Research, Society and Development, v. 11, n. 5, e44111528339, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i5.28339

# Microbial Biosurfactant: Production, Characterization and Application as a Food Emulsions

## Biossurfactante Microbiano: Produção, Caracterização e Aplicação em Emulsões Alimentares

## Biosurfactante Microbiano: Producción, Caracterización y Aplicación en Emulsiones Alimentarias

Received: 03/22/2022 | Reviewed: 03/31/2022 | Accept: 04/06/2022 | Published: 04/12/2022

**Isabela Regina Alvares da Silva Lira**
ORCID: https://orcid.org/0000-0002-1649-9622
Catholic University of Pernambuco, Brazil
E-mail: isabelaalvares24@gmail.com
**Emília Mendes da Silva Santos**
ORCID: https://orcid.org/0000-0001-7566-0079
Catholic University of Pernambuco, Brazil
E-mail: emiliamendes.farma@gmail.com
**Jenyffer Medeiros Campos Guerra**
ORCID: https://orcid.org/0000-0002-8788-0746
Catholic University of Pernambuco, Brazil
E-mail: jenyffermcampos@gmail.com.
**Hugo Moraes Meira**
ORCID: https://orcid.org/0000-0001-8886-4855
Advanced Institute of Technology and Innovation, Brazil
E-mail: hugo_morais15@hotmail.com
**Leonie Asfora Sarubbo**
ORCID: https://orcid.org/0000-0002-4746-0560
Catholic University of Pernambuco, Brazil
E-mail: leonie.sarubbo@unicap.com.br.
**Juliana Moura de Luna***
ORCID: https://orcid.org/0000-0003-4619-6857
Catholic University of Pernambuco, Brazil
E-mail: juliana.luna@unicap.br

**Abstract**
The aim of the present study was to select the better biosurfactant-producing microorganism between two species of Candida, grown in different media. The determination of its physicochemical composition, the structural characterization and the properties of the biosurfactant in terms of toxicity were performed. Formulations of mayonnaise were tested for stability and the growth of pathogens. *C. guilliermodii* grown in a medium with 5.0% molasses, 5.0% corn steep liquor and 5.0% waste frying oil was selected for the production of biosurfactant, with a reduction in surface tension to 28.6 mN/m, a yield of 21 g/l and a critical micelle concentration of 0.7 g/l. The isolated biosurfactant was not toxic for the vegetable seeds tested and had a glycolipid nature. All mayonnaise formulations remained stable, with the absence of pathogenic microorganisms. The present results, the novel biosurfactant produced herein has potential application in food systems, such as mayonnaise emulsions.
**Keyword:** Biotensioative agents; Agroindustrial waste*; Candida*; Bioemulsifiers.

**Resumo**
O objetivo do presente estudo foi selecionar o melhor microrganismo produtor de biossurfactante entre duas espécies de *Candida*, cultivadas em diferentes meios. A determinação de sua composição físico-química, a caracterização estrutural e as propriedades do biossurfactante em termos de toxicidade foram realizadas. As formulações de maionese foram testadas quanto à estabilidade e ao crescimento de patógenos. *C. guilliermodii* cultivada em meio com 5,0% de melaço, 5,0% de licor de maceração de milho e 5,0% de óleo residual de fritura foi selecionado para a produção de biossurfactante, com redução da tensão superficial para 28,6 mN/m, rendimento de 21 g/l e uma concentração micelar crítica de 0,7 g/l. O biossurfactante isolado não foi tóxico para as sementes de hortaliças testadas e apresentou natureza glicolipídica. Todas as formulações de maionese permaneceram estáveis, com ausência de microrganismos patogênicos. Com os presentes resultados, o novo biossurfactante aqui produzido tem potencial aplicação em sistemas alimentícios, como emulsões de maionese.
**Palavras-chave:** Agentes biotensioativos; Resíduos agroindustriais; *Cândida*; Bioemulsificantes.

**Resumen**
El objetivo del presente estudio fue seleccionar el mejor microorganismo productor de biosurfactantes entre dos especies de *Candida*, cultivadas en diferentes medios. Se realizó la determinación de su composición fisicoquímica, la caracterización estructural y las propiedades del biosurfactante en cuanto a toxicidad. Las formulaciones de mayonesa se probaron para la estabilidad y el crecimiento de patógenos. Se seleccionó *C. guilliermodii* cultivada en un medio con 5,0% de melaza, 5,0% de licor de maceración de maíz y 5,0% de aceite de fritura usado para la producción de biosurfactante, con una reducción de la tensión superficial a 28,6 mN/m, un rendimiento de 21 g/l y una concentración micelar crítica de 0,7 g/l. El biosurfactante aislado no resultó tóxico para las semillas vegetales ensayadas y tenía naturaleza glicolipídica. Todas las formulaciones de mayonesa se mantuvieron estables, con ausencia de microorganismos patógenos. Según los presentes resultados, el nuevo biosurfactante producido aquí tiene una aplicación potencial en los sistemas alimentarios, como las emulsiones de mayonesa.
**Palabras clave:** Agentes biosurfactantes; Residuos agroindustriales; *Cándida*; Bioemulsionante.

