
	Squalene synthase	-7.4	Arg76
	Flo11	-7.9	Cys179, Asn147, Thr135
	Flo1	-6.2	Asp82, Thr77, Asp78

Table 18. Continued.

Natural phenolics	Protein targets	Binding energy (kcal/mol)	Hydrogen bond interactions
Cinnamic acid	Chitin synthase III	-6.1	Gly200, Glu203, Leu201
	Squalene synthase	-6.0	Gly187, Tyr283, Thr191
	Flo11	-6.2	Trp144
	Flo1	-5.4	Asp78
Vanillin	Chitin synthase III	-6.3	Asp685, Asn690, Gly200, Leu201
	Squalene synthase	-5.3	Thr191
	Flo11	-5.5	Thr75, Glu131
	Flo1	-4.9	Asp82, Asp125
Vanillic acid	Chitin synthase III	-6.9	Asp685, Asn690, Glu203, Leu201, Gly200
	Squalene synthase	-5.7	Thr191, Tyr283
	Flo11	-5.5	Met145
	Flo1	-5.5	Gln45, Thr132, Thr131, Trp122
Ferulic acid	Chitin synthase III	-6.8	Gly200, Leu201, Glu203, Lys681
	Squalene synthase	-6.0	Thr191, Tyr283
	Flo11	-6.2	Thr135, Asn147, Tyr133
	Flo1	-5.9	Asp78, Asp125, Asp82
Caffeic acid	Chitin synthase III	-7.1	Asp685, Tyr688
	Squalene synthase	-6.0	Thr191, Gly187, Tyr283
	Flo11	-6.2	Trp144, Asn147, Cys179
	Flo1	-5.9	Asp125
p-Coumaric acid	Chitin synthase III	-6.6	Asp685, Lys681, Leu201
	Squalene synthase	-5.9	Gly187, Thr191, Tyr283
	Flo11	-6.2	-
	Flo1	-5.4	Asp125, Thr132, Tyr46
Syringic acid	Chitin synthase III	-6.7	Gly200, Leu201, Glu203
	Squalene synthase	-5.6	Asp83, Arg235, Tyr178
	Flo11	-5.4	Asn147, Met145
	Flo1	-4.9	Gln179, Ser253
Gallic acid	Chitin synthase III	-6.9	Asn690, Gly200, Leu201, Lys197
	Squalene synthase	-5.6	Tyr283, Thr191, Phe295
	Flo11	-5.6	Met145, Asn147
	Flo1	-5.0	Asn135, Thr131, Thr132, Gln45, Tyr46

It was apparent from the docking results obtained that phenolics with a higher number of hydroxyl groups had a lower docking score, when compared with phenolics with less hydroxyl groups (Table 18 and Appendix 6). Phenolics with a higher number of hydroxyl groups

included polydatin, quercetin, (−)-epicatechin, and resveratrol which ranged between 3 to 6 (Appendix 6).

5.3.3. Molecular docking of the phenolic ligands to *S. cerevisiae* Flo11

For the phenolics-Flo11 docking studies, the lowest binding free energy was recorded in polydatin at -8.3 kcal/mol, while in resveratrol, quercetin, and (−)-epicatechin, the binding free energy was -7.9, -8.0, and -7.6 kcal/mol, respectively (Table 18). The binding free energy of the remaining phenolic compounds was in the range of -6.2 to -5.4 kcal/mol. The common residues that formed hydrogen bond interactions included Thr135, Asn147, and Tyr133, while the common hydrophobic interactions were formed with Ala134, Gln136, Cys179, Asn180, and Pro146 (Figure 10). In terms of the number of hydrogen bonds interactions, polydatin formed six, while quercetin formed four bonds with the protein target (Figure 10). Resveratrol, (−)-epicatechin, caffeic acid, and ferulic acid formed three hydrogen bonds, the rest of the phenolics formed either two or one, except *p*-coumaric acid where only hydrophobic bonds were formed; some of the phenolics-Flo11 interactions are shown in Figure 10. The molecular docking poses with the lowest binding free energy for the phenolics-Flo11 complex is shown in Appendix 7. The Flo11 protein is crucial for cell-cell interaction, invasive growth, and adhesion on a solid surface, therefore, its inhibition is crucial in mitigating biofilm establishment (Guo *et al.* 2000). The interactions between phenolics and proteins can occur as covalent and noncovalent interactions. The covalent interaction of molecules is usually irreversible and may be influenced by a host of conditions, such as alkaline conditions and the availability of phenolic oxidases (Yilmaz *et al.* 2022). Noncovalent interactions are reversible and include hydrogen bonding, ionic bonds, hydrophobic interactions, and electrostatic interactions. When considered together, these interactions may change the biological characteristics and functions of the proteins (Yilmaz *et al.* 2022). Based on UniProtKB protein database, *S. cerevisiae* Flo11 protein is similar in structure to glucoamylase from *Saccharomyces diastaticus*, a subspecies of *S. cerevisiae* whose vegetative cells and ascospores have been reported to spoil carbonated fermented beverages (Suiker *et al*. 2021).

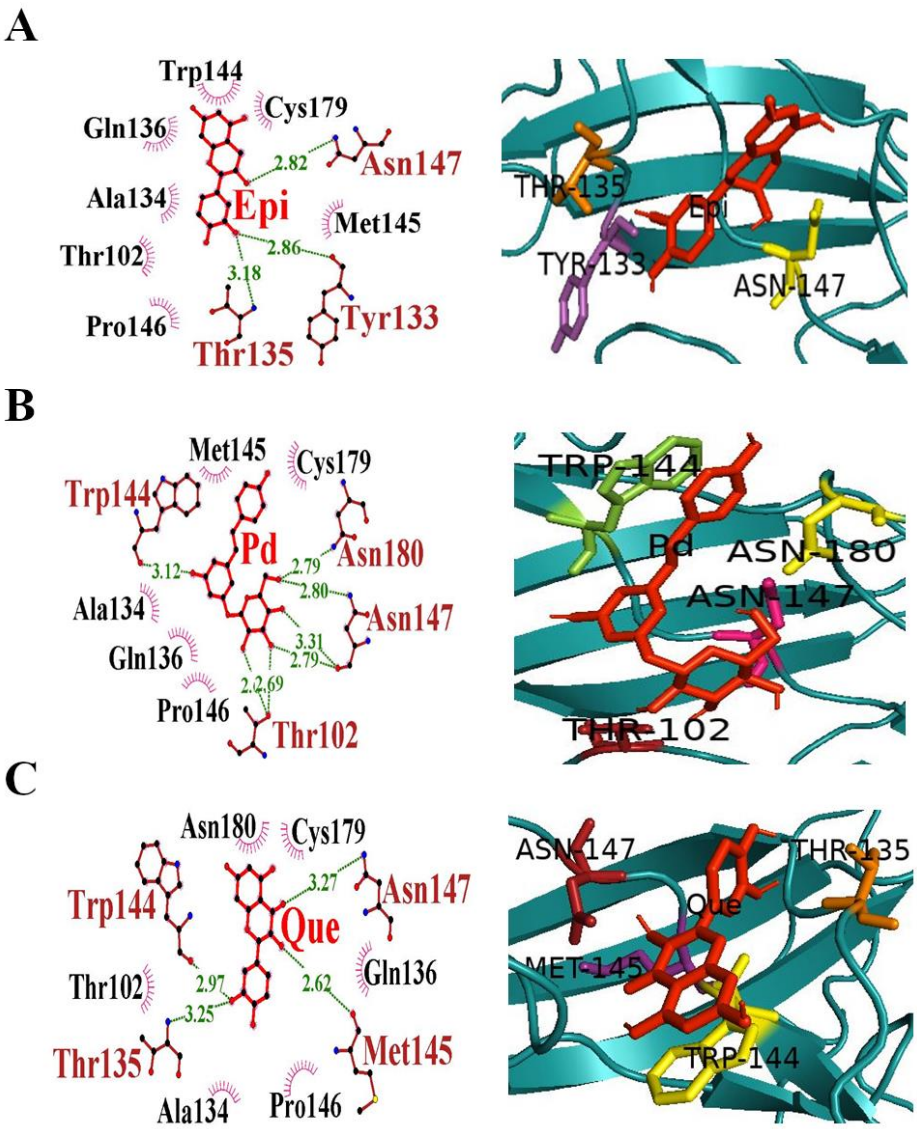


Figure 10. 2D and 3D interaction of (−)-epicatechin (**A**), polydatin (**B**), quercetin (**C**) with *S. cerevisiae* Flo11 (PDB ID: 4UYR). Epi=(−)-epicatechin, Pd=polydatin, Que=quercetin. 2D image show hydrogen and hydrophobic interactions. 3D image show hydrogen bond residues.

5.3.4. Molecular docking of the phenolic ligands to *S. cerevisiae* squalene synthase

The molecular docking studies of the phenolics ligands on the squalene synthase receptor had the highest affinity in polydatin, which was -8.7 kcal/mol. (−)-Epicatechin, quercetin, and resveratrol had binding energies of -8.1, -7.8, and -7.4 kcal/mol, respectively (Table 18). The rest of the phenolic ligands had their binding energy ranging from -6.0 to -5.3 kcal/mol (Table 18). Hydrogen bond interactions between the phenolic ligands and squalene synthase binding

site included Arg76, Phe295, Asn222, Thr191, and Tyr283 amino acid residues (Figure 11 and Table 18).

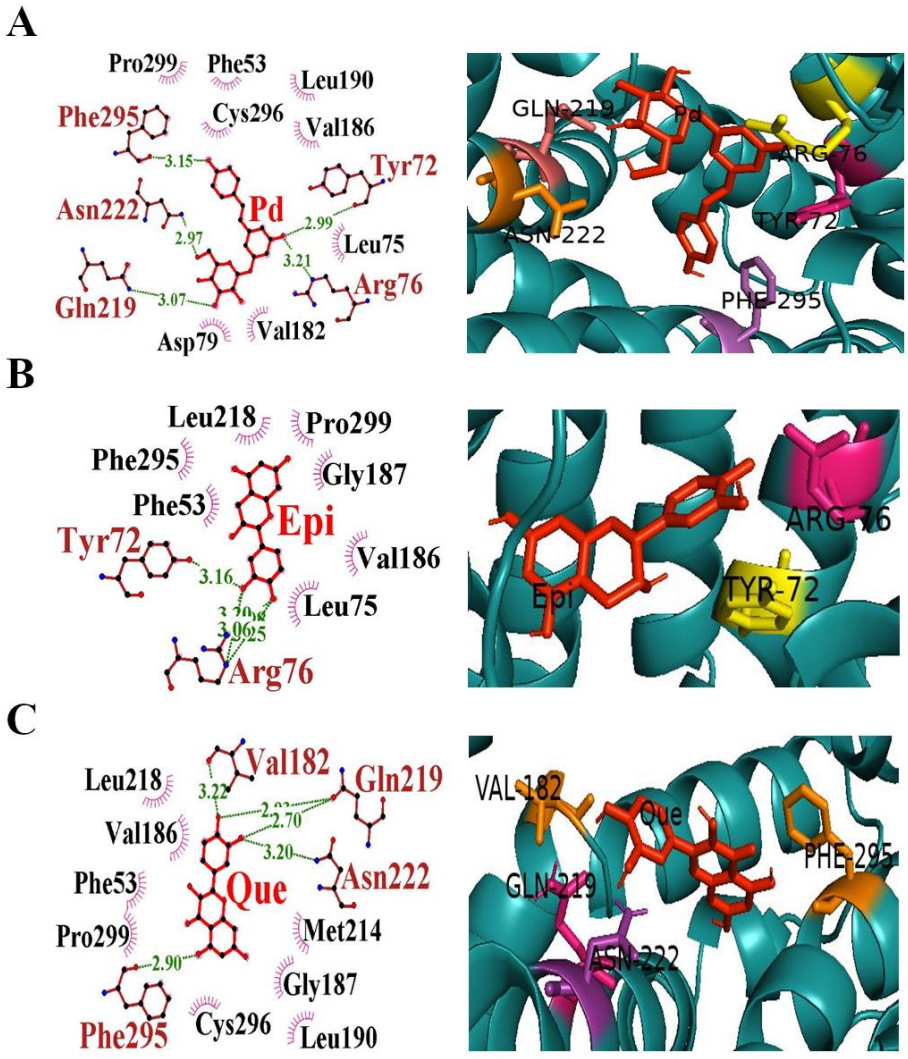


Figure 11. 2D and 3D interaction of polydatin (**A**), (−)-epicatechin (**B**), quercetin (**C**) with *S. cerevisiae* squalene synthase protein. Pd=polydatin, Epi=(−)-epicatechin, Que=quercetin. 2D image shows hydrogen and hydrophobic interactions. 3D image show hydrogen bond residues.

Hydrophobic interactions were found between the ligand and Pro299, Leu190, Val186, Leu75, and Phe53 residues (Figure 11 and Table 18**)**. In terms of the number of hydrogen bonds crucial for the bonding strength, polydatin and quercetin had five each. The rest of the phenolic ligands had hydrogen bonds ranging from 1 to 3. It was interesting to note the presence of Tyr and Phe residues among the residues that form hydrogen bonds. In one study, site-directed mutagenesis of eukaryotic squalene synthase identified conserved Tyr, Phe, and Asp residues

essential for function (Tansey and Shechter 2001); the aromatic rings of Phe and Tyr were crucial in the isoprenoid metabolic pathway for carbocation intermediates stabilization (Tansey and Shechter 2001). A protein basic local alignment sequence similarity search by NCBI-BLAST and *S. cerevisiae* squalene synthase revealed similarity with squalene synthase sequences from other food related yeasts such as *Saccharomyces arboricola* H-6 (96%), *Kluyveromyces lactis* (69%) and *Kluyveromyces marxianus* DMKU3-1042 (69%).

5.3.5. Molecular docking of the phenolic ligands to *S. cerevisiae* chitin synthase III

Chitin synthase 3 is an integral membrane protein whose trafficking from the trans-Golgi network to the cell surface is heavily dependent on chitin synthase 5 and chitin synthase 6 (Sanchatjate and Schekman 2006). The phenolics-chitin synthase III docking studies resulted in quercetin, polydatin, (−)-epicatechin, caffeic acid, and resveratrol having binding score ranging from -10.4 to -7.1 kcal/mol (Table 18). Polydatin had the lowest binding score of -10.4 kcal/mol (Table 18). The key residues that formed hydrogen bonds with the phenolic ligands included Lys681, Gly200, Lys197, Leu201, and Tyr688 (Figure 12), while hydrophobic interaction was common with Glu203 and Asn690 (Figure 12). Polydatin, (−)-epicatechin, vanillin, and quercetin formed more than 5 hydrogen bonds with their bond lengths ranging from 2.79 to 3.33 Å (Figure 12). The rest of the phenolic ligands formed hydrogen bonds ranging from 2 to 5 in number (Figure 12). The higher the number of hydrogen bonds and the lower the bond length, the more favorable is the binding of the ligands on the receptor binding pockets **(**Kortemme *et al*. 2003). In this regard, the compounds with a fairly higher number of hydrogen bonds, such as polydatin, (−)-epicatechin, and quercetin, had a robust binding in the receptors binding pockets than those with fewer hydrogen bonds. This was confirmed by the relatively favorable binding scores of these compounds (Table 18).

