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# A FUNCTIONAL PROPERTY OF A DOMESTIC APPLE ISOLATE: PULCHERRIMIN PRODUCTION BY *METSCHNIKOWIA PULCHERRIMA* ELM-GS-3 VIA WASTE VALORIZATION

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## ABSTRACT
*Metschnikowia pulcherrima* ELM-GS-3 was isolated from damaged Granny Smith apples and identified via MALDI-TOF MS and ITS sequencing (97.89% similarity, NCBI database). Pulcherrimin production was confirmed on $FeCl_3$-supplemented media by maroon-red pigment formation and microscopic analysis. Food waste-derived media, including potato peel, onion skin, watermelon rind extracts, and diluted molasses, were evaluated for biomass and pigment production. Onion skin extract yielded the highest biomass (9.78±0.1 g/L) and pulcherrimin (7.63±0.6 g/L), followed by molasses and watermelon rind. FTIR analysis confirmed iron presence, while SEM revealed an amorphous microporous 3D structure. Absorbance peaked at 420 nm in alkali solution, consistent with low solubility except in alkaline conditions. The pigment's low solubility profile, except in alkali, aligns with its stability characteristics observed in the literature. This study demonstrates the potential of food waste in pulcherrimin production and the biotechnological relevance of *M. pulcherrima* ELM-GS-3.
**Keywords:** *Metschnikowia pulcherrima*, pulcherrimin, onion skin, waste, fermentation, ITS sequencing

# YERLİ BİR ELMA İZOLATININ FONKSİYONEL ÖZELLİĞİ: ATIKLARIN DEĞERLENDİRİLMESİ YOLUYLA *METSCHNIKOWIA PULCHERRIMA* ELM-GS-3 TARAFINDAN PULKERİMİN ÜRETİMİ

## ÖZ
*Metschnikowia pulcherrima* ELM-GS-3, hasar görmüş Granny Smith elmalarından izole edilmiş ve MALDI-TOF MS ile ITS dizileme yöntemleri kullanılarak tanımlanmıştır (%97.89 benzerlik, NCBI veritabanı). Pulcherrimin üretimi, $FeCl_3$ içeren besiyerinde bordo-kırmızı pigment oluşumu ve mikroskobik analiz ile doğrulanmıştır. Patates kabuğu, soğan kabuğu, karpuz kabuğu ekstreleri ve seyreltilmiş melas gibi gıda atıklarından türetilmiş besiyerleri, biyokütle ve pigment üretimi açısından değerlendirilmiştir. En yüksek biyokütle (9.78±0.1 g/L) ve pulcherrimin (7.63±0.6 g/L) üretimi soğan kabuğu ekstresinde gözlemlenmiş, bunu sırasıyla melas ve karpuz kabuğu takip etmiştir. FTIR analizi pigmentin demir içerdiğini doğrularken, SEM analizi amorf ve mikroporoz 3D bir yapı ortaya koymuştur. Pigmentin alkali çözeltide maksimum absorpsiyon dalga boyu 420 nm olarak belirlenmiş

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ve düşük çözünürlük profili, literatürde bildirilen stabilite özellikleriyle uyumlu bulunmuştur. Bu çalışma, gıda atıklarının pulcherrimin üretiminde potansiyelini ve *M. pulcherrima* ELM-GS-3'ün biyoteknolojik önemini ortaya koymaktadır.,
**Anahtar kelimeler:** *Metschnikowia pulcherrima*, pulkerimin, soğan kabuğu, atık, fermantasyon, ITS dizileme

## INTRODUCTION

The increasing global focus on sustainability and environmental preservation has highlighted the need to find innovative ways to reduce food waste and utilize it as a resource for value-added products (Lin et al., 2014). Food waste and by-products are generated in significant quantities during food processing, and if not managed properly, they contribute to environmental pollution and greenhouse gas emissions (Gustavsson et al., 2011; Tiwari and Khawas, 2021; Sarker et al., 2024). In recent years, there has been growing interest in the valorization of such waste streams to produce value-added products (Galanakis, 2012; Mishra et al., 2023). Food wastes and by-products are rich in carbohydrates, fibers, and micronutrients that can serve as essential carbon and nitrogen sources for microbial growth. These substrate sources contain abundant polysaccharides, vitamins, and minerals, which promote microbial activities and can be harnessed in bioprocessing and biorefinery applications to produce value-added compounds such as biofuels, organic acids, and biopolymers (Kosseva, 2013; Kampen, 2014; Nair et al., 2017). Studies have reported that different types of agro-industrial wastes can serve as effective substrates for producing microbial pigments, thereby reducing production costs and promoting sustainable practices (Panesar et al., 2015; Ramesh et al., 2022). This approach aligns with the principles of a circular bioeconomy, which aims to create sustainable production systems while reducing environmental burdens (Koutinas et al., 2014: Vea et al., 2018).

Microbial pigments are of particular interest due to their diverse applications in the food, pharmaceutical, and cosmetic industries, where natural colorants are preferred over synthetic alternatives (Dufossé, 2006: Kalra et al., 2020; Lyu et al., 2022; Di Salvo et al., 2023). Among the various microorganisms capable of pigment production, yeasts have emerged as promising candidates due to their fast growth rate, ability to utilize diverse substrates, and stability under different environmental conditions (Bernard et al., 2024).

Pulcherrimin-producing *Metschnikowia* strains are widespread among yeast communities that colonize ripening fruits, floral nectar, and tree sap fluxes. They are also frequently detected in fruit juices and during the fermentation of wine (SlÁviková et al., 2009; Graça et el., 2015; Sipiczki, 2020). It is a non-pathogenic yeast species known for its ability to produce pulcherrimin, a maroon-red pigment formed by the chelation of iron with pulcherriminic acid (Tatay-Núñez et al., 2024). Pulcherrimin has demonstrated various biological activities, including antimicrobial effects, which are attributed to its ability to sequester iron, thereby inhibiting the growth of competing microorganisms and showing significant photoprotection against UVA-induced damage and cell death, antioxidant and cytoprotective activities (Sipiczki, 2006; Kántor et al., 2015; Pawlikowska et al., 2020; Charron-Lamoureux et al., 2023; Kregiel et al., 2024).