## 1. Introduction

Biosurfactants are a structurally diverse group of surface-active molecules produced by microorganisms. They can accumulate on cellular surfaces or can be released into the extracellular medium. These amphiphilic molecules are preferred over their chemical homologues because of their biodegradability, low toxicity and efficiency in extreme temperature and pH conditions (Campos et al., 2014; Geetha et al., 2018; Silva et al., 2022; Mallik & Banerjee, 2022). Biosurfactant properties such as emulsifying, antiadhesive and antimicrobial behavior are important in the food, pharmaceutical and oil industries where they are also used as hydrocarbon dissolution agents (Marcelino et al., 2020; Singh, 2018; Farias et al., 2021; Pinto et al., 2022).

Bioemulsifiers are used in the food industry is due to their emulsifying, foaming, humectant and solubilizing properties. These products can be used as emulsifiers in the processing of raw material, the control of the clustering of fat globules, the stabilization of aerated systems and the improvement of the consistency of fatty products (Pacwa-Płociniczak et al., 2011). Emulsification is particularly useful in the food industry and bioemulsifiers have the capacity to form stable emulsions, thereby improving the texture and creaminess of many products (Campos et al., 2015; Pinto et al., 2022).

Yeasts of the genus *Candida* stand out among species of bioemulsifier-producing microorganisms due to their usefulness in food products (Bourdichon et al., 2012). These yeasts have *Generally Regarded As Safe* (GRAS) status and therefore do not pose risks in terms of toxicity or pathogenicity, enabling their use in the food (Campos et al., 2014: Daylin et al., 2017; Silva et al., 2022). Bioemulsifiers produced by *Candida utilis* has been used in salad dressings (Lee & Kim, 2001) and cookie (Ribeiro et al., 2020), and *Candida bombicola* has been used in cupcake (Silva et al., 2020). Others yeasts recently reported for the production of biosurfactants include *Candida sphaerica* (Luna et al., 2016), *Starmerella bombicola* (Wang et al., 2019) and *C. lipolytica* (Santos et al., 2017), which have potential as producers of compounds with surfactant activities. Thus, the aim of the present study was to produce a non-toxic biosurfactant of *C. guilliermondii* UCP 0992 and *C. lipolytica* UCP 0988 with emulsifying properties for application as an additive in food systems.

## 2. Methodology

### 2.1 Microorganisms

Two species of *Candida* (*C. guilliermondii* UCP 0992 and *C. lipolytica* UCP 0988) were tested as biosurfactant producers. Both strains were acquired from the culture bank of the Environmental Science Research Center of the Catholic University of Pernambuco.

**2.2 Maintenance medium**

The yeasts were maintained at 5 °C in yeast mold agar (YMA) with the following composition (p/v): yeast extract (0.3%), malt extract (0.3%), tryptone (0.5%), d-glucose (1%) and agar (5%), dissolved in distilled water (100 ml). Subcultures were performed monthly to maintain cell viability.

**2.3 Growth medium for inoculum**

Yeast mold broth (YMB) was used for the growth of the inoculum, which has the same composition as yma with the exception of agar (p/v): yeast extract (0.3%), malt extract (0.3%), tryptone (0.5%) and d-glucose (1%). all components were solubilized in distilled water and the medium was sterilized in an autoclave at 121 °c for 20 minutes.

**2.4 Preparation of inoculum**

The inoculum was transferred to a tube containing a YMA medium to obtain a young culture. Next, the sample was transferred to flasks containing 50 ml of YMB medium, followed by incubation with constant stirring at 200 rpm and 28 °C for 24 h. After this period, cell counts were performed in a Neubauer chamber until obtaining the desired final concentration of cells ($10^8$ CFU/ml).

**2.5 Biosurfactant production media**

Different media were tested for the production of biosurfactants: A) 5.0% glucose, 0.1% yeast extract, 0.1% urea and 5.0% waste frying oil; B) 5.0% molasses, 0.1% yeast extract, 0.1% urea and 5.0% waste frying oil; C) 5.0% molasses, 5.0% corn steep liquor and 5.0% waste frying oil; and D) 2.5% molasses, 2.0% corn steep liquor and 2.5% waste frying oil.