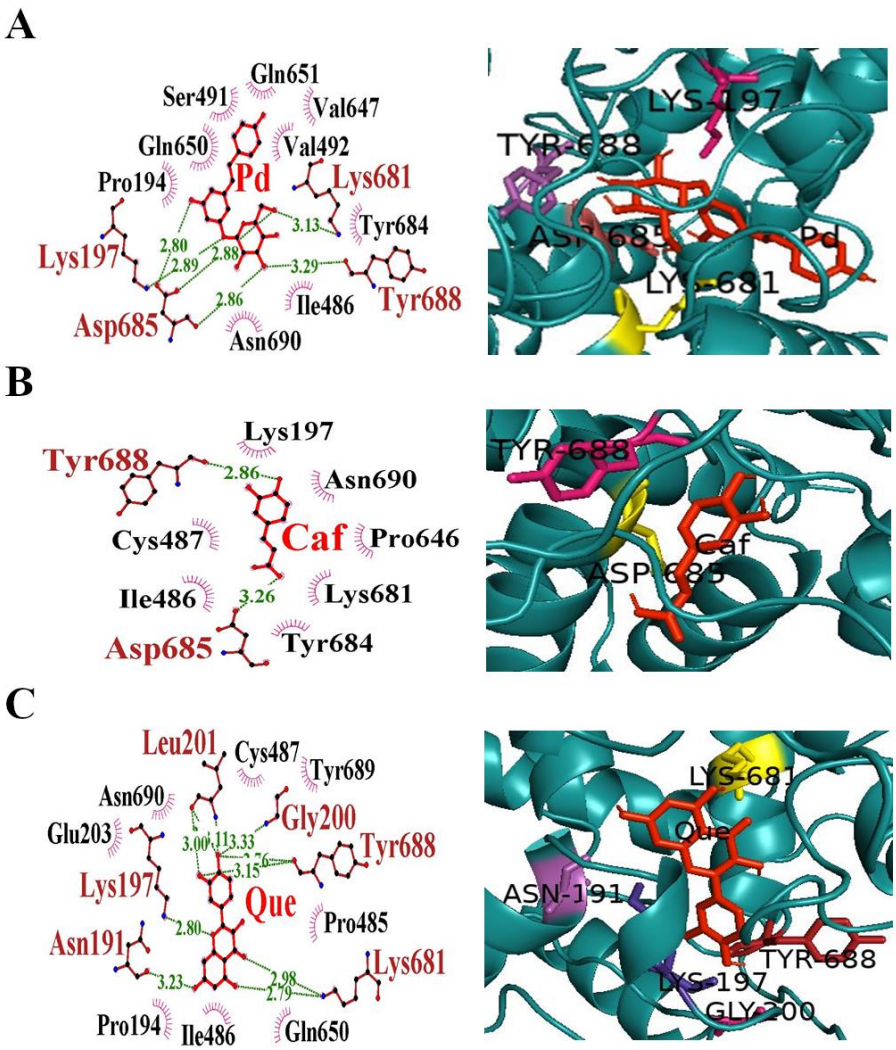


Figure 12. 2D and 3D interaction of polydatin (**A**), caffeic acid (**B**), quercetin (**C**) with *S. cerevisiae* chitin synthase III protein (PDB ID: 4WJW). Pd=(−) polydatin, Caf=caffeic acid, Que=quercetin. 2D image shows hydrogen and hydrophobic interactions. 3D image show hydrogen bond residues.

5.3.6. Molecular docking of the phenolic ligands to *S. cerevisiae* Flo1

Polydatin, (−)-epicatechin, and quercetin had the lowest binding scores against Flo1 protein target with values of -7.2, -7.1, and -7.0 kcal/mol, respectively. The rest of the compounds ranked lower in their binding potential with scores ranging between -6.2 to -4.9 kcal/mol (Table 18). (−)-Epicatechin formed 10 hydrogen bonds, while quercetin and polydatin formed 8 and 6 hydrogen bonds, respectively. The rest of the phenolic ligands formed between 1 to 5 hydrogen bonds (Table 18). Key residues involved in hydrogen bond interactions included

Thr132, Asp78, and Tyr46 (Table 18 and Figure 13). Some of the common residues involved in hydrophobic interactions included Phe127, Ile81, and Trp122 (Figure 13).

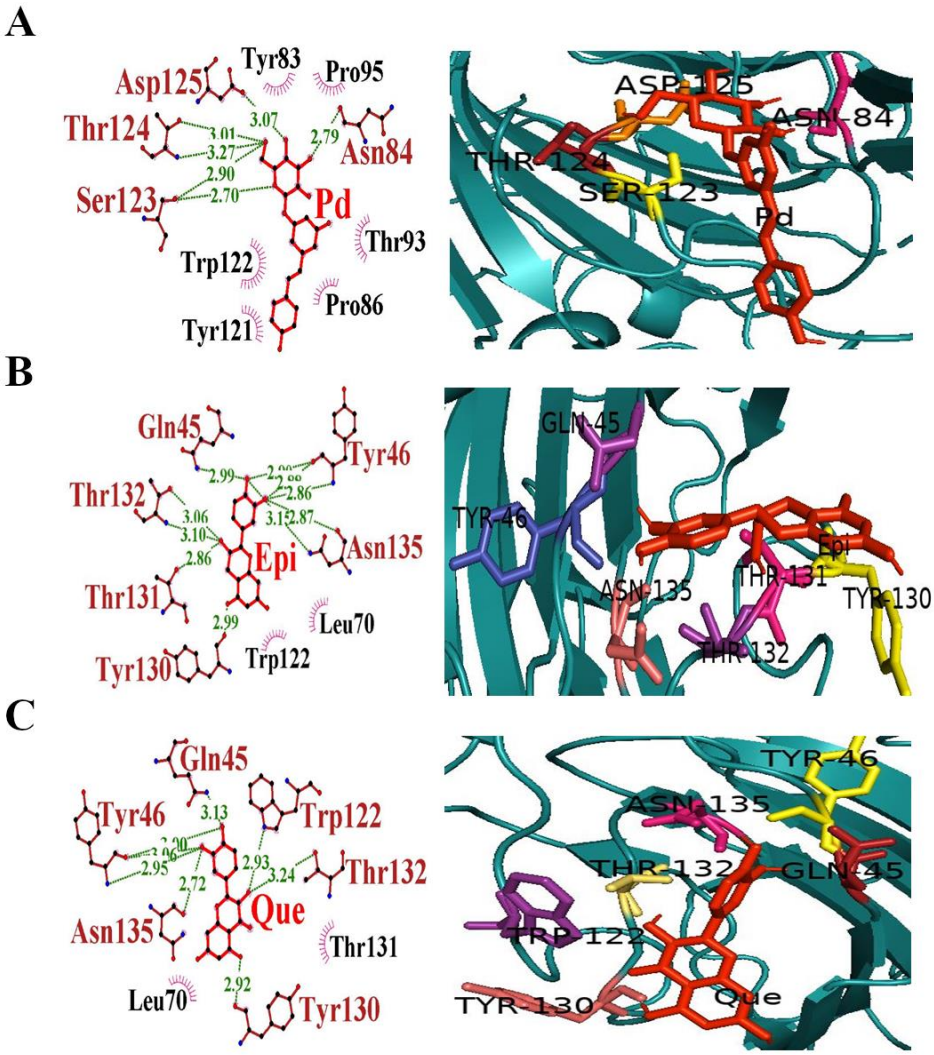


Figure 13. 2D and 3D interaction of polydatin (**A**), (−)-epicatechin (**B**), quercetin (**C**) with *S. cerevisiae* Flo1 protein. Pd=polydatin, Epi=(−)-epicatechin, Que=quercetin. 2D image shows hydrogen and hydrophobic interactions. 3D image show hydrogen bond residues.

Flo1 protein is crucial for the flocculation of yeasts cells, which is important for yeasts' survival under adverse conditions (Goossens *et al.* 2011). Among the 30-member family of flocculins, Flo1 is believed to be the most effective due to the middle elongated tandem repeat sequences that facilitate the N-terminal carbohydrate-binding domain from one cell to capture the N-oligosaccharides of the adjacent cell (Verstrepen *et al.* 2005). A protein basic local alignment sequence similarity search by NCBI-BLAST and *S. cerevisiae* Flo1 showed 68%

sequence similarity to the wild yeast *Saccharomyces paradoxus* Flo9. Efficient inhibition of Flo1 protein would therefore weaken the yeasts' adherent properties and lessen its survivability.

6. SUMMARY

The introduction of phenolic compounds in the mainstream food preservation platforms is becoming more of a possibility than a mirage due to the numerous studies that have demonstrated the appealing properties of these bioactive compounds. Many experiments proved that these natural compounds have numerous benefits to human health and potential antimicrobial activity. The utilization of natural phenolics in the food industry will also be an opportunity to minimize the usage of chemical food preservatives often associated with medical conditions such as cancer and hypertension.

The use of antimicrobials in combination is a robust strategy capable of stretching the growth inhibitory spectrum of individual compounds, retard or arrest the development of antimicrobial resistance, and dilute unsavory properties of the individual compounds capable of affecting the organoleptic properties of food. The effective compound combinations can be adopted in food preservation strategies, probably with better outcomes than using singular antimicrobial preservatives.

Most studies of natural phenolics entail the extraction and characterization of their actions against food spoilage and pathogenic bacteria. However, only a few studies have explored the potentialities of different classes of natural phenolics against food spoilage yeasts. This study aimed to determine the antimicrobial action of phenolic compounds alone or in combinations against food-related yeasts, i.e., *Debaryomyces hansenii*, *Wickerhamomyces anomalus*, *Schizosaccharomyces pombe*, and *Saccharomyces cerevisiae*. Experiments were performed towards the planktonic and biofilm growth, and adhesion capacity of yeasts in the presence of phenolics, food preservatives and in case of their combinations. Molecular docking analysis was also performed with selected phenolics against *S. cerevisiae* protein targets known to play role in cellular structure maintenance, biofilm formation and adhesion. Computational investigation of natural phenolics is important since it expands the scope of our understanding of the possible mechanisms of inhibition of these bioactive compounds against yeasts. Such information can be an essential cog in the judicious and rational design of semi-synthetic anti-yeast compounds with enhanced properties, such as improved solubility, stability, and lipophilicity. Overall, the present work can find its importance in the food industrial realms, and

these findings can be adopted to prolong the shelf-life of foods. The results achieved in this study are as follows:

1. **Planktonic growth inhibitory, antibiofilm and/or antiadhesion property of many individual phenolic compounds has been identified towards food spoilage yeasts.**

In the antiplanktonic growth assay of the individual phenolics, 15 compounds belonging to different phenolic classes were investigated through a broth microdilution assay. Cinnamic acid had the lowest MIC against all the spoilage yeasts at 0.5 mg/mL. Most of the phenolic compounds had their MIC above 2 mg/mL. At the highest screened concentration for the antiplanktonic microbial growth of 2 mg/mL, MIC was achieved by *p*-coumaric acid, vanillin, and ferulic acid against *S. cerevisiae* and *S. pombe*. Quercetin, (−)- epicatechin, resveratrol, and 4-hydroxybenzaldehyde, had MIC at 2 mg/mL against *S. cerevisiae, S. pombe, D. hansenii*, and *W. anomalus*, respectively. Vanillin had MIC of 1 mg/mL against *W. anomalus* and *D. hansenii*. Ideally, of all the screened natural phenolics, cinnamic acid, and vanillin exhibited the most favorable inhibitory potential against the studied yeasts. The planktonic microbial growth of *S. cerevisiae* was remarkably tolerant against most of the phenolic compounds, probably due to the expression of inhibitor-resistant transporters, microbial degradation of some of the phenolic compounds into inactive compounds, and biotransformation of some of the phenolics into less active compounds. All the phenolic compounds had significant inhibition against *S. pombe* planktonic growth at 0.25 mg/mL, except 4-hydroxybenzaldehyde, with cinnamic acid having more than 60% planktonic growth inhibition. Vanillin was equally active against *S. pombe* planktonic growth, with growth inhibition of 80% at 1 mg/mL. Polydatin and 4-hydroxybenzaldehyde also had favorable activity against the planktonic growth of *S. pombe* with over 70% inhibition at 1 mg/mL. All the screened phenolic compounds had significant inhibition against the free-floating cells of *D. hansenii* and *W. anomalus* at 0.5 mg/mL.

The antibiofilm activity of the 15 phenolic compounds was investigated at 0.5 mg/mL. Vanillin had high activity against *D. hansenii*, *W. anomalus*, and *S. pombe* biofilm formation with an inhibition of 97%, 77%, and 82%, respectively. Cinnamic acid was highly effective against the biofilm formation of *D. hansenii*, *W. anomalus*, and *S. pombe*, with inhibition of more than 80% at the studied concentration. Most of the phenolic compounds had low activity against the biofilm formation of *S. cerevisiae*, however, it was interesting to note the impressive

antibiofilm activity of 4-hydroxybenzaldehyde against *S. cerevisiae* at 64%. Ferulic acid and protocatechuic acid had a slight promoting effect against *S. cerevisiae* biofilm formation at the studied concentration. Ten phenolic compounds, namely cinnamic acid, vanillin, gallic acid, (−)-epicatechin, polydatin, resveratrol, syringic acid, *p*-coumaric acid, 4-hydroxybenzaldehyde, and ferulic acid, had more than 50% antibiofilm activity against *D. hansenii*. Fluorescence microscopy of the biofilm formation in the presence and absence of the phenolic compounds showed fragmented biofilms for the phenolics-containing samples and intact biofilms for the control samples lacking phenolics.