This study aims to explore the potential of using onion (*Allium cepa* L.) skin, watermelon (*Citrullus lanatus*) rinds, potato (*Solanum tuberosum* L.) peels, and molasses as substrates for pulcherrimin production by *Metschnikowia pulcherrima* ELM-GS-3. The study also involves a comprehensive characterization of the produced pulcherrimin to determine its structural and chemical properties. The specific objectives of the research include (i) identification of *M. pulcherrima* ELM-GS-3, (ii) evaluation of food waste and by-product substrates for microbial biomass and pigment production, and (iii) characterization of the produced pulcherrimin using various analytical techniques. By demonstrating the feasibility of using food by-products for pulcherrimin production, this study contributes to the

development of sustainable bioprocesses and supports the shift towards a bio-based economy.

## MATERIALS AND METHODS

### Isolation of *Metschnikowia pulcherrima* ELM-GS-3

The yeast strain *Metschnikowia pulcherrima* ELM-GS-3 was isolated from damaged Granny Smith apples showing black spots surrounded by a reddish halo which were obtained from a local producer in Çorum, Türkiye. The apples were first washed with sterile distilled water, and the damaged portions were excised under sterile conditions. The excised tissue was macerated in sterile 0.85% saline solution and allowed to incubate at 30°C for 24 hours to promote the growth of indigenous microorganisms. The resulting suspension was streaked onto Yeast Extract Peptone Dextrose (YPD) agar plates and incubated at 28°C for 48 hours. Colonies displaying characteristic white to cream coloration were selected and purified by repeated streaking on fresh YPD agar plates.

### Identification of *Metschnikowia pulcherrima* ELM-GS-3 by MALDI-TOF MS

Sample preparation was conducted according to Karasu-Yalcin et al., (2021) by using protein extraction method. To extract the cells using this method, 1 mL aliquots of liquid culture were taken and centrifuged at 13 000 g for 2 minutes. The cell pellet was rinsed twice with sterile distilled water and air-dried for 20 minutes. Following this, the cells were lysed with 70% formic acid (volume used was proportional to the size of the cell pellet, approximately 30 L) and acetonitrile was added in an equal volume. The supernatant was spotted onto MTP 384 Ground Steel Target (#8280784 Bruker, Germany) following extensive vortexing and centrifugation (13 000 g, 2 minutes). For each aliquot, a total of four spots for sampling (1 L each) were analyzed. After the sample spot had air-dried, it was covered with 1 L of matrix (10 mg/mL α-cyano-4-hydroxy-cinnamic acid [α-CHCA], Bruker) and allowed to air-dry once more prior to being analyzed with the Autoflex Speed (Bruker Daltonik GmbH, Germany) using MALDI-TOF MS. The instrument is equipped with a 355 nm nitrogen laser, which was discharged in linear positive mode at the sample spots at a frequency of 55 Hz. Each spectrum was generated by aggregating 100 samples' profiles.

MALDI TOF/TOF MS (Autoflex Speed from Bruker Daltonics, Germany) in combination with the MALDI Biotyper 3.1 software program was used for identification based on the analysis of mass spectra. The mass spectrometer was calibrated with a bacterial test standard from Bruker (Bruker Daltonics GmbH, Germany). This calibration kit comprises a typical protein extract of E. coli DH5 alpha spiked with two additional pure proteins (RNAse A and myoglobin) to cover an overall mass range of 3.6 to 17 kDa. Before each analysis, the calibration procedure was performed again. MS-signals were acquired for each sample in linear positive mode between 2000 and 20 000 Da m/z by summing 500 laser-shot spectra in accordance with the manufacturer's automatic technique MBT_FC.par. The voltages of IS1 and IS2 ion sources were 19.99 kV and 19.80 kV, respectively. The lens had a voltage of 6500 kV and an extraction pulse of 200 nanoseconds. The laser intensity was between 50 and 60%.

Identification was conducted in Scientific Industrial and Technological Application and Research Center of Bolu Abant Izzet Baysal University, Turkey. Mass spectra were analyzed using Biotyper software (version 3.1; Bruker Daltonics) and the Biotyper database version DB-6903, which contained 6903 reference MALDI-TOF MS profiles (6120 of bacteria, 776 of fungi, and 7 of archaea). Using a score, the Biotyper software quantified the degree of similarity between experimental profiles obtained from microorganism isolates and reference profiles. The value of the score is determined by the similarity between the observed and stored datasets. A score greater than 2.3 (green) indicates an exceptional species-level identification, while a score greater than 2.0 indicates a good species-level identification. The score between 1.7 and 2.0 (yellow) indicates a reliable identification of the genus. In contrast, a score value of less than 1.7 (red color) indicates that there is no substantial

similarity between the unknown profile and the database (Karasu-Yalcin et al. 2021).

**Identification of *Metschnikowia pulcherrima* ELM-GS-3 by Sequence Analysis of the Internal Transcribed Spacer (ITS) Regions**

For DNA isolation, the EurX GeneMATRIX Plant & Fungi DNA isolation kit (Poland) was used. After DNA isolation, the quantity and purity of the obtained DNA were assessed using the Thermo Scientific Nanodrop 2000 (USA) spectrophotometer. For the PCR analysis, the universal primers ITS1 and ITS4 were used to amplify the target gene regions for species identification. The primer sequences were as follows: ITS1 5' TCCGTAGGTGAACCTGCGG 3' and ITS4 5' TCCTCCGCTTATTGATATGC 3'. PCR conditions included an initial denaturation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 45 seconds, annealing at 57°C for 45 seconds, and extension at 72°C for 60 seconds. A final extension was performed at 72°C for 5 minutes, and the temperature was then lowered to 4°C to complete the PCR.

The amplification products obtained with the Kyratec thermocycler were analyzed by electrophoresis on a 1.5% agarose gel prepared with 1x TAE buffer, run at 100 volts for 90 minutes, and visualized under UV light using ethidium bromide staining. A single-step PCR was performed to amplify a region of approximately 700 base pairs, using Solis Biodyne (Estonia) FIREPol® DNA Polymerase Taq polymerase enzyme. The presence of a single band on the agarose gel indicated successful PCR amplification. For PCR product purification, the single-band samples were purified using the MAGBIO "HighPrepTM PCR Clean-up System" (AC-60005) according to the manufacturer's procedures. Sanger sequencing was carried out at Macrogen's laboratory in the Netherlands, using the ABI3730XL Sanger sequencing device (Applied Biosystems, Foster City, CA) and the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). The reads obtained with ITS1 and ITS4 primers were assembled into a contig to create a consensus sequence using the CAP contig assembly algorithm in BioEdit software.