**2.6 Production of biosurfactant**

Fermentations for the production of biosurfactant were performed in 1000 ml Erlenmeyer flasks containing 500 ml of production medium and incubated with the suspension of $10^6$ CFU/ml. The inoculum was added and the media were kept under orbital stirring at 200 rpm for 144 h at a temperature of 28 °C. After the incubation period, the media were submitted to centrifugation with stirring at 4500 rpm for 20 minutes for the obtainment of the cell-free broth. Aliquots were withdrawn after fermentation for the determination of surface tension, biomass, pH and yield of biosurfactant.

**2.7 Growth kinetics**

From the previously established medium, aliquots were withdrawn at 2, 4, 6, 8, 12, 24, 30, 36, 48, 60, 72, 96, 120 and 144 h throughout fermentation. The samples were submitted to filtration, followed by centrifugation at 4500 rpm and 9 °C for 15 min. Next, cell-free broths were used for the determination of surface tension, pH and the emulsification index. For the determination of biomass in dry weight, 50 ml of the culture were centrifuged at 5000 rpm for 15 minutes and the supernatant was discarded. The biomass was dried in a forced-air oven at 105 °C for 24 h and weighed.

**2.8 Surface tension determination**

Surface tension was measured in cell-free metabolic broth (crude biosurfactant) using a Sigma 700 tensiometer (KSV Instruments, Helsinki, Finland) (Lira et al., 2021).

**2.9 Isolation of biosurfactant**

The method by adding methanol extraction after 144 h of *C. guilliermondii* culturing, the broth was centrifuged at 2000 rpm for 20 min for the removal of the cells and submitted to the extraction process (Lira et al., 2021).

**2.10 Nuclear magnetic resonance spectroscopy**

The extracted biosurfactant was re-dissolved in deuterated chloroform ($CDCl_3$) and the respective $^1H$ NMR spectra were recorded at 25 °C using an Agilent 300 Mz spectrometer operating at 300.13 MHz. Chemical displacements (δ) were given on a scale of ppm in relation to tetramethylsilane (TMS).

**2.11 Infrared spectroscopy**

The biosurfactant extract recovered from the supernatant of the *C guillermondii* isolate was characterized by Fourier transform infrared (FTIR) spectroscopy using the Spectrum 400 IR-NIR (Perkin Elmer) with a resolution of 4 $cm^{-1}$ in the region of 400 to 4000 wavenumbers ($cm^{-1}$).

**2.12 Gas chromatography (GC)**

The sample of fatty acids (hydrophobic fraction) of the biosurfactant was analyzed in a gas chromatograph (Hewlett Packard model HP 5890 Série II) with an injector temperature of 220 ºC. Chromatographic separation was performed in a DB-5 column (30 m x 0.32 mm x 0.5 µm) with a flame ionization detector (FID) at 290 ºC, using nitrogen as the carrier gas.

**2.13 Phytotoxicity test**

The phytotoxicity of the biosurfactant was evaluated in a static assay involving seed germination and root growth of three vegetable plants: cabbage (*Brassica oleracea*), tomato (*Solanum lycopersicum*) and maroon cucumber (*Cucumis anguria*), based on Tiquia (Tiquia et al., 1996). Test solutions were prepared in distilled water with biosurfactant concentrations of 0.35, 0.7 and 1.4 g/l, respectively. After five days of incubation in the dark, seed germination, root growth (≥ 5 mm) and the germination index were calculated.

**2.14 Toxicity Test with *Artemia salina***

The toxicity assay was performed with the isolated biosurfactant using brine shrimp (*Artemia salina*) as the bioindicator. Brine shrimp eggs were obtained from the local market. The larvae were used within one day after hatching. After dilutions of the biosurfactant solution at 0.35, 0.7 and 1.4 g/l, respectively in seawater, the assays were performed in a 50 ml beaker with 10 larvae in 20 ml of seawater + 20 ml of the biosurfactant at the different concentrations. The mortality rate was calculated after 24 h.

**2.15 Toxicity test with onion *(Allium cepa L.)***

Toxicity of the isolated biosurfactant was also evaluated using onion (*Allium cepa* L.) as the indicator. The biosurfactant was used at concentrations of 0.35, 0.7 and 1.4 g/l, respectively. The cell-free broth was also tested. The root growth inhibition test was conducted using the method described by Jardim (2004) with some modifications. These tests were performed in triplicate.