Antiadhesion studies were also performed on polystyrene surface to which the most bioactive phenolics against yeasts' biofilms were involved. Apart from vanillin and cinnamic acid, other phenolic compounds used in the assay were polydatin and syringic acid against *D. hansenii*, *p*-coumaric acid and 4-hydroxybenzoic acid against *W. anomalus*, ferulic acid and 4-hydroxybenzaldehyde against *S. pombe*, and (−)-epicatechin and 4-hydroxybenzaldehyde against *S. cerevisiae*. All the assayed compounds had significant adhesion inhibition against the spoilage yeasts. The highest antiadhesion activity of the phenolic compounds was observed in *S. pombe*, where cinnamic acid and vanillin had 91% and 81% inhibition, respectively. In *D. hansenii*, and *W. anomalus*, vanillin and cinnamic acid had more than 50% adhesion inhibition. *S. cerevisiae* exhibited phenolic tolerance since most of the phenolic compounds had less than 30% activity against the yeast, except cinnamic acid, which had 51% antiadhesion activity against *S. cerevisiae*. Natural phenolics have been attributed to the downregulation of genes that encode adhesins. In addition, these bioactive compounds can inhibit the biosynthesis of cell wall components crucial for yeasts' attachment on the abiotic surface. From the results of the adhesion inhibition, it was apparent that different phenolic compounds have different points of intervention as antibiofilm agents, which is also dependent on the yeast being investigated. Vanillin and cinnamic acid seem to start inhibiting biofilms in *S. pombe* from the initial stage of adhesion. However, in other compounds such as 4-hydroxybenzaldehyde against *S. cerevisiae*, it seems this compound has minimal intervention against adhesion of biofilms on the abiotic surface and only becomes potent at subsequent stages of the biofilm formation.

2. Antiplanktonic activity of phenolic-phenolic and phenolic-food preservative combinations against food spoilage yeasts has been studied. Synergistic interactions were identified in several combinations.

The activity of vanillin and cinnamic acid combination against yeasts' planktonic microbial growth was evaluated through a checkerboard method. Based on the checkerboard assay results, synergistic interaction was present in all the food spoilage yeasts under investigation. In *D. hansenii* and *W. anomalus*, the FICI was ≤0.31, while in *S. pombe* and *S. cerevisiae*, the FICI was ≤0.28. There was a reduction of ≥32-fold in the MIC of vanillin in *S. pombe* and *S. cerevisiae* and ≥16-fold in *W. anomalus* and *D. hansenii*. Cinnamic acid recorded a ≥4-fold reduction in the MIC in all the food spoilage yeasts. Anyway, combination of vanillin and cinnamic acid had a great inhibitory effect against the free-floating cells of spoilage yeasts; this may have been due to the multi-target effect of the combination on yeast growth. The combination was quite stable, and the original potency possessed by the individual compounds has been improved. To our knowledge, this is the first study in which vanillin and cinnamic acid have been combined and subjected to an antifungal assay.

Vanillin and cinnamic acid were also combined separately with three food preservatives, namely, potassium sorbate, sodium benzoate, and sodium diacetate, to create binary antifungal solutions that were investigated for their antimicrobial activity against the four studied yeasts. Synergistic interaction was present in all vanillin-food preservative combinations in *S. pombe* with a FICI of 0.41 and 0.46. In *S. cerevisiae*, synergistic interaction was present in vanillin-sodium benzoate and vanillin-potassium sorbate with a FICI index of 0.31, while in *W. anomalus*, synergism was present in vanillin-sodium benzoate (FICI 0.33). In *D. hansenii*, all vanillin-synthetic combinations were indifferent (FICI range 2.02-2.64).

In cinnamic acid-food preservative combinations, synergistic interaction was observed in the cinnamic acid-sodium diacetate combination against *S. pombe* with a FICI of ≤0.34. The cinnamic acid-sodium benzoate and cinnamic acid-potassium sorbate combinations in *S. pombe* were additive in activity with a FICI index of 0.67 and 0.83, respectively. In *W. anomalus*, cinnamic acid-sodium benzoate and cinnamic acid-potassium sorbate combinations were additive (FICI 0.58), while cinnamic acid-sodium diacetate was antagonistic in activity (FICI 4.16). In *S. cerevisiae*, cinnamic acid-sodium diacetate was additive in activity (FICI 0.9), while cinnamic acid-sodium benzoate and cinnamic acid-potassium sorbate were indifferent, with

each having a FICI of 2.82. In *D. hansenii*, higher resistance against the cinnamic-food preservative combinations was quite evident, with cinnamic acid-sodium benzoate (FICI > 9.28) and cinnamic acid-sodium diacetate (FICI 5.28) being antagonistic. The cinnamic acid-potassium sorbate combination in *D. hansenii* was indifferent (FICI 1.78). The phenolic-food preservative combinations were quite active even at low concentrations compared to singular food preservative compounds. In general, the vanillin-food preservatives had a more favorable activity against the planktonic growth of tested yeasts than the cinnamic acid-food preservatives. In addition, possible alterations in solubility, lipophilicity, stability, and stereochemistry of the compounds in combination can affect its bioactivity against microbial targets. A reduction in the MIC of food preservatives in combinations is important, as this may allow low concentrations to be used in food products.

3. **Biofilm formation of food spoilage yeasts has been studied in the presence of phenolic-phenolic and phenolic-food preservative combinations; vanillin-cinnamic acid in combination was identified as an effective strategy.**

The vanillin-cinnamic acid combination was assayed against biofilm formation of spoilage yeasts. The combination had synergistic interaction in all the studied yeasts (FICI range ≤0.19-0.5). The MIC of the two phenolic compounds was reduced by a range of 4 to ≥16 in combined use. The vanillin-cinnamic combination provides a possible solution for the management of highly resistant fungal biofilms in food industry.

The phenolic-food preservative combinations with a FICI less than 1 for the planktonic microbial growth inhibition were investigated for their antibiofilm activity against the studied food spoilage yeasts. Most of the phenolic-food preservative combinations were indifferent to the biofilm formation, except the vanillin-sodium diacetate combination in *S. pombe* that was additive in activity (FICI 0.75). There was a remarkable reduction in the MIC of most food preservatives in the presence of phenolic compounds. In *W. anomalus*, potassium sorbate, and sodium benzoate had a 16-fold MIC reduction when combined with vanillin. In *S. pombe*, sodium benzoate had a 64-fold reduction in the MIC with cinnamic acid and a 32-fold reduction with vanillin. In *S. cerevisiae*, a 32-fold MIC reduction was present for sodium benzoate and potassium sorbate when combined with vanillin.

4. **Antiadhesion activity of phenolic-phenolic and phenolic-food preservative combinations against food spoilage yeasts has been studied. The vanillin-cinnamic acid combination was effective against yeast adherence on abiotic surface and might influence the initial stage of biofilm formation.**

The vanillin-cinnamic acid combination was synergistic in all the spoilage yeasts, except in *D. hansenii*, where additivity was observed. The combination of vanillin and cinnamic acid also had a reducing effect on the MIC of individual compounds to the range of 2 to 16. In phenolic-food preservative combination tests, the vanillin-potassium sorbate combination had synergistic interaction in *W. anomalus* and *S. pombe*, with FIC index values of 0.25 and 0.38, respectively. Most of the phenolic-food preservative combinations in *S. pombe* were additive in activity with a FICI range of 0.53-0.75, while in *S. cerevisiae*, all the phenolic-food preservatives combinations were indifferent, with a FICI range of 1.5-2.5. In *W. anomalus*, the phenolic-food preservative combinations were indifferent to adhesion inhibition, except the vanillin-potassium sorbate combination. The presence of phenolic compounds in the combination led to reduced MICs of the food preservatives. The antiadhesion studies of natural compounds is under-examined, though a critical part of microbes' establishment and biofilm formation on a solid surface. The vanillin-cinnamic acid and phenolic-food preservative combinations with synergistic outcomes on yeast adhesion, can be used to impede the establishment of yeasts on solid surfaces.

5. **Binding potential of phenolics to protein targets with different physiological functions has been evaluated.**

Syringic acid, gallic acid, vanillic acid, caffeic acid, ferulic acid, cinnamic acid, *p*-coumaric acid, polydatin, resveratrol, (−)-epicatechin, quercetin, and vanillin phenolics were subjected to *in silico* molecular docking studies. The protein targets, i.e., chitin synthase III, squalene synthase, Flo11 and Flo1, selected for the docking studies were from *S. cerevisiae*, and are important in flocculation, maintenance of structural integrity, adhesion, and biofilm formation physiological functions. Most of the phenolic ligands demonstrated promising binding potential to the protein targets when hydrogen bonds, hydrophobic bond interaction, and the free energy of binding were analyzed. It was apparent from the docking results that more hydroxylated phenolics had higher binding affinity based on negative docking score when

compared with less hydroxylated phenolics. The phenolics with higher number of hydroxyl groups included polydatin, quercetin, (−)-epicatechin, and resveratrol.

In case of docking of phenolics with Flo11 protein target, the lowest free energy pose was found to be for polydatin at -8.3 kcal/mol. The lowest free energy poses in resveratrol, quercetin, and (−)-epicatechin, was in the range of -8.0 to -7.6 kcal/mol. The rest of the phenolic compounds had their binding score ranging from -6.2 to -5.4 kcal/mol. The different phenolic compounds had some common residues involved in hydrogen and hydrophobic bond interactions. For the hydrogen bond interactions, the common residues were Thr135, Asn147, and Met145, while in hydrophobic interactions, they included Ala134, Gln136, Cys179, and Asn180. The inhibition of Flo11 protein is important in preventing biofilm establishment and eventual growth. The phenolics-squalene synthase molecular docking had the ligand pose that generated the lowest binding score in polydatin, which had -8.7 kcal/mol. The best docking pose of (−)-epicatechin, quercetin, and resveratrol against squalene synthase had binding score ranging from -8.1 to -7.4 kcal/mol. Other phenolics had their best docking poses with binding free energy ranging from -6.0 to -5.3 kcal/mol. The common residues forming hydrogen bonds were Arg76, Phe295, Thr191, and Tyr283, while in hydrophobic bonds they were Pro299, Leu190, Val186, and Phe53. Previous studies on squalene synthase have demonstrated the importance of Phe and Tyr residues in carbocation intermediates stabilization in the isoprenoid metabolic pathway. The phenolics-chitin III synthase docking studies had the best ligand poses with free binding energy ranging from -10.4 to -7.1 kcal/mol. The common residues forming hydrogen bonds were Lys681, Gly200, Lys197, Leu201, and Tyr688, while the hydrophobic bonds were Glu203, Lys197, and Asn690. The phenolics-Flo1 docking studies had the best ligand poses having binding free energy ranging from -7.2 to -6.2 kcal/mol, with polydatin having the lowest binding energy. The common residues involved in hydrogen bond interaction included Thr132, Asp78, and Tyr46, while the hydrophobic interactions involved common residues such as Phe127, Ile81, and Ser80. Efficient inhibition of the Flo1 protein can weaken the yeasts' adherence properties and lessen its survivability under adverse conditions.

Since most of the identified interacting residues are not located in the active site of the targeted proteins, a non-competitive model of inhibition seems to be a plausible explanation of the phenolics mechanism of inhibition of the selected targets. Non-competitive inhibition has been seen in other antifungal compounds such as echinocandins and caspofungin against 1,3-

beta-glucan synthase, which is important for cell wall formation in fungi. More studies will be helpful to unravel the mode of inhibition of our protein targets by the phenolics. It is imperative to note also the apparent dichotomy between the *in silico* and *in vitro* outcomes. This phenomenon is not unusual since the *in silico* model quantifies the ligand-target interaction without considering the multiple biological factors that exist before the ligand binds to the target. Anyway, it is challenging to model the convolution typical of biological systems using a computer program or software, as they are non-linear systems prone to a certain degree of unpredictability.

The present study provided information about the behavior of food spoilage yeasts in the presence of phenolics. The findings may change the food preservation strategies and create a paradigm shift from the traditional chemical-based methods to an innocuous food preservation stratagem. Namely, the results achieved can be utilizable in food preservation methodology developments. Food spoiler fungi has recently become a well-studied research area in the food industry. In line with this, our findings on the great anti-yeast potential of some of the phenolics, such as vanillin and cinnamic acid, can be adopted in the management of spoilage yeast activities in the food industry and in domestic food preservation. However, further studies are needed since the interaction between phenolic compounds and preservatives can be different in complex antioxidant mixtures, e.g., in food systems, from the results of the *in vitro* experiments performed here.

7. ÖSSZEFOGLALÁS

Az élelmiszereket szennyező élesztők megváltoztathatják az ételek minőségét és érzékszervi tulajdonságait. A legtöbb ilyen élesztő biofilmképző, melynek sejtjei fokozott rezisztenciát mutatnak antibiotikumokkal és más környezeti tényezőkkel szemben. A tartósítószerek fontosak a megelőzésben, ugyanakkor a fogyasztók részéről egyre nagyobb az igény a természetes eredetű tartósítószer adalékanyagok alkalmazása iránt. Ilyen adalékanyagok lehetnek a növények által termelt különféle fenolos vegyületek is. Élelmiszeripari kutatások már leírták egyes természetes eredetű fenolok antimikrobiális hatását, és néhány vegyület biofilm-gátló aktivitása is ismert. Szükség van azonban élelmiszerekkel kapcsolatos élesztők elleni új vegyületek hatásának tesztelésére és átfogó elemzésére. Fenolos vegyületek romlást okozó élesztők elleni aktivitásáról ráadásul eddig meglehetősen kevés adat állt rendelkezésünkre.

Kutatásunkban fenolos vegyületek (4-hidroxibenzoesav, galluszsav, vanillinsav, sziringsav, protokatekusav, fahéjsav, *p*-kumársav, kávésav, ferulasav, polidatin, rezveratrol, kvercetin, (−)-epikatekin, vanillin, 4-hidroxibenzaldehid) és tartósítószerek (nátrium-benzoát, kálium-szorbát, nátrium-diacetát) antimikrobiális hatását vizsgáltuk *Debaryomyces hansenii*, *Wickerhamomyces anomalus* (korábban *Pichia anomala*), *Schizosaccharomyces pombe* és *Saccharomyces cerevisiae* élelmiszer eredetű élesztőkön. Az élesztők planktonikus és biofilm növekedési formáit, és a sejtek letapadását elemeztük a fenolok és tartósítószerek egyedi és kombinációs alkalmazásában. Ezen kívül molekuláris dokkolás modellezést is végeztünk kiválasztott fenolokkal olyan *S. cerevisiae* fehérjecélpontok ellen, amelyekről ismert, hogy szerepet játszanak a sejtszerkezet fenntartásában, a biofilm képződésben és/vagy az adhézióban.