**Inoculum Preparation**

Inoculum was prepared in 100 mL of sterile Tryptic Soy Broth (Merck, Germany) medium in a cotton-plugged conical flask to prepare an inoculum from the strain grown on a YM agar slant, including (g $L^{-1}$): yeast extract, 3; malt extract, 3; peptone, 5; glucose, 10; and agar, 15, at 28°C for 48 hours. The flask was incubated for 48 hours at 30°C with 100 rpm agitation in a shaking incubator (Lab Companion, South Korea) (Mujdeci, 2021).

**Fermentation Process**

Fermentation was initially conducted in a synthetic fermentation medium to confirm pulcherrimin production by *M. pulcherrima* ELM-GS-3 strain. For this purposes, 150 mL of sterile synthetic minimal broth [1% glucose (w/v), 0.3% $(NH_4)_2SO_4$ (w/v), 0.1% $KH_2PO_4$ (w/v), 0.05% $MgSO_4 \times 7H_2O$ (w/v), 0.05% yeast extract (w/v), 0.05% $FeCl_3$ (w/v)] was inoculated with 5% (v/v) of the inoculum in cotton-plugged Erlenmeyer flasks. The flasks were then incubated at 28°C with 130 rpm agitation for 7 days (Kregiel et al., 2022).

The subsequent stage of the fermentation experiments involved the production of pulcherrimin using potato peels, watermelon rinds, and onion skins, as well as molasses, as substrate sources in the fermentation medium. For this purpose, the modified method described by Mujdeci (2022) was used. Molasses was sourced from a sugar factory in Corum City, Turkey, and diluted at a ratio of 1:10 (v v-1). Watermelon rinds, onion skins, and potato peels were used as fermentation media after separate preprocessing. In this method, 2 L of distilled water were added to 1000 g of peel. After boiling the mixture at 100°C for 30 min, the extract was separated by filtration using cellulose filter papers. Before sterilization, 0.05% $FeCl_3$ (w/v) was added to each extract and the mixtures were used as fermentation media. Sterilization of the fermentation media was carried out at 121°C for 15 min using autoclave.

The fermentation was carried out in 500 mL Erlenmeyer flasks containing 200 mL of the fermentation medium. The medium was inoculated with a 10% (v/v) inoculum of *M. pulcherrima* ELM-GS-3, prepared by culturing the yeast in YPD broth for 24 hours at 28°C. The flasks were incubated at 28°C for 7 days with constant shaking at 150 rpm.

**Extraction and Purification of Pulcherrimin**

During the experiments, 10 mL of each culture was sampled from the fermentation medium for every 24 hours and centrifuged at 4°C and 2599 × g for 20 minutes. The resulting precipitate containing yeast cells and red pigment was treated with 50 mL of 99.8% methanol per 10 g of wet yeast biomass at 4°C and incubated overnight. Following incubation, the yeast cells were centrifuged at 4°C and 2599 × g for 20 minutes and washed twice with 25 mL of distilled water. The yeast biomass was then resuspended in 2M NaOH and centrifuged again (4°C, 2.599 × g, 20 minutes). The pH of the supernatant was adjusted to 1.0 using 4M HCl, followed by incubation at 100°C for 30 minutes. The resulting pigment precipitate was collected by centrifugation (4°C, 2599 × g, 30 minutes) and washed three times with 25 mL of distilled water. To obtain pure pulcherrimin, the steps of dissolution in NaOH and precipitation with HCl were repeated three times. Finally, the red pigment was collected by centrifugation and stored at −20°C, as described by Kregiel et al. (2022). The concentration of pulcherrimin in each fermentation medium was determined as the dry weight of pulcherrimin per liter of fermentation medium (Mujdeci, 2022).

**Characterization of Pulcherrimin**

For spectrophotometric analysis, the purified pulcherrimin was dissolved in methanol, and its wavelength spectrum was recorded using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan) over the range of 200-800 nm. The the wavelength giving maximum absorption (λmax) was determined to confirm the presence of pulcherrimin. The structural characteristics of the extracted pulcherrimin were further analyzed using Fourier-transform infrared spectroscopy (FTIR) (Thermo Scientific/Nicolet IS10, Thermo Fisher Scientific, Waltham, MA, USA). The pigment was mixed with KBr to form pellets, and the spectra were recorded in the range of 4.000-600 $cm^{-1}$, with 40 scans at a resolution of 4.0 $cm^{-1}$. The molecular structures of pulcherrimin particles were assessed using a scanning electron microscope (SEM) (Quanta FEG 450, FEI, Amsterdam, Netherlands).

For solubility assessment, 0.1 g of purified pulcherrimin samples was dissolved in 10 mL of distilled water, 1 mol $L^{-1}$ KOH, and 1 mol $L^{-1}$ NaOH solutions, as well as various organic solvents such as chloroform, ethyl acetate, ethanol, methanol, acetic acid, ether, petroleum ether, hexane, and acetone. After stirring at 25°C for 1 hour, the solutions were filtered through coarse filter paper. The absorbance measurements of the filtrates were carried out at λmax, as described by Mujdeci (2021).

## RESULTS

**Identification of *Metschnikowia pulcherrima* ELM-GS-3**

Among the yeast strains isolated from damaged Grany Smith apples (data not shown), pinkish, round colonies with smooth edges (ELM-GS-3) were identified as *M. pulcherrima* with a log score value of 1.954 as a result of identification by MALDI-TOF MS. Fig. 1 illustrates raw MALDI-TOF MS profile of *M. pulcherrima* (a) and matching result of experimental profiles and BioTyper database (b). Validation of the identified species was performed by sequence analysis. PCR products obtained by amplification from ITS regions were sequenced and compared with the DNA sequence databases of The National Center for Biotechnology Information (NCBI). The species was confirmed as *Metchikowia pulcherrima* with 97.89% similarity.

To determine if the identified *M. pulcherrima* ELM-GS-3 strain produces pulcherrimin, cells were inoculated into $FeCl^{-3}$ containing YM agar and the synthetic minimal broth described in Section 2.5. Maroon-red pigment formation was observed (Fig. 2a, b). Additionally, cells grown in the fermentation medium were examined under a light microscope, and intracellular red pigment formation was documented through photography (Fig. 2c).