### 2.16 Application of biosurfactant as a food additive

The emulsifying property of the biosurfactant was tested in the formulation of seven different types of mayonnaise sauce obtained from commercial samples of the following ingredients (w / v): 40% sunflower oil, 40.3% water, 10% vinegar, 4% powdered egg (Naturovos LTDA, Brazil), 2% sugar, 2% salt, 1% mustard flour and 0.2% guar gum and 0.5% instant starch (Unilever LTDA, Brazil). The concentration of the isolated biosurfactant ranged from 0.2 to 0.8% (w/v). The formulas were then stored at 4 ºC for one month for future analyses of their appearance, pH and viscosity (Shepherd et al., 1995). The tests were carried out in duplicate.

The best formulation out of the seven was chosen to carry on with the experiments. New emulsions were formulated with the same ingredients but in different proportions, as described in: Formula1- Starch; Formula 2 - Starch + Isolated *Candida* biosurfactant; Formula 3- Starch + Guar gum; Formula 4- Guar gum; Formula 5 – Isolated *Candida* biosurfactant + Guar gum and Formula 7-Isolated biosurfactant.

The sauces were then mixed for one minute at room temperature and then stored at 8 °C for one month in order to be analyzed regarding appearance, pH and viscosity again. The emulsion stability of the sauces was analyzed at each week.

## 3. Results

### 3.1 Selection of culture medium and biosurfactant-producing microorganism

In the present study involving two strains of *Candida* grown in different production media, the biosurfactants produced by *C. lipolytica* led to surface tensions ranging from 33 to 52 mN/m. The biosurfactant produced by *C. guilliermondii* grown in 5% molasses, 5% corn steep liquor and 5% waste frying oil reduced the surface tension of water from 72 to 28.6 mN/m. This condition was therefore selected and the biosurfactant produced by *C. guilliermondii* demonstrated excellent surface tension-reducing capacity (Table 1).

**Table 1.** Surface tension as parameter for selection of microorganism and biosurfactant production medium: A) 5.0% glucose, 0.1% yeast extract, 0.1% urea and 5.0% waste frying oil; B) 5.0% molasses, 0.1% yeast extract, 0.1% urea and 5.0% waste frying oil; C) 5.0% molasses, 5.0% corn steep liquor and 5.0% waste frying oil; and D) 2.5% molasses, 2.0% corn steep liquor and 2.5% waste frying oil.

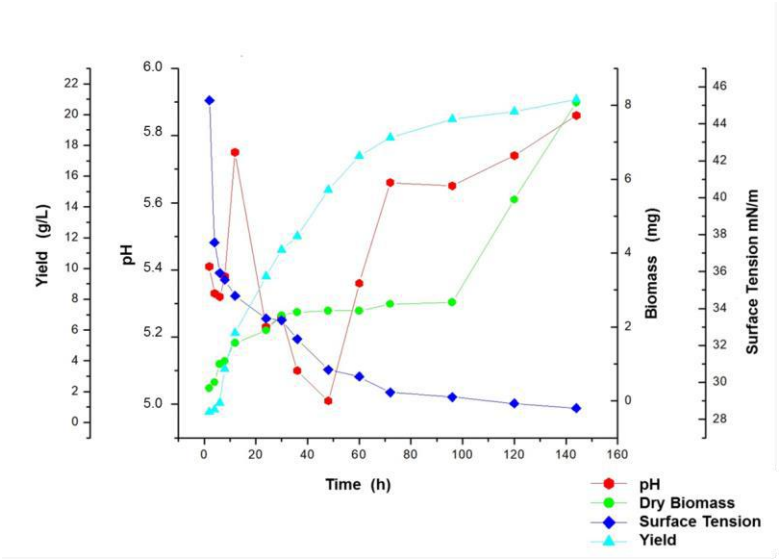
| **Production media** | ***Microorganism*** | |
|---|---|---|
| | ***C. lipolytica*** | ***C. guilhermondii*** |
| **A** | 36.23 ± 0.01 mN/m | 38.08 ± 0.09 mN/m |
| **B** | 35.51 ± 0.10 mN/m | 35.80 ± 0.05 mN/m |
| **C** | 52.13± 0.12 mN/m | 28.60 ± 0.02 mN/m |
| **D** | 33.05 ± 0.03 mN/m | 33.02 ± 0.71 mN/m |

Source: Authors.

### 3.2 Growth curves and biosurfactant production

Figure 1 shows the kinetics of the biosurfactant produced by *C. guilliermondii* UCP 0996 in a medium containing 5% molasses, 5% corn steep liquor and 5% waste frying oil.

**Figure 1**. Growth kinetics, pH, surface tension, dry biomass and yield of biosurfactant produced by *C. guilliermondii* UCP0996 grown in medium containing 5% molasses, 5% corn steep liquor and 5% waste frying oil.




Source: Authors.