A munka során a következő eredményeket értük el: 1) Azonosítottuk számos egyedi fenol vegyület élesztő planktonikus és biofilm növekedést és/vagy adhéziót gátló képességét. A legjobb aktivitásokat vanillin és fahéjsav esetében figyeltük meg. 2) Planktonikus növekedés tesztekben a vanillin-fahéjsav és egyes fenol-tartósítószer kombinációkra szinergiát azonosítottunk. 3) Megállapítottuk, hogy a vanillin-fahéjsav kombináció hatékony stratégia a vizsgált élesztő biofilmek gátlására. 4) A vanillin-fahéjsav kombináció hatásos volt az élesztők megtapadása ellen is abiotikus felületen, ami befolyásolhatja biofilmek kialakulásának kezdeti szakaszát. 5) A molekuláris dokkolás szimulációban a legtöbb fenol ígéretes kötési potenciált

mutatott a fehérje célpontjaihoz. A több hidroxilcsoportot tartalmazó fenoloknak nagyobb volt a kötési affinitása.

A hatékony fenolok, fenol-fenol és vagy fenol-tartósítószer kombinációk alkalmasak lehetnek természetes összetevőjű tartósítószerek és felületfertőtlenítők kifejlesztéséhez. További vizsgálatokra is szükség van azonban, mivel a fenolos vegyületek és a tartósítószerek közötti kölcsönhatás komplex antioxidáns keverékekben, például élelmiszerrendszerekben, eltérhet az *in vitro* kísérletek eredményeitől.

8. REFERENCES

Aboagye, I.A., Oba, M., Koenig, K.M., Zhao, G.Y., Beauchemin, K.A. (2019) Use of gallic acid and hydrolyzable tannins to reduce methane emission and nitrogen excretion in beef cattle fed a diet containing alfalfa silage. *Journal of Animal Science* 97, 2230-2244.

Adams, T.B., Cohen, S.M., Doull, J., Feron, V.J., Goodman, J.I., Marnett, L.J., Munro, I.C., Portoghese, P.S., Smith, R.L., Waddell, W.J., Wagner, B.M. (2004) The FEMA GRAS assessment of cinnamyl derivatives used as flavor ingredients. *Food and Chemical Toxicology* 42, 157-185.

Agamah, F.E., Mazandu, G.K., Hassan, R., Bope, C.D., Thomford, N.E., Ghansah, A., Chimusa, E.R. (2020) Computational/*in silico* methods in drug target and lead prediction. *Briefings in Bioinformatics* 21, 1663-1675.

Alalwan, H., Rajendran, R., Lappin, D.F., Combet, E., Shahzad, M., Robertson, D., Nile, C.J., Williams, C., Ramage, G. (2017) The anti-adhesive effect of curcumin on *Candida albicans* biofilms on denture materials. *Frontiers in Microbiology* 8, 659.

Albertyn J., Pohl C.H., Viljoen B.C. (2014) *Rhodotorula*. *Encyclopedia of Food Microbiology* (Second Edition), pp 291-295.

Albuquerque, B.R., Heleno, S.A., Oliveira, M.B.P.P., Barros, L., Ferreira, I.C.F.R. (2021) Phenolic compounds: current industrial applications, limitations and future challenges. *Food and Function* 12, 14-29.

Alderees, F., Mereddy, R., Webber, D., Nirmal, N., Sultanbawa, Y. (2018) Mechanism of action against food spoilage yeasts and bioactivity of *Tasmannia lanceolata*, *Backhousia citriodora* and *Syzygium anisatum* plant solvent extracts. *Foods* 7, 179.

Alves, C.T., Ferreira, I.C.F.R., Barros, L., Silva, S., Azeredo, J., Henriques, M. (2014) Antifungal activity of phenolic compounds identified in flowers from North Eastern Portugal against *Candida* species. *Future Microbiology* 9, 139-146.

Alves, M.J., Ferreira, I.C.F.R., Froufe, H.J.C., Abreu, R.M.V., Martins, A., Pintado, M. (2013) Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. *Journal of Applied Microbiology* 115, 346-357.

Anantharaju, P.G., Gowda, P.C., Vimalambike, M.G., Madhunapantula, S.V. (2016) An overview on the role of dietary phenolics for the treatment of cancers. *Nutrition Journal* 15, 99.

André, S., Vallaeys, T., Planchon, S. (2017) Spore-forming bacteria responsible for food spoilage. *Research in Microbiology* 168, 379-387.

Annunziata, G., Jimenez-García, M., Tejada, S., Moranta, D., Arnone, A., Ciampaglia, R., Tenore, G.C., Sureda, A., Novellino, E., Capó, X. (2020) Grape polyphenols ameliorate muscle decline reducing oxidative stress and oxidative damage in aged rats. *Nutrients* 12, 1280.

Benbettaieb, N., Nyagaya, J., Seuvre, A.M., Debeaufort, F. (2018) Antioxidant activity and release kinetics of caffeic and *p*-coumaric acids from hydrocolloid-based active films for healthy packaged food. *Journal of Agriculture and Food Chemistry* 66, 6906-6916.

Betoni, J.E.C., Mantovani R.P., Barbosa, L.N., Di Stasi L.C., Fernandes J.A. (2006) Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. *Memórias do Instituto Oswaldo Cruz* 101, 387-390.

Bhattacharya, A., Sood, P., Citovsky, V. (2010) The roles of plant phenolics in defence and communication during *Agrobacterium* and *Rhizobium* infection. *Molecular Plant Pathology* 11, 705-719.

Bhattacharya, D., Ghosh, D., Bhattacharya, S., Sarkar, S., Karmakar, P., Koley, H., Gachhui, R. (2018) Antibacterial activity of polyphenolic fraction of Kombucha against *Vibrio cholerae*: targeting cell membrane. *Letters in Applied Microbiology* 66, 145-152.

Boesch-Saadatmandi, C., Loboda, A., Wagner, A.E., Stachurska, A., Jozkowicz, A., Dulak, J., Döring, F., Wolffram, S., Rimbach, G. (2011) Effect of quercetin and its metabolites isorhamnetin and quercetin-3-glucuronide on inflammatory gene expression: role of miR-155. *Journal of Nutritional Biochemistry* 22, 293-299.

Bojsen, R., Regenberg, B., Folkesson, A. (2014) *Saccharomyces cerevisiae* biofilm tolerance towards systemic antifungals depends on growth phase. *BioMed Central Microbiology* 14, 305.

Boo, Y.C. (2019) Can plant phenolic compounds protect the skin from airborne particulate matter? *Antioxidants* 8, 379.

Brogi, S., Ramalho, T.C., Kuca, K., Medina-Franco, J.L., Valko, M. (2020) Editorial: *In silico* methods for drug design and discovery. *Frontiers Chemistry* 8, 612.

Bulik, D.A., Olczak, M., Lucero, H.A., Osmond, B.C., Robbins, P.W., Specht C.A. (2003) Chitin synthesis in *Saccharomyces cerevisiae* in response to supplementation of growth medium with glucosamine and cell wall stress. *Eukaryotic Cell* 2, 886-900.

Caesar, L.K., Cech, N.B. (2019) Synergy and antagonism in natural product extracts: when 1 + 1 does not equal 2. *Natural Products Reports* 36, 869-888.

Calvo-Castro, L., Syed, D.N., Chamcheu, J.C., Vilela, F.M.P, Pérez, A.M., Vaillant, F., Rojas, M., Mukhtar, H. (2013) Protective effect of tropical highland blackberry juice (*Rubus adenotrichos* Schltdl.) against UVB-mediated damage in human epidermal keratinocytes and in a reconstituted skin equivalent model. *Photochemisty and Photobiology* 89,1199-1207.

Carvalho, R.S, Carollo, C.A, de Magalhães, J.C, Palumbo, J.M.C, Boaretto, A.G, Nunes e Sá, I.C, Ferraz, A.C, Lima, W.G, de Siqueira, J.M, Ferreira, J.M.S. (2018) Antibacterial and antifungal activities of phenolic compound-enriched ethyl acetate fraction from *Cochlospermum regium* (mart. Et. Schr.) Pilger roots: Mechanisms of action and synergism with tannin and gallic acid. *South African Journal of Botany* 114, 181-187.

Chait, R., Craney, A., Kishony, R. (2007) Antibiotic interactions that select against resistance. *Nature* 446, 668-671.

Chao, P.C., Hsu, C.C., Yin, M.C. (2009) Anti-inflammatory and anti-coagulatory activities of caffeic acid and ellagic acid in cardiac tissue of diabetic mice. *Nutrition and Metabolism* 6, 33.

Cherng, Y.G., Tsai, C.C., Chung, H.H., Lai, Y.W., Kuo, S.C., Cheng, J.T. (2013) Antihyperglycemic action of sinapic acid in diabetic rats. *Journal of Agriculture and Food Chemistry* 61, 12053–12059.

Cheynier, V., Comte, G., Davies, K. M., Lattanzio, V., Martens, S. (2013) Plant phenolics: Recent advances on their biosynthesis, genetics, and ecophysiology. *Plant Physiology and Biochemistry* 72, 1-20.

Chiva-Blanch, G., Urpi-Sarda, M., Ros, E., Arranz, S., Valderas-Martinez, P., Casas, R., Sacanella, E., Llorach, R., Lamuela-Raventos, R.M., Andres-Lacueva, C., Estruch, R. (2012) Dealcoholized red wine decreases systolic and diastolic blood pressure and

increases plasma nitric oxide: short communication. *Circulation Research* 111, 1065-1068.

Chlebicz, A., Śliżewska, K. (2018) Campylobacteriosis, salmonellosis, yersiniosis, and listeriosis as zoonotic foodborne diseases: a review. *International Journal of Environmental Research and Public Health* 15, 863.

Chung, J.E., Tan, S., Gao, S.J., Yongvongsoontorn, N., Kim, S.H., Lee, J.H., Choi, H.S., Yano, H., Zhuo, L., Kurisawa, M., Ying, J.Y. (2014) Self-assembled micellar nanocomplexes comprising green tea catechin derivatives and protein drugs for cancer therapy. *Nature Nanotechnology* 9, 907-912.

Codo, T.N.M., Marquardt, P., Ahyi, V., Fester, K., Spiegler, V., Vissiennon, C. (2022) Anti-inflammatory potential of phenolic compounds isolated from *Entada africana* Guill. & Perr. used in the republic of Benin. *Frontiers in Pharmacology* 13, 931240.

Corrêa, J.A.F., dos Santos, J.V.G., Evangelista, A.G., Pinto, A.C.S.M., de Macedo, R.E.F., Luciano, F.B. (2021) Combined application of phenolic acids and essential oil components against *Salmonella Enteritidis* and *Listeria monocytogenes in vitro* and in ready-to-eat cooked ham. *LWT Food Science and Technology* 149, 111881.

Cosme, P., Rodríguez, A.B., Espino, J., Garrido, M. (2020) Plant phenolics: Bioavailability as a key determinant of their potential health-promoting applications. *Antioxidants* 9, 1263.

da Cruz Cabral, L., Pinto, V.F., Patriarca, A. (2013) Application of plant derived compounds to control fungal spoilage and mycotoxin production in foods. *International Journal of Food Microbiology* 166, 1-14.

da Fonseca, S.T.D., Teixeira, T.R., Ferreira, J.M.S., Lima, L.A.R.D.S., Luyten, W., Castro, A.H.F. (2022) Flavonoid-rich fractions of *Bauhinia holophylla* leaves inhibit *Candida albicans* biofilm formation and hyphae growth. *Plants* 11, 1796.

de-la-Fuente-Blanco, A., Ferreira, V. (2020) Gas chromatography olfactometry (GC-O) for the (semi)quantitative screening of wine aroma. *Foods* 9, 1892.

de Llano, G.D., Moreno-Arribas, M.V., Bartolomé, B. (2020) Cranberry polyphenols and prevention against urinary tract infections: relevant considerations. *Molecules* 25, 3523.

Das, A.K., Islam, M.N., Faruk, M.O., Ashaduzzaman, M., Dungani, R. (2020) Review on tannins: Extraction processes, applications and possibilities. *South African Journal of Botany*, 135, 58-70.

De Vita, D., Simonetti, G., Pandolfi, F., Costi, R., Di Santo, R., D'Auria, F.D., Scipione, L. (2016) Exploring the anti-biofilm activity of cinnamic acid derivatives in *Candida albicans*. *Bioorganic* and *Medicinal Chemistry Letters* 26, 5931-5935.

Dewick, P.M. (2001) The shikimate pathway: aromatic amino acids and phenylpropanoids. In Medicinal Natural Products: A Biosynthetic Approach, 2nd ed., pp. 137-186. Chichester: John Wiley.

Díaz-Gómez, R., Toledo-Araya, H., López-Solís, R., Obreque-Slier, E. (2014) Combined effect of gallic acid and catechin against *Escherichia coli*. *LWT Food Science and Technology* 59, 896-900.

Dixon, R.A., Pasinetti, G.M. (2010) Flavonoids and isoflavonoids: from plant biology to agriculture and neuroscience. *Plant Physiology* 4, 453-457.

Dudonne, S., Poupard, P., Coutiere, P., Woillez, M., Richard, T., Merillon, J.M., Vitrac, X. (2011) Phenolic composition and antioxidant properties of poplar bud (*Populus nigra*) extract: individual antioxidant contribution of phenolics and transcriptional effect on skin aging. *Journal of Agriculture and Food Chemistry* 59, 4527-4536.

Durazzo, A., Zaccaria, M., Polito, A., Maiani, G., Carcea, M. (2013) Lignan content in cereals, buckwheat and derived foods. *Foods* 2, 53-63.

Dürre P. (2014) Physiology and sporulation in *Clostridium*. *Microbiology Spectrum* 2, TBS-0010-2012.

Działo, M., Mierziak, J., Korzun, U., Preisner, M., Szopa, J., Kulma, A. (2016) The potential of plant phenolics in prevention and therapy of skin disorders. *International Journal of Molecular Sciences* 17, 160.

Eberhardt, J., Santos-Martins, D., Tillack, A.F., Forli, S. (2021) AutoDock Vina 1.2.0: New docking methods, expanded force field, and python bindings. *Journal of Chemical Information and Modeling* 61, 3891-3898.

Escott, C., Del Fresno, J.M., Loira, I., Morata, A., Suárez-Lepe, J.A. (2018) *Zygosaccharomyces rouxii*: control strategies and applications in food and winemaking. *Fermentation* 4, 69.

Evensen, N.A., Braun, P.C. (2009) The effects of tea polyphenols on *Candida albicans*: inhibition of biofilm formation and proteasome inactivation. *Canadian Journal of Microbiology* 55, 1033-1039.