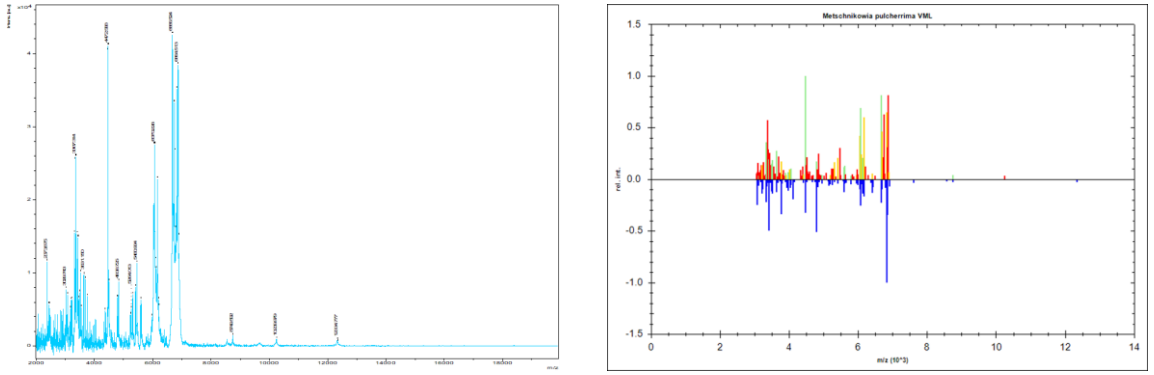


Figure 1. a: raw MALDI-TOF MS profile of *M. pulcherrima* ELM-GS-3, b: matching result of experimental profiles and BioTyper database

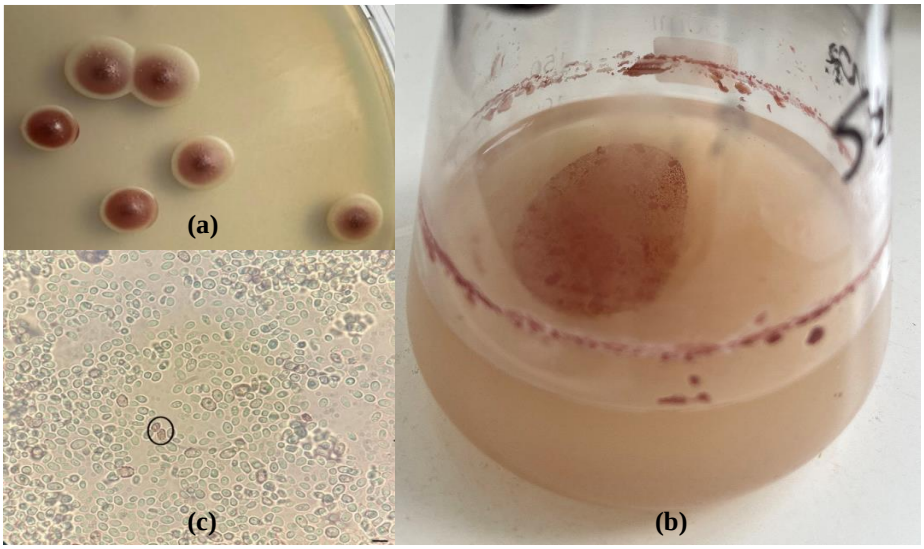


Figure 2. a: *M. pulcherrima* GS-3 colonies on YM Agar including 0.05% $FeCl_3$ (w/v), b: pigment production in synthetic minimal broth, c: light micrograph of the pulcherrimin produced *M. pulcherrima* ELM-GS-3 cells (in circle) at Day 7 of fermentation in glucose containing fermentation media (scale bar = 10 µm).

**Valorization of Food Wastes and By-products for Pulcherrimin Production**

The graph showing the change in biomass concentration of *Mechnikowia pulcherrima* ELM-GS-3 over time in potato peel, onion skin, and watermelon rind extracts, as well as diluted molasses medium, is presented in Fig. 3.

In experiments using potato peel extract and molasses solution as the fermentation medium, the highest biomass concentration was recorded as 3.70±0.1 g/L and 7.67±0.5 g/L, respectively. When watermelon rind extract was used, the biomass concentrations of *M. pulcherrima* ELM-GS-3 were relatively higher, recorded as 9.60±0.1 g/L. Onion skin extract was identified as the medium that most effectively supported the growth of *M. pulcherrima* ELM-GS-3, yielding 9.78±0.1 g/L biomass. Fig. 4 shows the biomass obtained on the 7$^{th}$ day of fermentation. As seen in the photograph, the biomass obtained from onion skin was dark maroon due to intracellular pigment production. In watermelon rind, all cells did not produce the pigment, and the

pulcherrimin-producing cells were clustered in the center of the pellet. In potato peel extract and molasses solution, a reddish color was observed due to pigment-producing cells.