The exponential growth phase occurred after four hours and continued through to 36 hours, at which point a stationary phase occurred, which was followed by another period of growth. Maximum biomass production was 8.0 g/l after 144 h. The onset of the stationary growth phase and biosurfactant production occurred after 72 h. The greatest biosurfactant yield (21 g/l) occurred in the stationary growth phase. Simultaneously, surface tension began to reduce, indicating that the novel substrates promote the biosynthesis of essential compounds for microbial growth and the production of biomolecules with active surface properties. Surface tension decreased from 45 mN /m to 28 mN/m during the growth curve stages, indicating excellent surfactant properties. The pH was altered little during the growth period (5.0 to 5.8), indicating that the microorganism adapted to the substrate and promoted the biosynthesis of essential compounds for growth, producing positive effects on the reduction in surface tension and the stability of production. The results show that biosurfactant production occurred since the onset of the exponential phase and throughout the stationary phase.

### 3.3 Phytotoxicity test

The toxicity of the biosurfactant from *C. guilliermondii* was tested in a short bioassay. Bioassays involving plants play an important role in predicting the effect of chemical products in an ecosystem.

The germination index, which combines measures of relative seed germination and relative root growth, was used to evaluate the toxicity of the biosurfactant to the seeds of *Brassica oleracea*, *Cucumis anguria* and *Solanum lycopersicum*. The results revealed that the solutions tested had no inhibitory effect on seed germination or root growth, indicating low toxicity of the biosurfactant. Moreover, leaf growth and the growth of secondary roots occurred under all conditions tested. *C. guilliermondii* biosurfactant was tested in a short bioassay, indicating the low toxicity of the produced biosurfactant.

### 3.4 Toxicity assay with *Artemia salina*

Brine shrimp, *Artemia salina*, is commonly used in ecotoxicology due to its simplicity in terms of laboratory handling and its short life cycle (Meyer et al., 1982). After exposure to the biosurfactant at 0.35, 0.7 and 1.4 g/l for 24 h, the *Artemia salina* larval survival rate was 100%.

**3.5 Toxicity test with onion *(Allium cepa L.)***

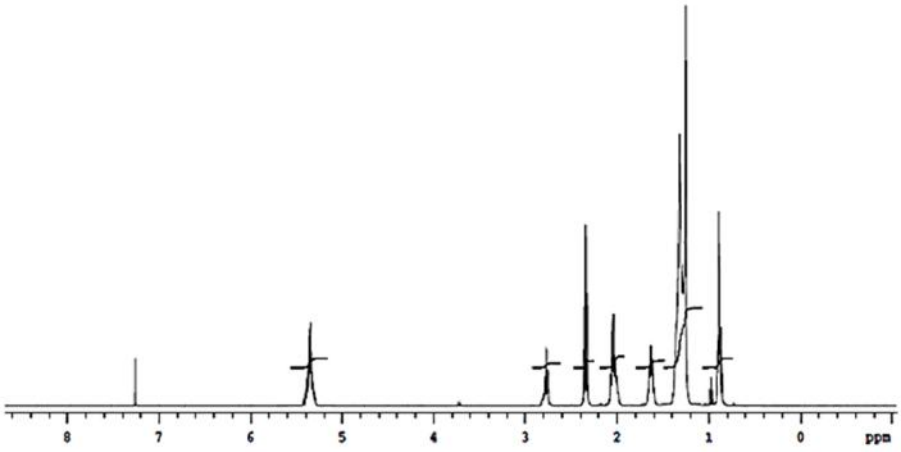
Plants such as the onion (*Allium cepa L.*) are widely used in ecotoxicological assays for the evaluation of the toxicity of different compounds (Jardim, 2004). The most commonly analyzed aspect is phytotoxicity, which is evidenced by the inhibition of germination and root growth when seeds are exposed to a harmful substance. This is a fast, easy test to determine the environmental impact of a compound (Benassi et al., 2019).

The results of subacute toxicity with *Allium cepa* were evaluated based on mean weight gain of onion plants in water and root growth after exposure to the biosurfactant at concentrations of 0.35, 0.7 and 1.4 g/l. Weight gain occurred in all samples exposed to the biosurfactant at the 1 x CMC as well as one control sample and one sample exposed to the biosurfactant at ½ x CMC and 2 x CMC, an average of 1.2 mg. (In the other samples, using metabolic fluid). The lack of weight gain in the other samples may be explained by the fact that onion plants must expend energy on self-defense when exposed to the biosurfactant.

**3.6 Structural analysis**

The structure of the biosurfactant was determined using nuclear magnetic resonance spectroscopy and gas chromatography. The hydrogen and carbon spectra are displayed in Figures.

