PH.D. THESIS

ANTIMICROBIAL ACTION OF NATURAL PHENOLICS AGAINST FOOD SPOILAGE YEASTS

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INTRODUCTION

Nature has gifted mankind with a variety of phenolic compounds. These compounds exist either in bound or in soluble form and can be divided into several sub-groups based on their chemical structure such as flavonoids, stilbenes, phenolic acids, tannins, and lignans. Natural phenolics are bioactive molecules with broad antimicrobial activity and beneficial effects on human health.

Natural phenolics have found application in the food, aquaculture, cosmetic, and sport industries. The introduction of phenolic compounds in 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. 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, hypertension, and allergies, among others.

Spoilage yeasts can cause food deterioration resulting in low food quality and shorter shelf life. Most of these yeasts are biofilm-forming showing increased resistance to antibiotics. Chemical food preservatives can support the prevention, however, there is a need for consumer-friendly natural approaches to be used in food preservative developments. Although there were investigations, including studies from our laboratory, on the effect of phenolics against pathogenic and spoilage bacteria, data concerning the inhibition of spoilage yeast activities remain scarce.

The use of anti-yeast compounds in combination is a robust strategy capable of stretching the anti-yeast spectrum of individual compounds, retard or arrest the development of antifungal resistance, and adulterate unsavoury properties of the individual compounds capable of affecting the organoleptic properties of food. Therefore, appealing anti-yeast compound combinations can be adopted in food preservation strategies, probably with better outcomes than when singular antifungal preservatives are used.

In silico investigation of the action of natural phenolics is also important since it expands the scope of our understanding of the possible mechanisms of inhibition of these bioactive compounds. Such information can be pivotal in the prudent rational design of semi-synthetic anti-yeast compounds with enhanced properties. The present work can be important in the food industrial realms, and the findings thereof can be adopted to prolong the shelf-life of foods.

AIMS

The main objective of this work 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.

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.

METHODS

Yeasts

Debaryomyces hansenii SZMC (SZMC=Szeged Microbiological Collection) 8045Mo, *Wickerhamomyces anomalus* (formerly *Pichia anomala*) SZMC 8061Mo, *Schizosaccharomyces pombe* SZMC 1280, *S. cerevisiae* SZMC 1279

Phenolics, food preservatives

Phenolics: 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, vanillin; food preservatives: sodium benzoate, potassium sorbate, sodium diacetate

Antiplanktonic growth tests of singular antimicrobials

- Determination of planktonic growth inhibition, including the minimum inhibitory concentration (MIC) of the tested antimicrobials by broth microdilution method

Biofilm formation tests

- Detection of formation of biofilm (with and without phenolics) by crystal violet staining
- Visualization of formed biofilms using fluorescence microscopy

Antiadhesion tests

- Detection of adhered cells on the microplate through crystal violet staining method

Compounds combination tests

- Checkerboard methodology

In silico tests

- Evaluation of phenolic ligands docking to *S. cerevisiae* targets through molecular docking method; AutoDockVina, AutoDock Tools

RESULTS

Influence of singular phenolics on planktonic growth of yeasts (Kimani *et al*. 2021)

In these studies, 15 phenolic compounds from different classes were screened for their antiplanktonic growth activity against selected food spoilage yeasts namely, *D. hansenii*, *W. anomalus, S. cerevisiae,* and *S. pombe*. The phenolics and their classes were gallic acid, vanillic acid, syringic acid, 4-hydroxybenzoic acid and protocatechuic acid (hydroxybenzoates), cinnamic acid, ferulic acid, *p*-coumaric acid and caffeic acid (hydroxycinnamates), resveratrol and polydatin (stilbenes), (−)-epicatechin and quercetin (flavonoids), vanillin and hydroxybenzaldehyde (phenolic aldehyde). Most of the phenolic compounds had less than 90% growth inhibition against the spoilage yeasts at the highest screened concentration. At 2 mg/mL MIC 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.

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. Polydatin was equally effective against *S. cerevisiae* demonstrating higher planktonic growth inhibition of more than 45% at 1 mg/mL. 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. 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.