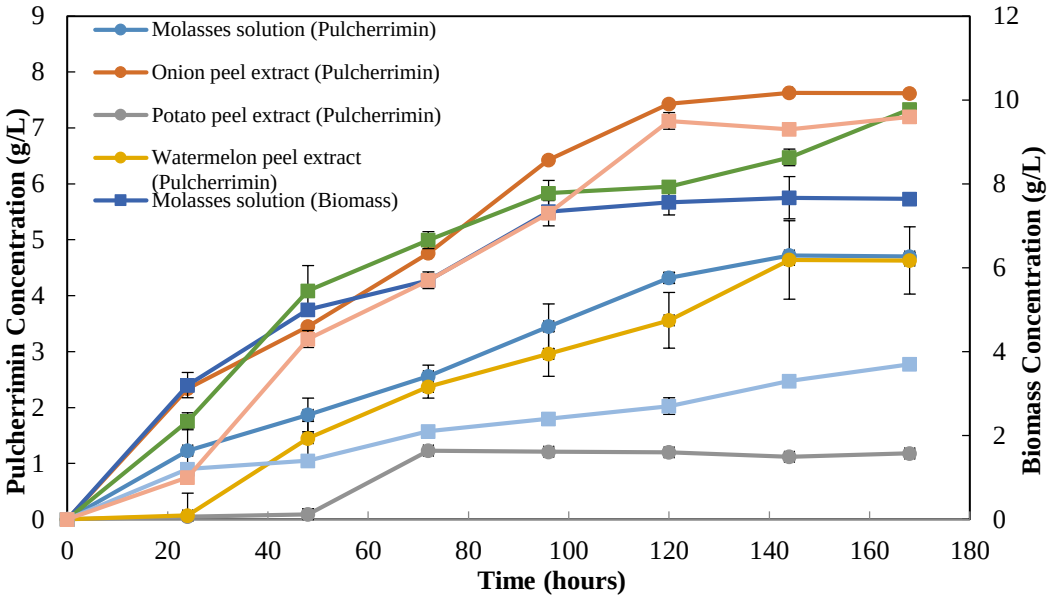


Figure 3. Variations of biomass and pulcherrimin concentrations with time

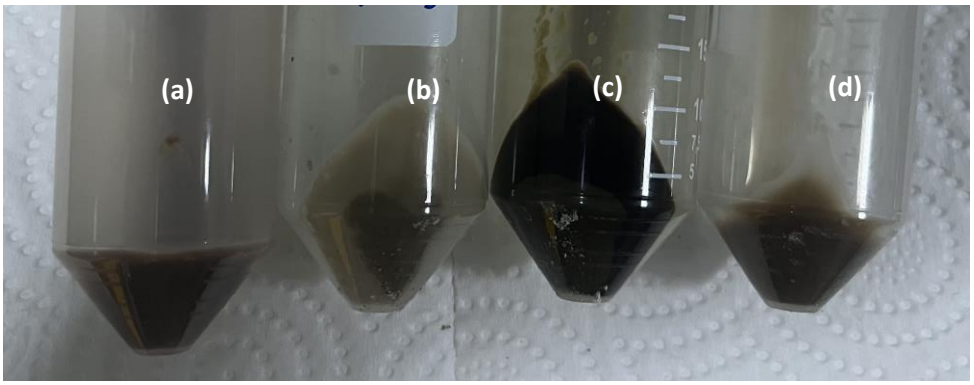


Figure 4. Biomass precipitated from cultures in a: potato peel extract, b: watermelon rindextract, c: onion skin extract, and d: molasses solution

The pulcherrimin concentrations produced by *M. pulcherrima* ELM-GS-3 over time are shown in Fig. 3. While it was found that potato peel extract was not suitable for pulcherrimin production, the highest pulcherrimin production (7.63±0.6 g/L) was obtained with onion skin extract. The highest pulcherrimin concentrations obtained from molasses solution and watermelon rind extract were determined to be very close to each other and 4.72±0.0 and 4.64±0.7 g/L, respectively.

**Characterization of Pulcherrimin**

The first step in characterization of pulcherrimin included FTIR analysis. The FTIR transmittance spectra of the pulcherrimin obtained in this study and in the study by McDonald (1963) are demonstrated in Fig. 5. The indicated peaks (700–1700 $cm^{-1}$) correspond to the absorption bands of pulcherrimin, consistent with those previously reported for *Candida pulcherrima* (McDonald, 1963). The peak at 580 $cm^{-1}$ is attributed to the presence of the heaviest ion in the compound, iron (Fe) (Mažeika et al., 2021).

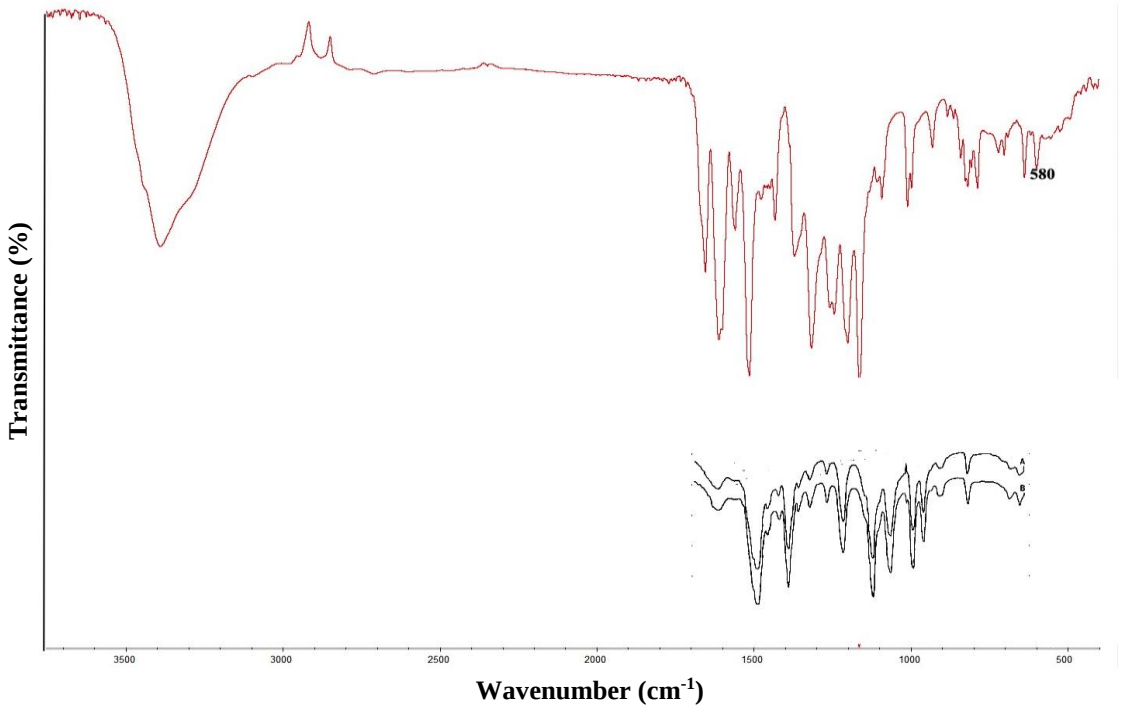


Figure 5. Fourier-transform infrared spectroscopy spectra of a: pulcherrimin produced by *M. pulcherrima* ELM-GS-3, b: pulcherrimin produced by *C. pulcherrima* (McDonald, 1963)

In this study, the second assay for pulcherrimin characterization involved investigating the molecular structure using SEM. The pulcherrimin sample exhibited an amorphous (irregular) shape with dimensions ranging from 75 to 150 nm. At higher magnification, the pulcherrimin sample demonstrated a 3D network structure with the presence of microporosity. The pores in the pulcherrimin particles are indicated by arrows in Fig. 6.