Ferreira, L.G., Dos Santos, R.N., Oliva, G., Andricopulo, A.D. (2015) Molecular docking and structure-based drug design strategies. *Molecules* 20, 13384-13421.

Fitzgerald, D.J., Stratford, M., Narbad, A. (2003) Analysis of the inhibition of food spoilage yeasts by vanillin. *International Journal of Food Microbiology* 86, 113-122.

Fleet, G.H. (2007) Yeasts in foods and beverages: impact on product quality and safety. *Current Opinion in Biotechnology* 18, 170-175.

Francisco, A.T., Michael, N.C. (2000) Dietary hydroxybenzoic acid derivatives – nature, occurrence and dietary burden. *Journal of the Science of Food and Agriculture* 80, 1024-1032.

Fujii, T., Wakaizumi, M., Ikami, T., Saito, M. (2008) Amla (*Emblica officinalis* Gaertn.) extract promotes procollagen production and inhibits matrix metalloproteinase-1 in human skin fibroblasts. *Journal of Ethnopharmacology* 119, 53-57.

Garnier, L., Valence, F., Mounier, J. (2017) Diversity and control of spoilage fungi in dairy products: an update. *Microorganisms* 5, 42.

Giada, M.D.L.R. (2013) Food phenolic compounds: Main classes, sources and their antioxidant power. In: Morales-Gonzalez, J.A. (ed.) Oxidative stress and chronic degenerative diseases—a role for antioxidants. IntechOpen, London, pp. 87-112.

Glasenapp, Y., Lucas, C., Wöltje, T., Fohrer, J., Papenbrock, J. (2019) Anti-adhesion activity of tannins isolated from the mangrove *Laguncularia racemosa*. *Chemistry and Biodiversity* 16, 1800632.

Gonelimali, F.D., Lin, J., Miao, W., Xuan, J., Charles, F., Chen, M., Hatab, S.R. (2018). Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. *Frontiers in Microbiology* 9, 1639.

Goossens, K.V., Stassen, C., Stals, I., Donohue, D.S., Devreese, B., De Greve, H., Willaert, R.G. (2011) The N-terminal domain of the Flo1 flocculation protein from *Saccharomyces cerevisiae* binds specifically to mannose carbohydrates. *Eukaryotic Cell* 10, 110-117.

Gougouli, M., Koutsoumanis, K.P. (2017) Risk assessment of fungal spoilage: A case study of *Aspergillus niger* on yogurt. *Food Microbiology* 65, 264-273.

Groenewald, M., Boekhout, T., Neuvéglise, C., Gaillardin, C., van Dijck, P.W., Wyss, M. (2014) *Yarrowia lipolytica*: safety assessment of an oleaginous yeast with a great industrial potential. *Critical Reviews in Microbiology* 40, 187-206.

Guo, B., Styles, C.A., Feng, Q., Fink, G.R. (2000) A *Saccharomyces* gene family involved in invasive growth, cell-cell adhesion, and mating. *Proceedings of the National Academy of Sciences* 97, 12158-12163.

Hafizur, R.M., Hameed, A., Shukrana, M., Raza S.A., Chishti, S., Kabir, N., Siddiqui R.A. (2015) Cinnamic acid exerts anti-diabetic activity by improving glucose tolerance *in vivo* and by stimulating insulin secretion *in vitro*. *Phytomedicine* 22, 297-300.

Hanhineva, K., Törrönen, R., Bondia-Pons. I., Pekkinen, J., Kolehmainen, M., Mykkänen, H., Poutanen, K. (2010) Impact of dietary polyphenols on carbohydrate metabolism. *International Journal of Molecular Sciences* 11, 1365-1402.

Hapeshi, A., Benarroch, J.M., Clarke, D.J., Waterfield, N.R. (2019) Iso-propyl stilbene: a life cycle signal? *Microbiology* 165, 516-526.

Huang, X., Ji, Y., Guo, L., Xu, Q., Jin, L., Fu, Y., Wang, Y. (2022) Incorporating tannin onto regenerated cellulose film towards sustainable active packaging. *Industrial Crops and Products* 180, 114710.

Imran, A., Shehzad, M.T., Ali Shah, S. J., Laws, M., Rahman, K.M., Khan, I.A., Shafiq, Z., Iqbal, J. (2022) Development, molecular docking, and *in silico* ADME evaluation of selective ALR2 inhibitors for the treatment of diabetic complications via suppression of the polyol pathway. *American Chemical Society Omega*, 7, 26425-26436.

Iwaki, A., Ohnuki, S., Suga, Y., Izawa, S., Ohya, Y. (2013) Vanillin inhibits translation and induces messenger ribonucleoprotein (mRNP) granule formation in *Saccharomyces cerevisiae*: application and validation of high-content, image-based profiling. *Public Library of Science ONE* 8, 61748.

Jayant, S.R., Sandeep, B.R., Ravikumar, B., Babasaheb, S.S., Mohan, K. (2013) Vanillin inhibits growth, morphogenesis and biofilm formation by *Candida albicans*. *Journal of Biologically Active Products from Nature* 3, 130-138.

Jennings, S.M., Tsay, Y.H., Fisch, T.M., Robinson, G.W. (1991) Molecular cloning and characterization of the yeast gene for squalene synthetase. *Proceedings of the national Academy of Sciences* 88, 6038-6042.

Johnson, M.D., Perfect, J.R. (2010) Use of antifungal combination therapy: agents, order, and timing. *Current Fungal Infection Reports 4*, 87-95.

Jordá, T., Puig, S. (2020) Regulation of ergosterol biosynthesis in *Saccharomyces cerevisiae*. *Genes* 11, 795.

Jung, U.J., Lee, M.K., Park, Y.B., Jeon, S.M., Choi, M.S. (2006) Antihyperglycemic and antioxidant properties of caffeic acid in db/db mice. *Journal of Pharmacology and Experimental Therapeutics* 318, 476-483.

Kalschne, D.L., Womer, R., Mattana, A., Sarmento, C.M., Colla, L.M., Colla, E. (2015) Characterization of the spoilage lactic acid bacteria in "sliced vacuum-packed cooked ham". *Brazilian Journal of Microbiology* 46, 173-181.

Kamboh, A.A., Arain, M.A., Mughal, M.J., Zaman, A., Arain, Z.M., Soomro, A.H. (2015) Flavonoids: health promoting phytochemicals for animal production-a review. *Journal of Animal Health and Production* 3, 6-13.

Kanna, S., Venojarvi, M., Roy, S., Sharma, N., Trikha, P., Bagchi, D., Bagchi, M., Sen, C.K. (2003) Dermal wound healing properties of redox-active grape seed proanthocyanidins. *Free Radical Biology and Medicine* 33, 1089-1096.

Kilinc, K., Demir, S., Turan, I., Mentese, A., Orem, A., Sonmez, M., Aliyazicioglu, Y. (2019) *Rosa canina* extract has antiproliferative and proapoptotic effects on human lung and prostate cancer cells. *Nutrition and Cancer* 273-282.

Kolbeck, S., Ludwig, C., Meng, C., Hilgarth, M., Vogel, R. F. (2020) Comparative proteomics of meat spoilage bacteria predicts drivers for their coexistence on modified atmosphere packaged meat. *Frontiers in Microbiology* 11, 209.

Kores, K., Lešnik, S., Bren, U., Janežič, D., Konc, J. (2019) Discovery of novel potential human targets of resveratrol by inverse molecular docking. *Journal of Chemical Information and Modeling* 59, 2467-2478.

Korošec, B., Sova, M., Turk, S., Kraševec, N., Novak, M., Lah, L., Stojan, J., Podobnik, B., Berne, S., Zupanec, N., Bunc, M., Gobec, S., Komel, R. (2014) Antifungal activity of cinnamic acid derivatives involves inhibition of benzoate 4-hydroxylase (CYP 53). *Journal of Applied Microbiology* 116, 955-966.

Kortemme, T., Morozov, A.V., Baker, D. (2003) An orientation-dependent hydrogen bonding potential improves prediction of specificity and structure for proteins and protein–protein complexes. *Journal of Molecular Biology* 326, 1239-1259.

Koyama, T., Murata, J., Horikawa, M., Satake, H. (2022) Production of beneficial lignans in heterologous host plants. *Frontiers in Plant Science*, 13, 1026664.

Krisch, J., Chandrasekaran, M., Kadaikunnan, S., Alharbi, N.S., Vágvölgyi, C. (2016) Latest about spoilage by yeasts: focus on the deterioration of beverages and other plant-derived products. *Journal of Food Protection* 79, 825-829.

Kristan, K., Bratkovic, T., Sova, M., Gobec, S., Prezelj, A., Urleb, U. (2009) Novel inhibitors of beta-ketoacyl-ACP reductase from *Escherichia coli*. *Chemico-Biological Interactions* 178, 310-316.

Kumar, N., Goel, N. (2019) Phenolic acids: natural versatile molecules with promising therapeutic applications. *Biotechnology Reports* 24, 370.

Laskowski, R.A., Swindells, M.B. (2011) LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. *Journal of Chemical Information and Modeling* 51, 2778-2786.

Lattanzio, V. (2013) Phenolic compounds: introduction. In: Ramawat, K., Mérillon, J.M. (eds.) Natural Products. Springer, Berlin, Heidelberg, pp. 1543-1580.

Lee, H.B., Patriarca, A., Magan, N. (2015) *Alternaria* in food: ecophysiology, mycotoxin production and toxicology. *Mycobiology* 43, 93-106.

Lee, J.H., Park, J.H., Cho, H.S., Joo, S.W., Cho, M.H., Lee, J. (2013) Anti-biofilm activities of quercetin and tannic acid against *Staphylococcus aureus*. *Biofouling* 29, 491-499.

Liao, J., Wang, Q., Wu, F., Huang, Z. (2022) *In Silico* methods for identification of potential active sites of therapeutic targets. *Molecules* 27, 7103.

Liu, Y., Grimm, M., Dai, W., Hou, M., Xiao, Z.X., Cao, Y. (2020) CB-Dock: a web server for cavity detection-guided protein-ligand blind docking. *Acta Pharmacologica Sinica* 41, 138-144.

Lopez, C.A.V., Valencia, E.I., González, S.F.A., Sánchez, L.A.L., Garcia, A.L.E., Garcia, V.R. (2022) Antioxidant, anti-inflammatory and cytotoxic activity of phenolic compound family extracted from raspberries (*Rubus idaeus*): a general review. *Antioxidants* 11, 1192.

Lorán, S., Carramiñana, J.J., Juan, T., Ariño, A., Herrera, M. (2022) Inhibition of *Aspergillus parasiticus* growth and aflatoxins production by natural essential oils and phenolic acids. *Toxins* 14, 384.

Lorenzo, J.M., Munekata, P.E., Dominguez, R., Pateiro, M., Saraiva, J.A., Franco, D. (2018) Main groups of microorganisms of relevance for food safety and stability: general aspects and overall description. *Innovative Technologies for Food Preservation* 63, 53-107.

Lou, Z., Wang, H., Rao, S., Sun, J., Ma, C., Li, J. (2012) *p*-Coumaric acid kills bacteria through dual damage mechanisms. *Food Control* 25, 550-554.

Lutz, M., Fuentes, E., Ávila, F., Alarcón, M., Palomo, I. (2019) Roles of phenolic compounds in the reduction of risk factors of cardiovascular diseases. *Molecules* 24, 366.

Maeda, K., Kuzuya, M., Cheng, X.W., Asai, T., Kanda, S., Tamaya, M.N., Sasaki, T., Shibata, T., Ighuchi, A. (2003) Green tea catechins inhibit the cultured smooth muscle cell invasion through the basement barrier. *Atherosclerosis* 166, 23-30.

Mahoney, N., Molyneux, R.J. (2004) Phytochemical inhibition of aflatoxigenicity in *Aspergillus flavus* by constituents of walnut (*Juglans regia*). *Journal of Agriculture and Food Chemistry* 52, 1882-1889.

Martillanes, S., Rocha-Pimienta, J., Cabrera-Bañegil, M., Martín-Vertedor, D., Delgado-Adámez, J. (2017) Application of phenolic compounds for food preservation: food additive and active packaging. In: Soto-Hernandez, M., Palma-Tenango, M., del Rosario Garcia-Mateos, M. (eds.) Phenolic compounds-Biological Activity. IntechOpen, London, pp. 39-58.

Martin, H., Kavanagh, K., Velasco-Torrijos, T. (2021) Targeting adhesion in fungal pathogen *Candida albicans*. *Future Medicinal Chemistry* 13, 313-334.

Martins, C.V.B., Da Silva, D.L., Neres, A.T.M., Magalhaes, T.F.F., Watanabe, G.A., Modolo, L.V., Sabino, A.A., De Fátima, A., De Resende, M.A. (2009) Curcumin as a promising antifungal of clinical interest. *Journal of Antimicrobial Chemotherapy* 63, 337-339.

Ming, D., Wang, D., Cao, F., Xiang, H., Mu, D., Cao, J., Li, B., Zhong, L., Dong, X., Zhong, X., Wang, L., Wang, T. (2017) Kaempferol inhibits the primary attachment phase of biofilm formation in *Staphylococcus aureus*. *Frontiers in Microbiology* 8, 2263.

Mohammadzadeh-Aghdash, H., Sohrabi, Y., Mohammadi, A., Shanehbandi, D., Dehghan, P., Dolatabadi, J.E.N. (2018) Safety assessment of sodium acetate, sodium diacetate and potassium sorbate food additives. *Food Chemistry* 257, 211-215.

Morris, G.M., Huey, R., Lindstrom, W., Sanner, M.F., Belew, R.K., Goodsell, D.S., Olson, A.J. (2009) AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *Journal of Computational Chemistry* 30, 2785-2791.

Motyl, M., Dorso, K., Barret, J., Giacobbe, R. (2005) Basic microbiological techniques used in antibacterial drug discovery. *Current Protocols in Pharmacology* 31, 13A.3.1–13A.3.22.

Mpountoukas, P., Vantarakis, A., Sivridis, E., Lialiaris, T. (2008) Cytogenetic study in cultured human lymphocytes treated with three commonly used preservatives. *Food and Chemical Toxicology* 46, 2390-2393.

Mu, D., Xiang, H., Dong, H., Wang, D., Wang, T. (2018) Isovitexin, a potential candidate inhibitor of Sortase A of *Staphylococcus aureus*. *Journal of Microbiology and Biotechnology* 28, 1426-1432.

Mutha, R.E., Tatiya, A.U., Surana, S.J. (2021) Flavonoids as natural phenolic compounds and their role in therapeutics: an overview. *Future Journal of Pharmaceutical Sciences* 7, 25.