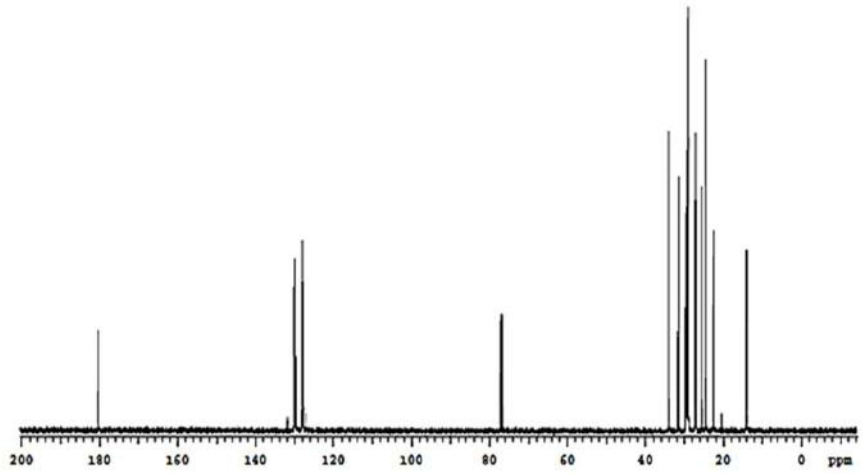
**Figure 2.** 1H-NMR spectrum recorded in CDCl3 of biosurfactant produced by *C. guilliermondii* in medium supplemented with 5% molasses, 5% corn steep liquor and 5% waste frying oil.




Source: Authors.

The $^{1}H$ NMR spectrum (Figure 2) had three well-defined regions, suggesting the presence of hydrogen closed to carboxylic acid groups at 7–8 ppm, close to double bonds at 5-6 ppm and aliphatic carbons at 1 to 3 ppm. The $^{13}C$ NMR spectrum (Figure 3) confirms the previous results, showing a characteristic choice of carboxylic acid at 180 ppm, double bonds between 120 and 140 ppm and aliphatic carbons in the 10–40 ppm region. The signals at 70-80 ppm were attributed to the residual solvent (chloroform). These results suggest that the biomolecule of the surfactant is a type of carboxylic acid metabolite likely bonded to carbohydrates (simple fatty acid), as described for other glycolipid biosurfactants produced by yeasts.

**Figure 3.** 13C-NMR spectrum recorded in CDCl3 of biosurfactant produced by *C. guilliermondii* in medium supplemented with 5% molasses, 5% corn steep liquor and 5% waste frying oil.

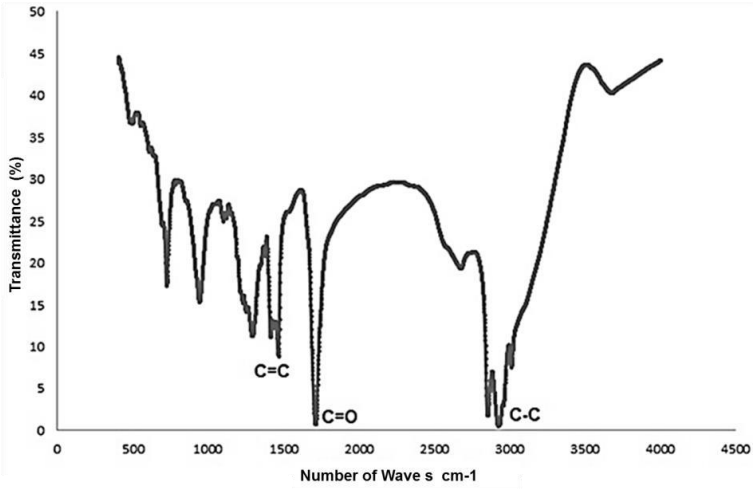



Source: Authors.

Figure 4 displays the FTIR spectra obtained for the isolated biosurfactant produced by *C. guilliermondii*, showing absorbance, regions of stretching from 1500 to 2000 $cm^{-1}$ and 2500 to 3000 $cm^{-1}$, indicating the possible presence of carbonyl groups (C=O) and simple bonds between carbons (C–C), respectively. The stretching at approximately 1400 $cm^{-1}$ corresponds to the double bond between carbons (C=C). Santos (Santos et al., 2016) describes a similar spectrum for a biosurfactant produced by *C. lipolytica* grown in a medium with 5% animal fat and 2.5% corn steep liquor.