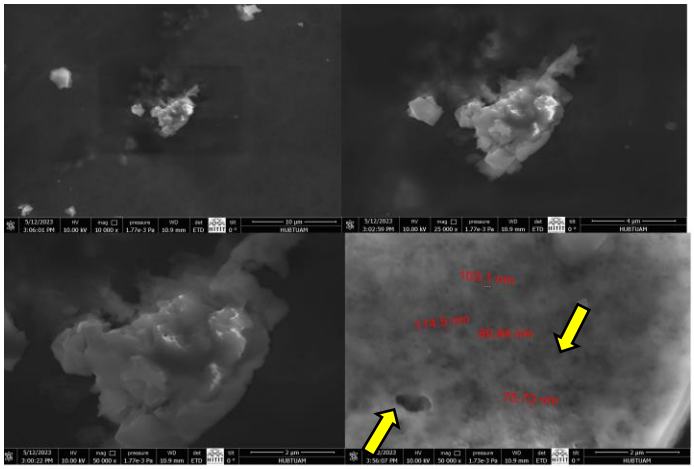

Figure 6. Scanning electronmicrograph of pulcherrimin nanoparticles at 10.0, 25.0, 50.0, and 100.0 K× magnifications

Maximum absorbance of alkali solution of pulchrerrimin was observed at 420 nm. Pulcherrimin exhibits low solubility in distilled water and most organic and inorganic solvents, except aqueous alkali, and shows resistance to degradation in concentrated acids which are distinctive characteristics of this pigment. In this study, significant similarity was observed with the literature. The pulcherrimin sample was insoluble in water and a range of organic solvents, including chloroform, ethyl acetate, ethanol, methanol, acetic acid, ether, petroleum ether, hexane, and acetone, but was soluble only in alkali.

## DISCUSSION

The present study demonstrated the potential to valorize food waste and by-products for pulcherrimin production using the yeast strain *Metschnikowia pulcherrima* ELM-GS-3, with successful isolation and characterization. The strain was identified through MALDI-TOF MS and ITS sequencing, confirming its identity as *M. pulcherrima*. This identification method, along with its pigmentation capability, aligns with previous studies reporting the natural ability of *M. pulcherrima* to produce pulcherrimin which is a pigment involved in microbial competition (Turkel and Ener, 2009; Sipiczki, 2020).

In this study, onion skin extract was the most favorable medium for biomass production and pulcherrimin synthesis, with the highest pulcherrimin yield compared to other substrates. Onion production, as one of the most widely consumed vegetable crops, is on the rise due to its valuable dietary, medicinal, and functional properties. According to the latest FAO data, global onion production has steadily increased, reflecting its high demand for dietary, medicinal, and functional uses. In recent years, China and India have led global production (FAOSTAT, 2022). Following these leaders are countries like the United States and Turkey, which also contribute significantly to global onion output. Given the vast global production of onions, finding ways to utilize onion skin is of great importance. In previous studies, onion skin was used as a substrate in fermentation media to produce some bio-products (Genemo et al., 2021; Taşar and Taşar, 2022; Hsueh et al., 2023). Genemo et al. (2021) examined bioethanol production from cabbage and onion peels, highlighting how optimizing the fermentation process with *Saccharomyces cerevisiae* could yield a sustainable energy source from agro-waste. Similarly, Hsueh et al. (2023) evaluated the feasibility of transforming onion peels into value-added products within a circular economy framework, emphasizing onion peels' rich bioactive compounds that could be harnessed for a range of applications. Mushimiyimana and Tallapragada (2017) further demonstrated that acid hydrolysis and subsequent fermentation offer a viable pathway for bioethanol production from onion skin and similar agro-wastes. Beyond bioethanol, Kim et al. (2019) explored a biorefining process converting onion waste carbohydrates into acetic acid, revealing onion waste's versatility as a fermentation substrate. Additionally, Ramesh et al. (2022) discussed pigment production via submerged fermentation, highlighting the role of natural substrates such as onion skins in generating bio-pigments under optimized culture conditions. Together, these studies underscore the potential of onion skin valorization to produce bioethanol, acetic acid, and pigments, reinforcing its contribution to bio-based circular economy initiatives and waste reduction. However, this is the first study in the literature that used onion peel to produce pulcherrimin.

Other substrates, such as watermelon rind and potato peel extracts, as well as molasses, were also investigated. While watermelon rind extract resulted in relatively high biomass production, the pulcherrimin yield was limited, suggesting that although watermelon rind provides some nutrients for cell growth, it lacks sufficient factors required for efficient pulcherrimin synthesis. Comparatively, Joshi et al. (2003) also decleraed variability in pigment production depending on the nutrient composition of the substrate, indicating that the carbon and nitrogen ratios, as well as micronutrient availability, play a significant role in pigment biosynthesis.

Pulcherrimin was characterized using several analytical techniques, including FTIR, SEM, and UV-visible spectroscopy. The FTIR analysis showed characteristic absorption peaks similar to those reported in the literature (McDonald, 1963), which is indicative of the consistency of pulcherrimin's structure across different studies. The presence of an absorption peak at 580 $cm^{-1}$, attributed to iron, is also in agreement with the findings of Mažeika et al. (2021), highlighting the involvement of metal ions in stabilizing the pigment structure. The results obtained from the SEM analysis showed an amorphous structure with a 3D network and microporosity, which was consistent with studies conducted by Mažeika et al. (2021).

UV-visible absorbance spectra of the purified pulcherrimin showed characteristics that align with those previously reported in the literature, confirming its chemical properties (McDonald, 1963). The pigment's low solubility in water and most organic solvents, except for aqueous alkali, is a known property of pulcherrimin (Kregiel et al., 2022), which could pose challenges for certain applications but also suggests its suitability for stable formulations. The stability in concentrated acids was also observed, which is a valuable property for industrial applications where chemical resistance is required.

The utilization of food waste as substrates for microbial pigment production presents significant environmental and economic benefits. Studies have shown that *M. pulcherrima* is capable of utilizing a wide range of low-cost medium under unsterilized conditions, making it highly versatile for biotechnological applications (Abomohra et al., 2020). In comparison, our study supports these findings and further emphasizes that food by-products can serve as cost-effective substrates for valuable pigment production.