Naczk, M., Shahidi, F. (2004) Extraction and analysis of phenolics in food. *Journal of Chromatography A* 1054, 95-111.

Nett, J.E., Andes, D. (2015) Fungal biofilms: *In vivo* models for discovery of anti-biofilm drugs. *Microbiology Spectrum* 3, E30.

Nguyen, L.T.H., Nguyen, U.T., Kim, Y.H., Shin, H.M., Yang, I.J. (2019) Astragali Radix and its compound formononetin ameliorate diesel particulate matter-induced skin barrier disruption by regulation of keratinocyte proliferation and apoptosis. *Journal of Ethnopharmacology* 228, 132-141.

Ogawa, K., Tashima, A., Sadakata, M., Morinaga, O. (2018) Appetite-enhancing effects of vanilla flavours such as vanillin. *Journal of Natural Medicines* 72, 798-802.

Ojo, M.A. (2022) Tannins in foods: Nutritional implications and processing effects of hydrothermal techniques on underutilized hard-to-cook legume seeds-a review. *Preventive Nutrition and Food Science* 27, 14-19.

Olszowy-Tomczyk, M. (2020) Synergistic, antagonistic and additive antioxidant effects in the binary mixtures. *Phytochemistry Reviews* 19, 63-103.

Osmond, B.C., Specht, C.A., Robbins, P.W. (1999) Chitin synthase III: synthetic lethal mutants and "stress related" chitin synthesis that bypasses the CSD3/CHS6 localization pathway. *Proceedings of the National Academy of Sciences* 96, 11206-11210.

Oufensou, S., Balmas, V., Azara, E., Fabbri, D., Dettori, M.A., Schüller, C., Zehetbauer, F., Strauss, J., Delogu, G., Migheli, Q. (2020) Naturally occurring phenols modulate vegetative growth and deoxynivalenol biosynthesis in *Fusarium graminearum*. *American Chemical Society Omega* 5, 29407-29415.

Oulahal, N., Degraeve, P. (2022) Phenolic-rich plant extracts with antimicrobial activity: an alternative to food preservatives and biocides? *Frontiers in Microbiology* 12, 753518.

Özvural, E.B., Huang, Q., Chikindas, M.L. (2016) The comparison of quality and microbiological characteristic of hamburger patties enriched with green tea extract using three techniques: Direct addition, edible coating and encapsulation. *LWT-Food Science and Technology* 68, 385-390.

Pacheco-Ordaz, R., Wall-Medrano, A., Goñi, M.G., Ramos-Clamont-Montfort, G., Ayala-Zavala, J.F., González-Aguilar, G.A. (2018) Effect of phenolic compounds on the growth of selected probiotic and pathogenic bacteria. *Letters in Applied Microbiology* 66, 25-31.

Paolini, A., Curti, V., Pasi, F., Mazzini, G., Nano, R., Capelli, E. (2015) Gallic acid exerts a protective or an anti-proliferative effect on glioma T98G cells via dose-dependent epigenetic regulation mediated by miRNAs. *International Journal of Oncology* 46, 1491-1497.

Panche, A.N., Diwan, A.D., Chandra, S.R. (2016) Flavonoids: an overview. *Journal of Nutritional Science* 5, 47.

Panzella, L. (2020) Natural phenolic compounds for health, food and cosmetic applications. *Antioxidants* 9, 427.

Payne, D.E., Martin, N.R., Parzych, K.R., Rickard, A.H., Underwood, A., Boles, B.R. (2013) Tannic acid inhibits *Staphylococcus aureus* surface colonization in an IsaA-dependent manner. *Infection and Immunity* 81, 496-504.

Pelinson, L.P., Assmann, C.E., Palma, T.V., da Cruz, I.B.M., Pillat, M.M., Mânica, A., Stefanello, N., Weis, G.C.C., de Oliveira, A.A., de Andrade, C.M., Ulrich, H., Morsch, V.M.M., Schetinger, M.R.C., Bagatini, M.D. (2019) Antiproliferative and apoptotic effects of caffeic acid on SK-Mel-28 human melanoma cancer cells. *Molecular Biology Reports* 46, 2085-2092.

Peng, Y., Ao, M., Dong, B., Jiang, Y., Yu, L., Chen, Z., Hu, C., Xu, R. (2021) Anti-inflammatory effects of curcumin in the inflammatory diseases: status, limitations and countermeasures. *Drug Design Development and Therapy* 15, 4503-4525.

Perez-Castillo, Y., Lima, T.C., Ferreira, A.R., Silva, C.R., Campos, R.S., Neto, J.B., Magalhães, H.I., Cavalcanti, B.C., Júnior, H.V., de Sousa, D.P. (2020) Bioactivity and molecular docking studies of derivatives from cinnamic and benzoic acids. *BioMed Research International* 2020, 6345429.

Piao, M.J., Yoo, E.S., Koh, Y.S., Kang, H.K., Kim, J., Kim, Y.J., Kang, H.H., Hyun, J.W. (2011) Antioxidant effects of the ethanol extract from flower of *Camellia japonica* via scavenging of reactive oxygen species and induction of antioxidant enzymes. *International Journal of Molecular Sciences* 12, 2618–2630.

Piper, J.D., Piper, P.W. (2017) Benzoate and sorbate salts: A systematic review of the potential hazards of these invaluable preservatives and the expanding spectrum of clinical uses for sodium benzoate. *Comprehensive Reviews in Food Science and Food Safety* 16, 868-880.

Pizzi, A. (2006) Recent developments in eco-efficient bio-based adhesives for wood bonding: opportunities and issues. *Journal of Adhesion Science and Technology* 20, 829-846.

Pok, P.S., García, L.V.A., Vicente, S.R.S.M., Pacín, A., Tolaba M., Alzamora, S.M., Resnik, S.L. (2020) Evaluation of citrus flavonoids against *Aspergillus parasiticus* in maize: aflatoxins reduction and ultrastructure alterations. *Food Chemistry* 318, 126414.

Pragasam, S.J., Venkatesan, V., Rasool, M. (2013) Immunomodulatory and anti-inflammatory effect of *p*-coumaric acid, a common dietary polyphenol on experimental inflammation in rats. *Inflammation* 36, 169-176.

Praparatana, R., Maliyam, P., Barrows, L.R., Puttarak, P. (2022) flavonoids and phenols, the potential anti-diabetic compounds from *Bauhinia strychnifolia Craib*. Stem. *Molecules* 27, 2393.

Rahman, M.M., Rahaman, M.S., Islam, M.R., Rahman, F., Mithi, F.M., Alqahtani, T., Almikhlafi, M.A., Alghamdi, S.Q., Alruwaili, A.S., Hossain, M.S., Ahmed, M., Das, R., Emran, T.B., Uddin, M.S. (2022) Role of phenolic compounds in human disease: current knowledge and future prospects. *Molecules* 27, 233.

Rampone, S., Pagliarulo, C., Marena, C., Orsillo, A., Iannaccone, M., Trionfo, C., Sateriale, D., Paolucci, M. (2021) *In silico* analysis of the antimicrobial activity of phytochemicals: towards a technological breakthrough. *Computer Methods and Programs in Biomedicine* 200, 105820.

Rani, R., Arora, S., Kaur, J. Manhas, R.K. (2018) Phenolic compounds as antioxidants and chemopreventive drugs from *Streptomyces cellulosae* strain TES17 isolated from rhizosphere of *Camellia sinensis*. *BMC Complementary Medicine and Therapies* 18, 82.

Raut, J.S., Shinde, R.B., Chauhan, N.M., Karuppayil, S.M. (2014) Phenylpropanoids of plant origin as inhibitors of biofilm formation by *Candida albicans*. *Journal of Microbiology and Biotechnology* 24, 1216-1225.

Reynolds, T.B., Fink, G.R. (2001) Bakers' yeast, a model for fungal biofilm formation. *Science* 291, 878-881.

Rodríguez-García, C., Sánchez-Quesada, C., Toledo, E., Delgado-Rodríguez, M., Gaforio, J.J. (2019) Naturally lignan-rich foods: a dietary tool for health promotion? *Molecules* 24, 917.

Romero-Rodríguez, A., Ruiz-Villafán, B., Martínez-de la Peña, C.F., Sánchez, S. (2023) Targeting the impossible: a review of new strategies against endospores. *Antibiotics* 12, 248.

Saibabu, V., Fatima, Z., Khan, L.A., Hameed, S. (2021) Mechanistic insights into the anticandidal action of vanillin reveal disruption of cell surface integrity and mitochondrial functioning. *Infectious Disorders Drug Targets* 21, 405-415.

Sanchatjate, S., Schekman, R. (2006) Chs5/6 complex: a multiprotein complex that interacts with and conveys chitin synthase III from the trans-Golgi network to the cell surface. *Molecular Biology of the Cell* 17, 4157-4166.

Sanhueza, L., Melo, R., Montero, R., Maisey, K., Mendoza, L., Wilkens, M. (2017) Synergistic interactions between phenolic compounds identified in grape pomace extract with

antibiotics of different classes against *Staphylococcus aureus* and *Escherichia coli*. *Public Library of Science ONE* 12, e0172273.

Sendamangalam, V., Choi, O.K., Kim, D., Seo, Y. (2011) The antibiofouling effect of polyphenols against *Streptococcus mutans*. *Biofouling* 27, 13-19.

Shahidi, F., Ambigaipalan, P. (2015) Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects - A review. *Journal of Functional Foods* 18, 820-897.

Shahzad, M., Sherry, L., Rajendran, R., Edwards, C.A., Combet, E., Ramage, G. (2014) Utilising polyphenols for the clinical management of *Candida albicans* biofilms. *International Journal of Antimicrobial Agents* 44, 269-273.

Sharma, A., Shahzad, B., Rehman, A., Bhardwaj, R., Landi, M., Zheng, B. (2019) Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. *Molecules* 24, 2452.

Shin, B., Park, C., Imlay, J.A., Park, W. (2018) 4-Hydroxybenzaldehyde sensitizes *Acinetobacter baumannii* to amphenicols. *Applied Microbiology and Biotechnology* 102, 2323-2335.

Silva, L.N., Zimmer, K.R., Macedo, A.J., Trentin, D.S. (2016) Plant natural products targeting bacterial virulence factors. *Chemical Reviews* 116, 9162-9236.

Siripatrawan, U., Noipha, S. (2012) Active film from chitosan incorporating green tea extract for shelf life extension of pork sausages. *Food Hydrocolloids* 27, 102-108.

Snyder, A.B., Churey, J.J., Worobo, R.W. (2016) Characterization and control of *Mucor circinelloides* spoilage in yogurt. *International Journal of Food Microbiology* 228, 14-21.

Soares, E.V. (2011) Flocculation in *Saccharomyces cerevisiae*: a review. *Journal of Applied Microbiology* 110, 1-18.

Son, M.J., Rico, C.W., Nam, S.H., Kang, M.Y. (2011). Effect of oryzanol and ferulic acid on the glucose metabolism of mice fed with a high fat diet. *Journal of Food Science* 76, H7-H10.

Srimahaeak, T., Petersen, M.A., Lillevang, S.K., Jespersen, L., Larsen, N. (2022) spoilage potential of contaminating yeast species *Kluyveromyces marxianus, Pichia kudriavzevii* and *Torulaspora delbrueckii* during cold storage of skyr. *Foods* 11, 1776.

Srinivasan, S., Muthukumaran, J., Muruganathan, U., Venkatesan, R.S., Jalaludeen, A.M. (2014) Antihyperglycemic effect of syringic acid on attenuating the key enzymes of carbohydrate metabolism in experimental diabetic rats. *Biomedicine and Preventive Nutrition* 4, 595-602.

Stefanović, O., Ličina, B., Vasić, S., Radojević, I., Čomić, L. (2018) Bioactive extracts of *Gentiana asclepiadea*: antioxidant, antimicrobial, and antibiofilm activity. *Botanica Serbica* 42, 223-229.

Suiker, I.M., Arkesteijn, G.J.A., Zeegers, P.J., Wösten, H.A.B. (2021) Presence of *Saccharomyces cerevisiae* subsp. *diastaticus* in industry and nature and spoilage capacity of its vegetative cells and ascospores. *International Journal of Food Microbiology* 347, 109173.

Sung, W.S., Lee, D.G. (2010) Antifungal action of chlorogenic acid against pathogenic fungi, mediated by membrane disruption. *Pure and Applied Chemistry* 82, 219-226.

Takó, M., Kerekes, E.B., Zambrano, C., Kotogán, A., Papp, T., Krisch, J., Vágvölgyi, C. (2020) Plant phenolics and phenolic-enriched extracts as antimicrobial agents against food-contaminating microorganisms. *Antioxidants* 9, 165.

Tang, Y., Zhu, W., Chen, K., Jiang, H. (2006) New technologies in computer-aided drug design: toward target identification and new chemical entity discovery. *Drug Discovery Today: Technologies* 3, 307-313.

Tangney, C.C., Rasmussen, H.E. (2013) Polyphenols, inflammation, and cardiovascular disease. *Current Atherosclerosis Reports* 15, 324.

Tansey, T.R., Shechter, I. (2001) Squalene synthase: structure and regulation. *Progress in Nucleic Acid Research and Molecular Biology* 65, 157-195.

Taofiq, O., Heleno, S.A., Calhelha, R.C., Fernandes, I.P., Alves, M.J., Barros, L., González-Paramás, A.M., Ferreira, I.C.F.R, Barreiro, M.F. (2018) Mushroom-based cosmeceutical ingredients: microencapsulation and *in vitro* release profile. *Industrial Crops and Products* 124, 44-52.

Teka, T., Zhang, L., Ge, X., Li, Y., Han, L., Yan, X. (2022) Stilbenes: source plants, chemistry, biosynthesis, pharmacology, application and problems related to their clinical application-a comprehensive review. *Phytochemistry* 197, 113128.

Telles, A.C., Kupski, L., Furlong, E.B. (2017) Phenolic compound in beans as protection against mycotoxins. *Food Chemistry* 214, 293-299.

Tondi, G., Pizzi, A. (2009) Tannin-based rigid foams: characterization and modification. *Industrial Crops and Products* 29, 356-363.

Tong, Z., He, W., Fan, X., Guo, A. (2022) Biological function of plant tannin and its application in animal health. *Frontiers in Veterinary Science* 8, 803657.

Torawane, S., Suryawanshi, Y., Mokat, D. (2020) Controlled release of functional bioactive compounds from plants. *Encapsulation of Active Molecules and Their Delivery System* 103-110.