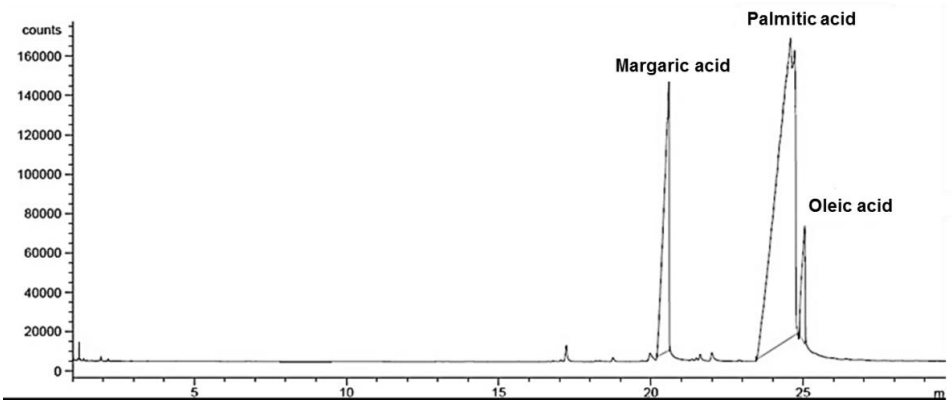
The GC analysis of the fatty acid composition of the isolated biosurfactant revealed C16:0 as the main component (75.3%), followed by C17:0 (19.6%) and C18:1 (5%) (Figure 5). The biosurfactant has diverse fatty acids in different proportions, demonstrating potential application in food formulations. Based on the results, the isolated biosurfactant has a glycolipid nature.

**Figure 4.** FTIR spectrum of biosurfactant produced by *C. guilliermondii* in medium supplemented with 5% molasses, 5% corn steep liquor and 5% waste frying oil.




Source: Authors.

**Figure 5.** Chromatogram of fatty acid profile of biosurfactant produced by *C. guilliermondii* in medium supplemented with 5% molasses, 5% corn steep liquor and 5% waste frying oil.




Source: Authors.

### 3.7 Application of biosurfactant as a food additive and mayonnaise sauces formulation

After one month of cold storage, the seven formulations with different concentrations of biosurfactant (0.2% to 0.8%) and guar gum were evaluated with regards to consistency. All the formulas presented to be stable during the storage period (refrigeration) and no phase separation could be observed. All the samples also presented a slight increase in pH (4.2-4.6), as opposed to the results presented by Kishk and Elsheshetawy (2013), where the pH decreased from 4.3 to 3.5 after four weeks of storage. All formulations exhibited good emulsion stability and firmness due to the stabilizing capacity of the gum and the emulsifying action of the biosurfactant. Therefore, all seven formulations were submitted to microbiological analysis.

Viscosity also remained constant, with small variations among the formulations (1415-1862 mPa.S). Mayonnaise is a non-Newtonian, pseudoplastic, thixotropic fluid with high consistency and rich in oils. Its viscosity diminishes with the increase in force applied, which leads to the alignment of molecules and a reduction in viscous friction. In other words, viscosity diminishes with the increase in the deformation rate (RPM) at a constant temperature (Sato, 2005).

The formula containing the biosurfactant from *C. guilliermondii* at a concentration of 0.5% with the addition of 0.5% guar gum was selected to prepare the formulation of six different mayonnaise sauces. The formulations showed different behaviors. In the first two weeks, formulas 1, 2 and 6 exhibited phase separations, with visible aqueous phase at the bottom of the container. This way, it was found that the isolated biosurfactant (formula 6) did not show the ability to stabilize the sauce for 30 days. This could be explained by the difficulty in stabilizing the formula due to a large number of microstructures made up of proteins, carbohydrates and lipids combinations found in food (McClements et al., 2017). Formulations 3,4 and 5 had the best stabilities being that Formula 5 present to be the most creamy and stable.

The pH of the formula samples ranged between 3.3 and 4.5 the formulas 3, 4 and 5 remained stable after the storage period. The viscosity data corroborates with the visual aspect of formula samples 1, 2 and 6 (214.6, 172.0 and 185.4 mPa.S, respectively). These low values of apparent viscosity are related to the deformation rates at a constant temperature. Formula 5 exhibited the highest apparent viscosity (1860 mPa.S) while formulas 3 and 4 displayed lower values (1525 and 1627 mPa.S, respectively), which indicates some degree of emulsion destabilization. One can conclude that the associated use of gum with biosurfactant demonstrated better visual aspect, texture and consistency compared to the other tested formulas.

## 4. Discussion

Analyzing the phytotoxicity obtained in this work for the *C. guillermondii* biosurfactant, pH and the concentration of crude extracts exert a direct influence on germination, as extracts may have substances, such as sugars, amino acids and

organic acids, that can affect metabolites. The non-germination in the samples submitted to the cell-free broth, which had a pH of approximately 5.0, indicates that acid pH leads to a reduction in plant development (Nascimento et al., 2018; Pacheco et al., 2017). Since the 80% GI value was used as an indicator of the disappearance of phytotoxicity (Meylheuc et al., 2001).