However, the observed differences in biomass and pigment production across substrates underline the importance of selecting appropriate waste materials for fermentation processes. This study suggests that optimizing the fermentation medium composition, including the carbon-to-nitrogen ratio and the presence of trace elements, can significantly enhance pulcherrimin production.

## CONCLUSION

In conclusion, this study provides valuable insights into the production of pulcherrimin using *Metschnikowia pulcherrima* ELM-GS-3, with a focus on using food waste substrates to enhance sustainability and cost-effectiveness. The comparison with literature indicates that onion skin is an excellent substrate for biomass and pigment production, with its nutrient content being favorable for both growth and secondary metabolite synthesis. The characterization of pulcherrimin was consistent with previous reports, confirming its structural and chemical properties. Future research should focus on optimizing fermentation conditions and investigating other types of food waste to improve pulcherrimin yield and reduce production costs. The valorization of food waste for pigment production aligns well with the goals of the bioeconomy and contributes to the development of sustainable bioprocesses.

## DECLARATION OF INTEREST

The author declares that she has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## COMPETING INTERESTS

The author have no relevant financial or non-financial interests to disclose.

## DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

## REFERENCES

Abomohra, A. E. F., Wang, Q., Huang, J., Saad-Allah, K. M. (2021). A sustainable approach for

bioconversion of food and lignocellulosic wastes into liquid biofuel using a new *Metschnikowia pulcherrima* isolate. *International Journal of Energy Research* 45(2): 3430-3441, doi: 10.1002/er.6028.

Bernard, A., Rossignol, T., Park, Y. K. (2024). Biotechnological approaches for producing natural pigments in yeasts. *Trends in Biotechnology* 42(12): 1644-1662, doi: 10.1016/ j.tibtech.2024.06.012.

Charron-Lamoureux, V., Haroune, L., Pomerleau, M., Hall, L., Orban, F., Leroux, J., Beauregard, P. B. (2023). Pulcherriminic acid modulates iron availability and protects against oxidative stress during microbial interactions. *Nature Communications* 14(1): 2536, doi: 10.1038/s41467-023-38222-0.

Di Salvo, E., Lo Vecchio, G., De Pasquale, R., De Maria, L., Tardugno, R., Vadalà, R., Cicero, N. (2023). Natural pigments production and their application in food, health and other industries. *Nutrients*, 15(8): 1923, doi: 10.3390/nu15081923.

Dufossé, L. (2006). Microbial production of food grade pigments. *Food technology and Biotechnology*, 44(3): 313-321, doi: 10.17113/ftb.

FAOSTAT (2022). Food and Agriculture Organization https://www.fao.org/faostat/ en/#data (Accessed 15 October 2024).

Galanakis, C. M. (2012). Recovery of high added-value components from food wastes: Conventional, emerging technologies and commercialized applications. *Trends in Food Science and Technology*, 26(2): 68-87, doi: 10.1016/ j.tifs.2012.03.003.

Genemo, T. G., Erebo, D. L., Gabr, A. K. (2021). Optimizing bio-ethanol production from cabbage and onion peels waste using yeast (*Saccharomyces cerevisiae*) as fermenting agent. *International Journal of Life Science Research Archive,* 1(1): 24-35, doi: 10.53771/ijlsra.2021.1.1.0012.

Graça, A., Santo, D., Esteves, E., Nunes, C., Abadias, M., Quintas, C. (2015). Evaluation of microbial quality and yeast diversity in fresh-cut apple. *Food Microbiology*, 51: 179-185, doi: 10.1016/j.fm.2015.06.003.

FAO (2011). Global food losses and food waste – Extent, causes and prevention. Rome, Italy.

Hsueh, C. C., Roxas, T. J., Chan, Y. H., Juan, C. N., Tayo, L. L., Chen, Y. Y., Chen, B. Y. (2023). Feasibility study of value-added production from onion peel agricultural wastes for circular economy. *Journal of the Taiwan Institute of Chemical Engineers*, 145: 104851, doi: 10.1016/ j.jtice.2023.104851.

Joshi, V. K., Attri, D., Bala, A., Bhushan, S. (2003). Microbial pigments. *Indian Journal of Biotechnology*, 2: 362-369, doi: 10.1007/978-1-4020-9942-7_8.

Kalra, R., Conlan, X. A., Goel, M. (2020). Fungi as a potential source of pigments: harnessing filamentous fungi. *Frontiers in Chemistry*, 8: 369, doi: 10.3389/fchem.2020.00369.

Kampen, W.H. (2014). Nutritional requirements in fermentation processes. In: *Fermentation and Biochemical Engineering Handbook*, Vogel, H.C, Todaro, C.M. (eds.), William Andrew Publishing, Norwich New York, pp. 35-57.

Kántor, A., Hutková, J., Petrová, J., Hleba, L., Kačániová, M. (2015). Antimicrobial activity of pulcherrimin pigment produced by *Metschnikowia pulcherrima* against various yeast species. *Journal of Microbiology, Biotechnology and Food Sciences*, *5*(3): 282-285, doi: 10.15414/jmbfs.2015/16.5.3.282-285.

Karasu-Yalcin, S., Soylemez-Milli, N., Eren, O., Eryasar-Orer, K. (2021). Reducing time in detection of *Listeria monocytogenes* from food by MALDI-TOF mass spectrometry. *Journal of Food Science and Technology*, 58: 4102-4109, doi: 10.1007/s13197-020-04869-6.

Kim, H. M., Choi, I. S., Lee, S., Yang, J. E., Jeong, S. G., Park, J. H., Park, H. W. (2019). Biorefining process of carbohydrate feedstock (agricultural onion waste) to acetic acid. *ACS Omega*, 4(27): 22438-22444, doi: 10.1021/acsomega.9b03093.

Kosseva, M.R. (2013). Recovery of commodities from food wastes using solid-state fermentation, In: *Food Industry Wastes: Assessment and Recuperation of Commodities*, Kosseva, M.R., Webb, C. (eds.), Academic Press, Cambridge, USA, pp. 77-102.

Koutinas, A. A., Vlysidis, A., Pleissner, D., Kopsahelis, N., Garcia, I. L., Kookos, I. K., Lin, C. S. K. (2014). Valorization of industrial waste and by-product streams via fermentation for the production of chemicals and biopolymers. *Chemical Society Reviews*, 43(8): 2587-2627, doi: 10.1039/c3cs60293a.