Torres-Fuentes, C., Suárez, M, Aragonès, G., Mulero, M., Ávila-Román, J., Arola-Arnal, A., Salvadó, M.J., Arola, L., Bravo, F.I., Muguerza, B. (2022) Cardioprotective properties of phenolic compounds: a role for biological rhythms. *Molecular Nutrition and Food Research* 66, e2100990.

Trott, O., Olson, A.J. (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry* 31, 455-461.

Turk, M., Montiel, V., Žigon, D., Plemenitaš, A., Ramos, J. (2007) Plasma membrane composition of *Debaryomyces hansenii* adapts to changes in pH and external salinity. Microbiology 153, 3586-3592.

Tyunin, A.P., Nityagovsky, N.N., Grigorchuk, V.P., Kiselev, K.V. (2018) Stilbene content and expression of stilbene synthase genes in cell cultures of *Vitis amurensis* treated with cinnamic and caffeic acids *Biotechnology and Applied Biochemistry* 65, 150-155.

Ullah, H., Hussain, Y., Santarcangelo, C., Baldi, A., Di Minno, A., Khan, H., Xiao, J., Daglia, M. (2022) Natural polyphenols for the preservation of meat and dairy products. *Molecules* 27, 1906.

Valerio, F., Di Biase, M., Huchet, V., Desriac, N., Lonigro, S.L., Lavermicocca, P., Sohier, D., Postollec, F. (2015) Comparison of three *Bacillus amyloliquefaciens* strains growth behaviour and evaluation of the spoilage risk during bread shelf-life. *Food Microbiology* 45, 2-9.

Velli, S.K., Sundaram, J., Murugan, M., Balaraman, G., Thiruvengadam, D. (2019) Protective effect of vanillic acid against benzo(a)pyrene induced lung cancer in Swiss albino mice. *Journal of Biochemistry and Molecular Toxicology* 33, e22382.

Velu, P., Vinothkumar, V., Babukumar, S., Ramachandhiran, D. (2017) Chemopreventive effect of syringic acid on 7,12-dimethylbenz(a)anthracene induced hamster buccal pouch carcinogenesis. *Toxicology Mechanisms and Methods* 27, 631-640.

Verstrepen, K.J., Jansen, A., Lewitter, F., Fink, G.R. (2005) Intragenic tandem repeats generate functional variability. *Nature Genetics* 37, 986-990.

Vilaça, R., Mendes, V., Mendes, M.V., Carreto, L., Amorim, M.A., de Freitas, V., Moradas-Ferreira, P., Mateus, N., Costa, V. (2012) Quercetin protects *Saccharomyces cerevisiae* against oxidative stress by inducing trehalose biosynthesis and the cell wall integrity pathway. *Public Library of Science One*. 7, e45494.

Vilela, C., Pinto, R.J.B, Coelho, J., Domingues, M.R.M, Daina, S., Sadocco, P., Santos, S.A.O, Freire, C.S.R. (2017) Bioactive chitosan/ellagic acid films with UV-light protection for active food packaging. *Food Hydrocolloids* 73, 120-128.

Volkamer, A., Kuhn, D., Rippmann, F., Rarey, M. (2012) DoGSiteScorer: a web server for automatic binding site prediction, analysis and druggability assessment. *Bioinformatics* 28, 2074-2075.

Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., Heer, F.T., de Beer, T.A.P., Rempfer, C., Bordoli, L., Lepore, R., Schwede, T. (2018) SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Research* 46, W296-W303.

Wu, Y., Bai, J., Zhong, K., Huang, Y., Qi, H., Jiang, Y., Gao, H. (2016) Antibacterial activity and membrane-disruptive mechanism of 3-p-trans-coumaroyl-2-hydroxyquinic acid, a novel phenolic compound from pine needles of *Cedrus deodara*, against *Staphylococcus aureus*. *Molecules* 21, 1084.

Xiao, Y., Zhang, S., Tong, H., Shi, S. (2018) Comprehensive evaluation of the role of soy and isoflavone supplementation in humans and animals over the past two decades. *Phytotherapy Research* 32, 384-394.

Xu, B., Ganesan, K., Mickymaray, S., Alfaiz, F.A., Thatchinamoorthi, R., Al Aboody, M.S. (2020) Immunomodulatory and antineoplastic efficacy of common spices and their

connection with phenolic antioxidants. *Bioactive Compounds in Health and Disease* 3, 15-31.

Yahfoufi, N., Alsadi, N., Jambi, M., Matar, C. (2018) The immunomodulatory and anti-inflammatory role of polyphenols. *Nutrients* 10, 1618.

Yamakoshi, J., Otsuka, F., Sano, A., Tokutake, S., Saito, M., Kikuchi, M., Kubota, Y. (2003) Lightening effect on ultraviolet-induced pigmentation of guinea pig skin by oral administration of a proanthocyanidin-rich extract from grape seeds. *Pigment Cell and Melanoma Research* 16, 629-638.

Yao, H., Liu, J., Xu, S., Zhu, Z., Xu, J. (2017) The structural modification of natural products for novel drug discovery. *Expert Opinion in Drug Discovery* 12, 121-140.

Yeung, A.W.K, Tzvetkov, N.T., Balacheva, A.A., Georgieva, M.G., Gan, R.Y, Jozwik, A., Pyzel, B., Horbańczuk, J.O., Novellino, E., Durazzo, A., Lucarini, M., Camilli, E., Souto, E.B., Atanasov, A.G., Santini, A. (2020) Lignans: quantitative analysis of the research literature. *Frontiers in Pharmacology* 11, 37.

Yilmaz, H., Gultekin Subasi, B., Celebioglu, H. U., Ozdal, T., Capanoglu, E. (2022) Chemistry of protein-phenolic interactions toward the microbiota and microbial infections. *Frontiers in Nutrition*, 9, 914118.

Yin, N., Ma, W., Pei, J., Ouyang, Q., Tang, C., Lai, L. (2014) Synergistic and antagonistic drug combinations depend on network topology. *Public Library of Science ONE*, 9, 93960.

Yoon, S.A., Kang, S.I., Shin, H.S., Kang, S.W., Kim, J.H., Ko, H.C., Kim, S.J. (2013) *p*-Coumaric acid modulates glucose and lipid metabolism via AMP-activated protein kinase in L6 skeletal muscle cells. *Biochemical and Biophysical Research Communication* 432, 553-557.

Yu, H.H., Chin, Y.W., Paik, H.D. (2021) Application of natural preservatives for meat and meat products against food-borne pathogens and spoilage bacteria: a review. *Foods* 10, 2418.

Zambrano, C., Kerekes, E.B., Kotogán, A., Papp, T., Vágvölgyi, C., Krisch, J., Takó, M. (2019) Antimicrobial activity of grape, apple and pitahaya residue extracts after carbohydrase treatment against food-related bacteria. *LWT-Food Science and Technology* 100, 416-425.

Zambrano, C., Kotogán, A., Bencsik, O., Papp, T., Vágvölgyi, C., Mondal, K.C., Krisch, J., Takó, M. (2018) Mobilization of phenolic antioxidants from grape, apple and pitahaya

residues via solid state fungal fermentation and carbohydrase treatment. *LWT-Food Science and Technology* 89, 457-465.

Zhang, T., Wei, X., Miao, Z. Hassan, H., Song., Y. (2016) Screening for antioxidant and antibacterial activities of phenolics from golden delicious apple pomace. *Chemistry Central Journal* 10, 47.

Zhao, J., Xia, H., Yu, T., Jin, L., Li, X., Zhang, Y., Shu, L., Zeng, L., He, Z. (2018) A colorimetric assay for vanillin detection by determination of the luminescence of o-toluidine condensates. *PLoS ONE* 13, e0194010.

Ziaullah, Rupasinghe, H.P.V. (2015) Application of NMR spectroscopy in plant polyphenols associated with human health. In: ur-Rahman, A., Choudhary, M.I. (eds.) Applications of NMR Spectroscopy Vol. 2. Elsevier, Waltham, USA, pp. 3-92.

9. ACKNOWLEDGEMENTS

I would like to sincerely thank the government of Hungary for hosting foreign students through its Stipendium Hungaricum Scholarship Programme, under the umbrella of Tempus Public Foundation.

I would like to thank the Director of the Institute of Biology Prof. Dr. Attila Gácser, for creating a harmonious environment where research in biology can be conducted.

I am profoundly appreciative and highly indebted to Prof. Dr. Csaba Vágvölgyi for selecting me four years ago, among many qualified applicants to pursue research work in the Department of Microbiology. Thank you for believing in me and giving me a chance to pursue my Ph.D. dream.

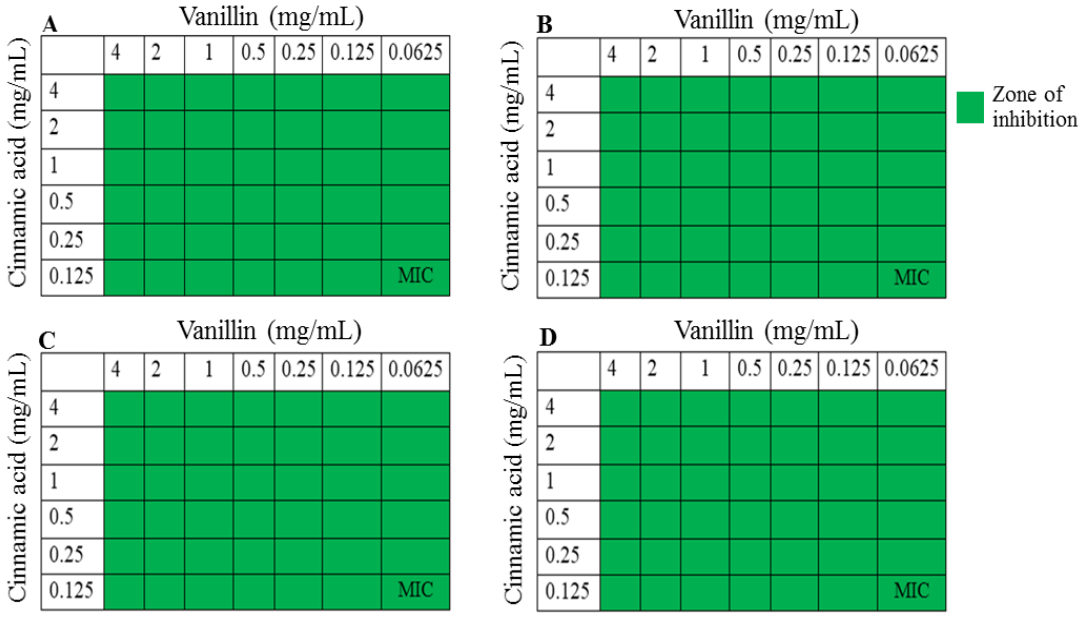
I would like to thank my supervisors Dr. Miklós Takó and Dr. Erika Beáta Kerekes for their expert advice and guidance throughout this arduous project, your rapport will always be remembered and highly appreciated.

Nothing would have been achieved without the cooperation and help of the whole research clue of lab 312. You were all kind, patient, helpful, and accommodating. I will always cherish the memories of all of you. I also sincerely thank Dr. Aruna Vigneshwari, Dr. Anuar Zhumakayev, and Dr. Chetna Tyagi for their great insights whenever I needed crucial information on research and coursework. Many thanks to all the unmentioned members of the Department of Microbiology who in their big or small way contributed to the success of this work.

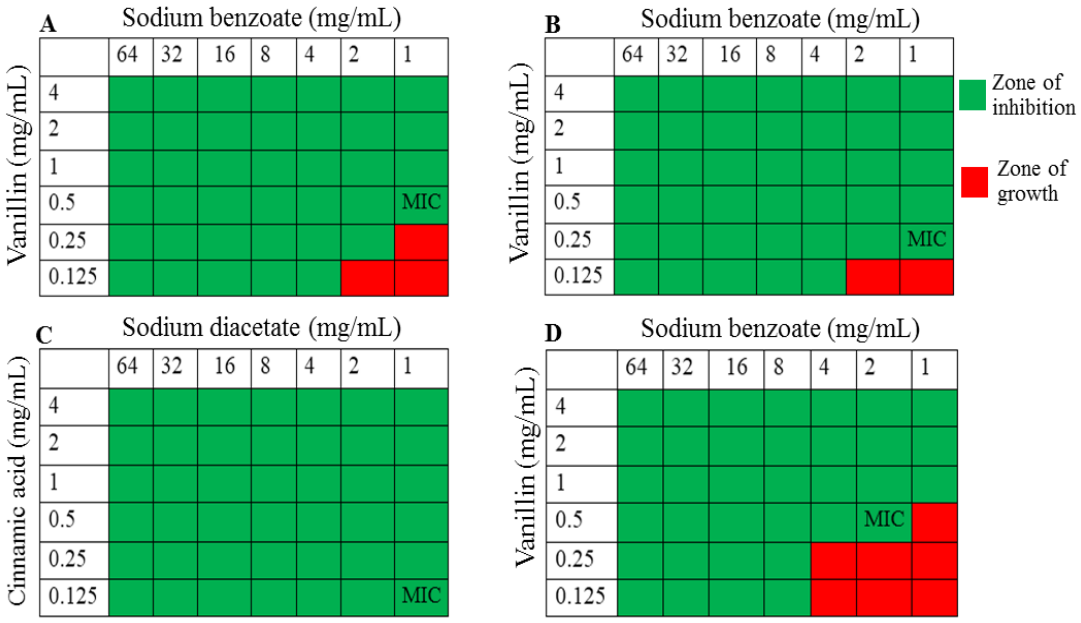
Finally, I would like to thank my wife Susan and my two sons Lemuel and Kristian for their immense patience towards their ‘runaway husband and dad,’ I do not know how I should repay you. I cannot forget my mother, brothers, and sisters; I am certain, you were praying for me.