The *S. pombe* planktonic growth was exceptionally inhibited by cinnamic acid having more than 60% growth inhibition at 250 μg/mL. Vanillin was equally highly active against *S. pombe* at 1 mg/mL with a planktonic growth of 80%. Polydatin and 4-hydroxybenzaldehyde demonstrated higher than 70% growth inhibition at 1 mg/mL against *S. pombe* growth. The planktonic growth of *D. hansenii* was significantly inhibited by vanillin, cinnamic acid, and 4-hydroxybenzaldehyde. At 250 μg/mL, vanillin, cinnamic acid, and 4-hydroxybenzaldehyde exhibited inhibitory effects

of 66%, 92% and 50%, respectively. At a concentration of 500 µg/mL, all the phenolic compounds had significant planktonic growth inhibition against *D. hansenii*.

W. anomalus was quite vulnerable to cinnamic acid with 80% planktonic growth inhibition at 250 µg/mL. Vanillin was also equally potent against *W. anomalus* having about 40% planktonic growth inhibition at 500 and 250 µg/mL. In addition, *p*-coumaric acid had >70% while 4-hydroxybenzaldehyde had >80% planktonic growth inhibition against *W. anomalus* at 1 mg/mL. Overall, the planktonic growth of the four food spoilage yeasts was highly sensitive to cinnamic acid and vanillin.

Influence of singular phenolics on biofilm formation of spoilage yeasts (Kimani *et al*. 2021)

The antibiofilm formation inhibitory activity of the 15 phenolic compounds against the studied spoilage yeasts was investigated at 500 µg/mL. Vanillin was highly potent as an antibiofilm agent against *D. hansenii* with a 97% biofilm inhibitory effect. Vanillin was also highly potent against the biofilm formation of *W. anomalus* and *S. pombe*, with a 77% and 82% biofilm inhibitory effect, respectively. There was a remarkably lower activity of vanillin against biofilm formation in *S. cerevisiae* which was below 50%. These results showed that the phenolic compounds' biofilm inhibitory activity differed depending on the studied food spoilage yeast. Cinnamic acid was highly active as an antibiofilm agent against *D. hansenii*, *W. anomalus*, *S. pombe* with a biofilm formation inhibition of more than 80%. In *S. cerevisiae*, the most active phenolic compound was 4-hydroxybenzaldehyde, which had a 64% biofilm formation inhibition. 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. Among the tested phenolics, only (−)-epicatechin and 4-hydroxybenzaldehyde had more than 50% biofilm formation inhibition in *S. cerevisiae*. Ferulic acid and protocatechuic acid had a slight invigorative effect on *S. cerevisiae* biofilm formation. 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. The biofilm inhibitory ability of the most bioactive compounds: vanillin, cinnamic acid and (−)-epicatechin, against the studied spoilage yeasts, was also investigated on the glass surface of a microscope

slide. After 24 h incubation, the biofilms formed in the presence and absence of the phenolic compounds were visualized by fluorescence microscopy. The resultant images from fluorescent microscopy showed fragmented biofilms for the samples containing the phenolic compounds and intact mature biofilms for the control samples, meaning that all the analyzed phenolic compounds had remarkable biofilm formation inhibition against the studied yeasts at 500 µg/mL.

Influence of singular phenolics on yeasts surface adhesion (Kimani *et al*. 2021)

The most effective phenolic compounds as antibiofilm agents in the studied spoilage yeasts 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*. From the antiadhesion study, all the assayed phenolic compounds had significant adhesion inhibition against the 4-h adhesion of spoilage yeast cells. This is exemplified in *D. hansenii*, where vanillin and cinnamic acid had more than 50% adhesion inhibition. 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. Cinnamic acid and vanillin were potent antiadhesive agents against *S. pombe* with adhesion inhibitions of 91% and 81%, respectively. 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*.

Influence of phenolic and phenolic-food preservative combinations on yeasts planktonic growth (Kimani *et al*. 2023)

Vanillin and cinnamic acid were combined to create an antimicrobial solution which was screened for antiplanktonic activity against the food spoilage yeasts using the checkerboard assay. Synergistic interaction was present in all the assayed spoilage yeasts. In *D. hansenii* and *W.*

anomalus, the fractional inhibitory concentration index (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 also recorded a ≥4-fold reduction in the MIC in all the strains. Multitarget effects of the vanillin-cinnamic acid combination could have contributed to the high anti-yeast planktonic activity.