Kregiel, D., Krajewska, A., Kowalska-Baron, A., Czarnecka-Chrebelska, K. H., Nowak, A. (2024). Photoprotective Effects of Yeast Pulcherrimin. *Molecules*, 29(20): 4873, doi: 10.3390/molecules29204873.

Kregiel, D., Nowacka, M., Rygala, A., Vadkertiová, R. (2022). Biological activity of pulcherrimin from the *Meschnikowia pulcherrima* Clade. *Molecules*, 27(6); 1855, doi: 10.3390/molecules27061855.

Lin, C. S. K., Koutinas, A. A., Stamatelatou, K., Mubofu, E. B., Matharu, A. S., Kopsahelis, N., Luque, R. (2014). Current and future trends in food waste valorization for the production of chemicals, materials and fuels: a global perspective. *Biofuels, Bioproducts and Biorefining*, 8(5): 686-715, doi: 10.1002/bbb.1506.

Lyu, X., Lyu, Y., Yu, H., Chen, W., Ye, L., Yang, R. (2022). Biotechnological advances for improving natural pigment production: A state-of-the-art review. *Bioresources and Bioprocessing*, 9(1): 8, doi: 10.1186/s40643-022-00497-4.

Mažeika, K., Šiliauskas, L., Skridlaitė, G., Matelis, A., Garjonytė, R., Paškevičius, A., Melvydas, V. (2021). Features of iron accumulation at high concentration in pulcherrimin-producing *Metschnikowia* yeast biomass. *JBIC Journal of Biological Inorganic Chemistry*, 26: 299-311, doi: 10.1007/s00775-021-01853-z.

MacDonald, J. C. (1963). The structure of pulcherriminic acid. *Canadian Journal of Chemistry*, 41(1): 165-172, doi: 10.1139/v63-021.

Mishra, K., Siwal, S. S., Nayaka, S. C., Guan, Z., Thakur, V. K. (2023). Waste-to-chemicals: Green solutions for bioeconomy markets. *Science of the Total Environment*, 887: 164006, doi: 10.1016/j.scitotenv.2023.164006.

Mujdeci, G. N. (2021). Natural melanin synthesized by *Aureobasidium pullulans* using food wastes and its characterization. *Applied Food Biotechnology*, 8(4): 307-318, doi: 10.22037/afb.v8i4.34599.

Müjdeci, G. N. (2022). Experimental modeling and optimization of melanin production by *Aureobasidium pullulans* NBRC 100716 in carrot peel extract. *Environmental Progress and Sustainable Energy*, 41(6): e13919, doi: 10.1002/ep.13919.

Mushimiyimana, I., Tallapragada, P. (2016). Bioethanol production from agro wastes by acid hydrolysis and fermentation process. *CSIR-NIScPR*, 75(06): 383-388.

Nair, R.B., Lennartsson, P.R., Taherzadeh, M.J. (2017). Bioethanol production from agricultural and municipal wastes, In: *Current Developments in Biotechnology and Bioengineering,* Wong, J.W.C., Tyagi, R.D., Pandey, A. (eds.), Elsevier, Amsterdam, Holland, pp. 157-190.

Panesar, R., Kaur, S., Panesar, P. S. (2015). Production of microbial pigments utilizing agro-industrial waste: a review. *Current Opinion in Food Science*, 1: 70-76, doi: 10.1016/j.cofs.2014.12.002.

Pawlikowska, E., Kolesińska, B., Nowacka, M., Kregiel, D. (2020). A new approach to producing high yields of pulcherrimin from *Metschnikowia* yeasts. *Fermentation*, 6(4): 114, doi: 10.3390/fermentation6040114.

Ramesh, C., Prasastha, V. R., Venkatachalam, M., Dufossé, L. (2022). Natural substrates and culture conditions to produce pigments from potential microbes in submerged fermentation. *Fermentation*, 8(9): 460, doi: 10.3390/fermentation8090460.

Sarker, A., Ahmmed, R., Ahsan, S. M., Rana, J., Ghosh, M. K., Nandi, R. (2024). A comprehensive review of food waste valorization for the sustainable management of global food waste. *Sustainable Food Technology*, 2: 48-69, doi: 10.1039/d3fb00156c.

Sipiczki, M. (2006). *Metschnikowia* strains isolated from botrytized grapes antagonize fungal and bacterial growth by iron depletion. *Applied and*

*Environmental Microbiology*, 72(10): 6716-6724, doi: 10.1128/AEM.01275-06.

Sipiczki, M. (2020). *Metschnikowia pulcherrima* and related pulcherrimin-producing yeasts: Fuzzy species boundaries and complex antimicrobial antagonism. *Microorganisms*, 8(7): 1029, doi: 10.3390/microorganisms8071029.

SlÁviková, E., Vadkertiová, R., Vránová, D. (2009). Yeasts colonizing the leaves of fruit trees. *Annals of Microbiology*, 59: 419-424, doi: 10.1007/BF03175125.

Taşar, Ö. C., Taşar, G. E. Use of onion peels as an economical substrate for microbial inulinase production under solid state fermentation. *Eurasian Journal of Biological and Chemical Sciences*, 5: 144-150, doi: 10.46239/ ejbcs.1163946.

Tatay-Núñez, J., Albi-Puig, J., Garrigós, V., Orejas-Suárez, M., Matallana, E., Aranda, A. (2024). Isolation of local strains of the yeast *Metschnikowia* for biocontrol and lipid production purposes. *World Journal of Microbiology and Biotechnology*, 40(3): 88, doi: 10.1007/s11274-024-03918-y.

Tiwari, A., Khawas, R. (2021). Food waste and agro by-products: a step towards food sustainability. In: *Innovation in the food sector through the valorization of food and agro-food by-products*, Baros, A.N. (ed), IntechOpen.

Türkel, S., Ener, B. (2009). Isolation and characterization of new *Metschnikowia pulcherrima* strains as producers of the antimicrobial pigment pulcherrimin. *Zeitschrift für Naturforschung C*, 64(5-6): 405-410, doi: 10.1515/znc-2009-5-618.

Vea, E. B., Romeo, D., Thomsen, M. (2018). Biowaste valorisation in a future circular bioeconomy. *Procedia CIRP*, 69: 591-596, doi: 10.1016/j.procir.2017.11.062.