10. APPENDICES

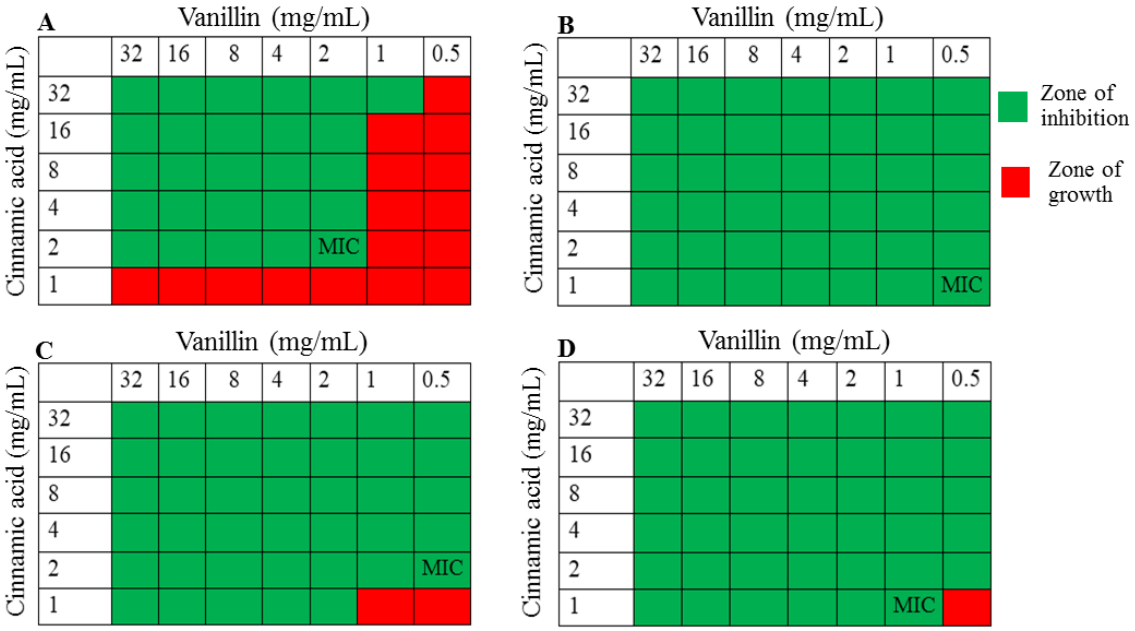
Appendix 1. Checkerboard layout of vanillin-cinnamic acid combinations used against the planktonic growth of *D. hansenii* (**A**), *S. cerevisiae* (**B**), *S. pombe* (**C**), and *W. anomalus* (**D**) spoilage yeasts. The MIC is the minimum inhibitory concentration considered in determining the fractional inhibitory concentration index. Lower combinations below the MIC did not inhibit growth.



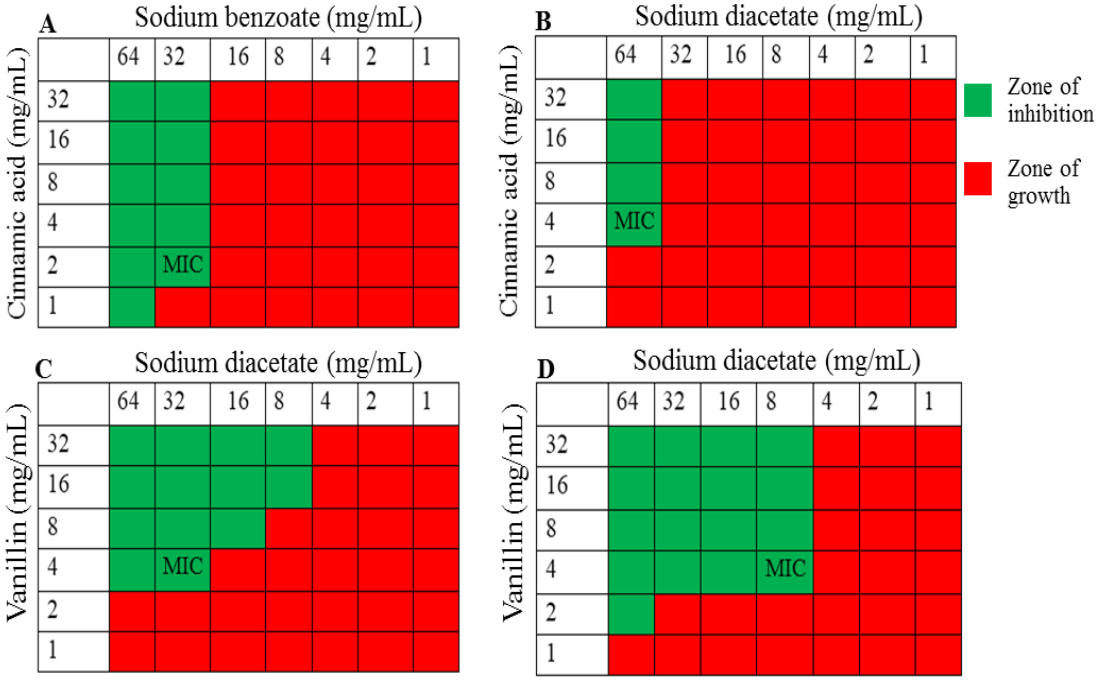
Appendix 2. Checkerboard layout of phenolic compound-food preservative combinations used against the planktonic growth of *S. pombe* (**A** and **C**), *W. anomalus* (**B**) and *S. cerevisiae* (**D**). The MIC is the minimum inhibitory concentration considered in determining the fractional inhibitory concentration index.



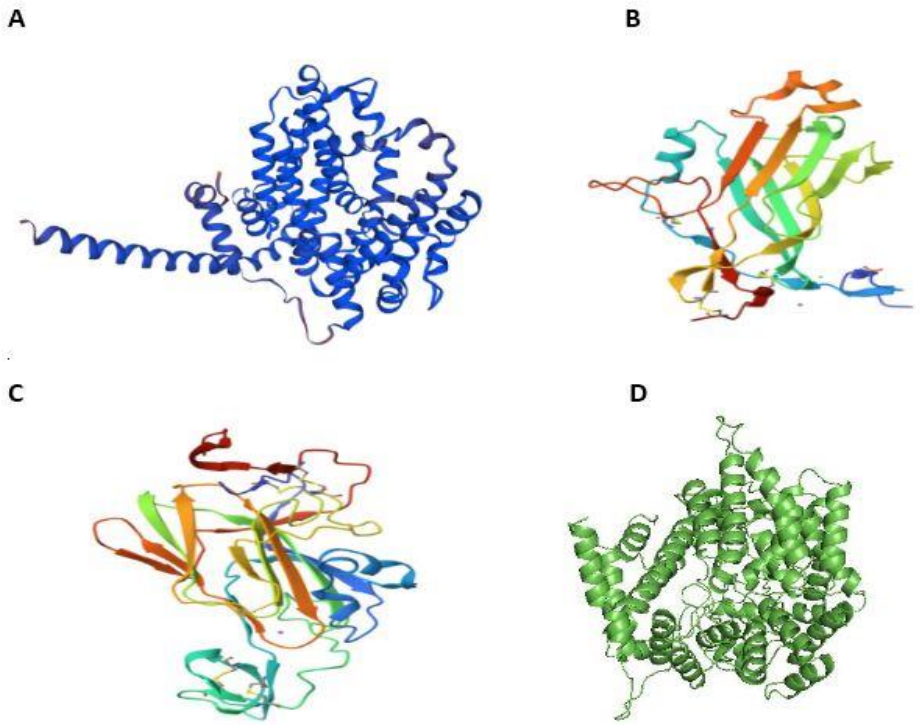
Appendix 3. Checkerboard layout of vanillin-cinnamic acid combination used against the biofilm growth of *D. hansenii* (**A**), *W. anomalus* (**B**), *S. cerevisiae* (**C**), and *S. pombe* (**D**). The MIC is the minimum inhibitory concentration considered in determining the fractional inhibitory concentration index.



Appendix 4. Checkerboard layout of phenolic compound-food preservative combinations used against the biofilm growth of *W. anomalus* (**A**), *S. pombe* (**B** and **D**) and *S. cerevisiae* (**C**). The MIC is the minimum inhibitory concentration considered in determining the fractional inhibitory concentration index.



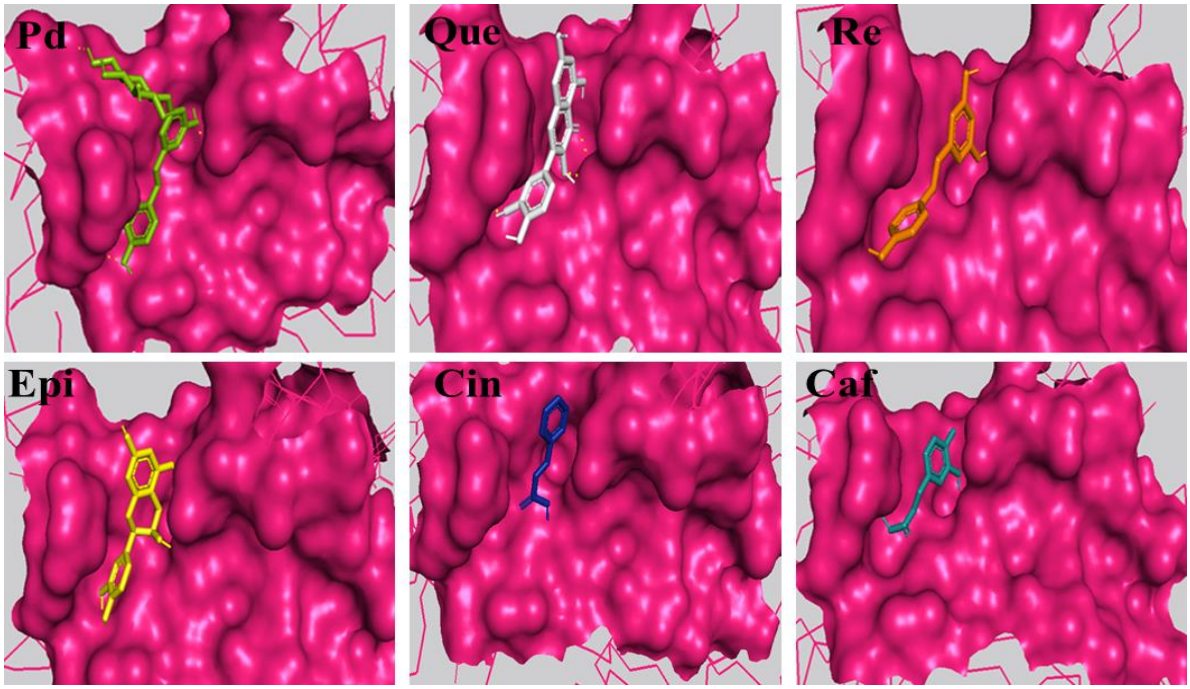
Appendix 5. The structures of the proteins used in the *in silico* investigation; squalene synthase (**A)**, Flo11 (**B**), Flo1 (**C**), Chitin synthase III (**D**).



Appendix 6. Physico-chemical properties of phenolic compounds used in the *in silico* studies.

Phenolic compound	Molecular formula	Molecular weight (g/mol)	Water solubility	-OH	No. of rotatable bonds
Polydatin	$C_{20}H_{22}O_8$	390.4	Soluble	6	5
Quercetin	$C_{15}H_{10}O_7$	302.24	Soluble	5	1
(−)-Epicatechin	$C_{15}H_{14}O_6$	290.27	Soluble	5	1
Resveratrol	$C_{14}H_{12}O_3$	228.24	Soluble	3	2
Cinnamic acid	$C_9H_8O_2$	148.16	Soluble	0	2
Vanillin	$C_8H_8O_3$	152.15	Soluble	1	2
Vanillic acid	$C_8H_8O_4$	168.15	Soluble	1	2
Ferulic acid	$C_{10}H_{10}O_4$	194.18	Soluble	1	3
Caffeic acid	$C_9H_8O_4$	180.16	Soluble	2	2
***p*-Coumaric acid**	$C_9H_8O_3$	164.16	Soluble	1	2
Syringic acid	$C_9H_{10}O_5$	198.17	Soluble	1	3
Gallic acid	$C_7H_6O_5$	170.12	Soluble	3	1

Appendix 7. The molecular docking poses with the lowest binding free energy for phenolic ligands of polydatin (**Pd**), quercetin (**Que**), resveratrol (**Re**), (−)-epicatechin (**Epi**), cinnamic acid (**Cin**), and caffeic acid (**Caf**) against Flo11 protein.



11. PUBLICATIONS UNDERLYING THE THESIS

Journal articles:

Kimani, B.G., Kerekes, E.B., Szebenyi, Cs., Krisch, J., Vágvölgyi, Cs., Papp, T., Takó, M. (2021) In vitro activity of selected phenolic compounds against planktonic and biofilm cells of food-contaminating yeasts. *Foods* 10, 1652.

Kimani, B.G., Takó, M., Veres, Cs., Krisch, J., Papp, T., Kerekes, E.B., Vágvölgyi, Cs. (2023) Activity of binary combinations of natural phenolics and synthetic food preservatives against food spoilage yeasts. *Foods* 12, 1338.

Abstracts:

Kimani, B.G., Anjeche, P.O., Kerekes, E.B., Szebenyi, Cs., Krisch, J., Papp, T., Vágvölgyi, Cs., Takó, M. (2022) Natural phenolics as anti-yeast substances: effects on growth and biofilm formation of food spoilage yeasts. In: FEMS Conference on Microbiology (FEMS Belgrade 2022): Abstract Book. pp. 169-170.

Kimani, B.G., Anjeche, P.O., Kerekes, E.B., Szebenyi, Cs., Krisch, J., Papp, T., Vágvölgyi, Cs., Takó, M. (2022) Combined action of natural phenolics and synthetic preservatives against food spoilage yeasts. In: Kiss, O. (ed.) 19th Wellmann International Scientific Conference: Book of Abstracts. p. 42.

Kimani, B.G., Anjeche, P.O., Kerekes, E.B., Szebenyi, Cs., Krisch, J., Papp, T., Vágvölgyi, Cs., Takó, M. (2021) A natural approach against the activity of food spoilage yeasts: focusing on plant phenolics. *Acta Microbiologica et Immunologica Hungarica* 68 (Supplement 1), 80.

Kimani, B.G., Anjeche, P.O., Kerekes, E.B., Szebenyi, Cs., Krisch, J., Papp, T., Vágvölgyi, Cs., Takó, M. (2021) Antimicrobial activity of natural phenolics against food spoilage yeasts. In: Kiss, O. (ed.) 18th Wellmann International Scientific Conference: Book of Abstracts. p. 41.

Kimani, B.G., Kerekes, E.B., Krisch, J., Papp, T., Mondal, K.C., Vágvölgyi, Cs., Takó, M. (2020) Inhibition of biofilm formation of food-contaminating bacteria and yeasts by naturally occurring phenolics. In: A Magyar Mikrobiológiai Társaság 2020. évi Nagygyűlése és a XIV. Fermentációs Kollokvium: Absztraktfüzet. pp. 18-19.

12. FINANCIAL SUPPORT

This work was supported by the National Research, Development and Innovation Office grant FK 134886, and the Széchenyi 2020 grants EFOP-3.6.1-16-2016-00008 and GINOP-2.3.2-15-2016-00052. The infrastructural background was established with the support of GINOP-2.3.3-15-2016-00006 grant. The work was also supported by the grant 2019-2.1.11-TÉT-2020-00148.