Vanillin and cinnamic acid were also combined separately with the three studied food preservatives to create binary antifungal solutions that were screened for antiplanktonic microbial activity against the studied spoilage yeasts. In the antiplanktonic activity tests, synergistic interaction was present in all the vanillin-food preservatives combinations in *S. pombe*, with a FICI range of 0.41-0.46. In *S. cerevisiae*, synergistic interaction was present in vanillin-sodium benzoate and vanillin-potassium sorbate with a FICI of 0.34. In *W. anomalus*, synergistic interaction was present in vanillin-sodium benzoate with a FICI of 0.33. In *D. hansenii*, all vanillin-food preservatives combinations were indifferent with a FICI range of 2.02-2.64. There was less antiplanktonic activity against the studied yeasts for the cinnamic acid-food preservatives combinations, with synergistic interaction only occurring 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 of 0.67 and 0.83, respectively. In *W. anomalus*, cinnamic acid-sodium benzoate and cinnamic acid-potassium sorbate combinations were additive in antiplanktonic activity with a FICI of 0.58, while cinnamic acid-sodium diacetate was antagonistic in activity, with a FICI of 4.16. 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. In *D. hansenii*, higher resistance against the cinnamic-synthetic 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, with a FICI of 1.78. When equal concentrations of vanillin/cinnamic acid-food preservatives combinations were compared with singular food preservatives for their planktonic cells' inhibitory activity against the studied yeasts, it was evident that vanillin/cinnamic-food preservatives combinations had a higher antiplanktonic activity, even at low concentrations compared to the singular food preservatives. This clearly demonstrated that the presence of

vanillin/cinnamic acid at low concentration had a promoting effect on the food preservatives anti-yeast activity.

Influence of phenolic and phenolic-food preservative combinations on yeasts biofilms (Kimani *et al*. 2023)

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 FICI range of ≤0.5. The combination also reduced the MIC of the two phenolic compounds by a range of 4 to ≥16. The phenolic-food preservative combinations with a FICI less than 1 in the antiplanktonic growth assay, were also 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, except the vanillin-sodium diacetate combination in *S. pombe* that was additive in activity, with a FICI of 0.75. However, it was interesting to note the remarkable reduction in the MIC of most food preservatives in the presence of phenolic compounds. In *W. anomalus,* for instance, 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 MIC with cinnamic acid and a 32-fold reduction when combined with vanillin. In *S. cerevisiae,* a 32-fold MIC reduction was recorded for sodium benzoate and potassium sorbate when combined with vanillin.

Influence of phenolic and phenolic-food preservative combinations on adhesion capacity of yeasts (Kimani *et al*. 2023)

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 all the spoilage yeasts, except in *D. hansenii* where additivity was observed (FICI 0.63). The combination of vanillin and cinnamic acid also had a reducing effect on the individual compounds' MICs to the range of 2 to 16. The phenolic-food preservative combinations were also evaluated for their ability to inhibit adhesion on the polystyrene surface. The vanillin-potassium sorbate combination had synergistic interaction in the case of *W. anomalus* and *S. pombe*, with FICI 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 preservative

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 led to the reduction of the food preservatives' MICs which ranged from 1 to 32.

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

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. 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 Met145, while the common hydrophobic interactions were formed with Ala134, Gln136, Cys179, Asn180, and Pro146. Based on the number of hydrogen bonds, polydatin had six, while quercetin had four bonds. Resveratrol, (−)-epicatechin, caffeic acid, and ferulic acid had three hydrogen bonds, the rest of the phenolics had either two or one, except in *p*-coumaric acid where only hydrophobic bonds were present.

The phenolics-chitin synthase III docking studies had quercetin, polydatin, (−)-epicatechin, caffeic acid, and resveratrol having binding free energy in the range of -10.4 to -7.1 kcal/mol. Polydatin had the lowest binding free energy of -10.4 kcal/mol. The common residues that formed hydrogen bonds with the phenolic ligands included Lys681, Gly200, Lys197, Leu201, and Tyr688, while hydrophobic interaction was common in Glu203, Lys197, and Asn690. Polydatin, (−)-epicatechin, vanillin, and quercetin had more than 5 hydrogen bonds, with their bond lengths ranging from 2.79 - 3.33 Å. The rest of the phenolic ligands had hydrogen bonds ranging from 2 to 5.

The molecular docking studies of the phenolics ligands on the squalene synthase receptor had the lowest binding energy in polydatin, which was -8.7 kcal/mol. (−)-Epicatechin, quercetin, and resveratrol had binding energy of -8.1, -7.8, and -7.4 kcal/mol, respectively. The rest of the phenolic ligands had their binding energy ranging from -6.0 to -5.4 kcal/mol. Hydrogen bond interactions between the phenolic ligands and squalene synthase were common in Arg76, Phe295, Gly187, Thr191, and Tyr283. Hydrophobic interactions were present in Pro299, Leu190, Val186, and Phe53. 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.

Polydatin, (−)-epicatechin, and quercetin had the lowest binding energy on the Flo1 protein target of -7.2, -7.1, and -7.0 kcal/mol, respectively, with the rest of the compounds having their binding energy ranging between -6.2 to -4.9 kcal/mol. (−)-Epicatechin had 10 hydrogen bonds, quercetin and polydatin had 8 and 6 hydrogen bonds, respectively, while the rest of the phenolic ligands had 1 to 5 hydrogen bonds. Some of the common residues involved in hydrogen bond interactions with the ligands included Thr132, Asp78, and Tyr46. Some of the common residues involved in hydrophobic interactions included Phe127, Ile81, and Ser80.

SUMMARY

1. Antiplanktonic, antibiofilm and/or antiadhesion property of many individual phenolic compounds has been identified towards food spoilage yeasts.
2. Antiplanktonic growth activity of phenolic-phenolic and phenolic-food preservative combinations against food spoilage yeasts has been studied. Synergistic interactions were identified in several combinations. To our knowledge, this is the first study to clearly show the immense potential of vanillin-cinnamic acid combination and several cinnamic acid/vanillin-food preservative combinations against food spoilage yeasts.
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. This is the first study, to our knowledge, to delineate the inordinate potential of cinnamic acid-vanillin compound combination against food spoilage yeasts.
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.
5. Binding potential of phenolics to protein targets with different physiological functions has been evaluated. 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.

ÖSSZEFOGLALÁS

Az élelmiszereket szennyező élesztők megváltoztathatják az ételek minőségét és érzékszervi tulajdonságait. 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 fenolos vegyületek is. Élelmiszeripari kutatások már leírták egyes fenolok antimikrobiális hatását, azonban élesztők elleni aktivitásukról eddig kevés adat állt rendelkezésünkre.

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 fenolokkal a sejtszerkezet fenntartásában, a biofilm képződésben és/vagy az adhézióban szerepet játszó *S. cerevisiae* fehérjecélpontok ellen.

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 fejlesztéséhez. 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.

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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. ***IF: 5.561***

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Yang, T., Rao, Z., **Kimani, B.G.**, Xu, M., Zhang, X., Yang, S-T. (2015) Two-step production of gamma-aminobutyric acid from cassava powder using *Corynebacterium glutamicum* and *Lactobacillus plantarum*. *Journal of Industrial Microbiology & Biotechnology* 42, 1157-1165. ***IF: 2.745***

Abstract:

Takó, M., Kovács, T., Perdana, M.I., **Kimani, B.G.**, Varga, M., Kerekes, E.B., Bethala, K., Matikhaye, S.B., Somkuwar, S.R., Gadpayale, J.V., Ramteke, P.W., Vágvölgyi, Cs. (2023) Antioxidative and antimicrobial properties of the medicinal plant *Amorphophallus shyamsalilianum*. In: Teparić, R., Leboš Pavunc, A., Kifer, D. (eds.) Power of Microbes in Industry and Environment 2023: Book of Abstracts. p. 104.

Cumulative impact factor: 21.749

DECLARATION

We declare that the contribution of Bernard Gitura Kimani was significant in the following publications, and most part of the thesis is based on the below mentioned publications:

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. ***IF: 5.561***

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. ***IF: 5.2***

The results reported in the Ph.D. dissertation and the publications were not used to acquire any Ph.D. degree previously and will not be used in future either.

Szeged, July 11, 2023

Dr. Miklós Takó

Dr. Erika Beáta Bencsik-Kerekes