The toxicity results obtained in our work can be compared in the literature, demonstrates the low toxicity of the biosurfactant under the conditions tested Luna et al. (2013) and Rufino et al. (2014) report similar results for other biosurfactants.

The results obtained by *C. guillermondii* are similar to descriptions in the literature characterizing biosurfactants isolated from yeasts grown in diesel oil (Chandran & Das, 2011). Santos et al. (2017) describes a surfactant biomolecule with a carboxylic acid structure produced by *C. lipolytica*. Another study described an NMR spectrum with similar signals, revealing a biomolecule with a glycolipid structure metabolized from the yeast *Pichia sorbitophila* (Bhatia & Saharan, 2016).

Analyzing the stabilization and formulation obtained in this work for the C. guillermondii biosurfactant with the work presented by Mnif and Ghribi (2016), the function of an emulsifier is to stabilize the emulsion by controlling the clustering of globules and stabilizing aerated systems). The use of biosurfactants as emulsifiers occurs during the processing of raw materials to control the clustering of globules, stabilize aerated systems and improve the consistency of fat-based products (Santos et al., 2016).

There are few reports in the literature on the use of biosurfactants in the formulation of food products (Luna et al., 2016) successfully used a carbohydrate-rich extracellular compound produced by *C. utilis* as an emulsifying agent in salad dressing. Campos et al. (2014, 2015, 2019) wherein he produced an emulsifying potential biosurfactant using *C. utilis* in the formulation of a mayonnaise. As well, another authors (Szczurek et al., 2013) also report the use of biosurfactants in emulsions and mayonnaise. However, recent studies with others microorganisms have been related using biosurfactants in food industry (Zouari et al., 2016; Ribeiro et al., 2020a; Silva et al., 2020; Ribeiro et al., 2020b; Pinto et al., 2022).

## 5. Conclusion

The biosurfactant of a glycolipid nature produced by *C. guilliermondii* in a low- cost medium demonstrated promising properties and innocuousness in toxicity tests and can therefore, be used in in mayonnaise formulations. The best stabilizer was guar gum, in combination with the biosurfactant, these two substances conferred stability to the emulsion. The results obtained in this research demonstrate the feasibility of future studies focused on scale-up production of the biosurfactant, aiming its future and promising application new ingredient in the food industry.

### Acknowledgments

This work was supported by the following Brazilian fostering agencies: State of Pernambuco Assistance to Science and Technology Foundation (FACEPE), National Council for Scientific and Technological Development (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001 and National Electrical Energy Agency (ANEEL).

## References

Benassi, L., Alias, C., Feretti, D., Gelatti, U., Piovani, G., Zerbini, I., Sorlini, S. (2019). Ecotoxicity and genotoxicity of steel slags: preliminary results. *Detritus*. 32-38.

Bourdichon, F., Casaregola, S., Farrokh, C., Frisvad, J. C., Gerds, M. L., Hammes, W. P., Harnett, J., Huys, G., Laulund, S., Ouwehand, A., Powell, I. B., Prajapati, J. B., Seto, Y., Schure, E., Ter, Boven, A. V., Vankerckhoven, V., Zgoda, A., Tuijtelaars, S., Hansen, E. B. (2012). Food fermentations: Microorganisms with technological beneficial use. *International Journal of Food Microbiology*. 154 (3), 87–97.

Bhatia, V., Saharan, B. S. (2016). Research Article Isolation and partial Structural & Functional Characterization of Glycolipid Biosurfactant producing *Pichia sorbitophila* WG1 from Rotten Grapes. *Journal of Chemical and Pharmaceutical Research*. 8(7), 357–367.

Campos, J. M., Stamford, T. L. M., Sarubbo, L. A. (2014). Production of a bioemulsifier with potential application in the food industry. *Applied Biochemistry and Biotechnology*. 172(6), 3234–3252.

Campos, J. M, Stamford, T. L. M., Rufino, R. D., Luna, J. M., Stamford, T. C. M., Sarubbo, L. A. (2015). Formulation of mayonnaise with the addition of a bioemulsifier isolated from *Candida utilis*. Toxicology Reports. 2, 1164–1170.

Chandran, P., Das, N. 2011. Characterization of s*ophorolipid* biosurfactant. *International Journal of Natural Sciences*. 2(1), 63–71.

Campos, J. M., Stamford, T. L. M., Sarubbo, L. A. (2019). Characterization and application of a biosurfactant isolated from *Candida utilis* in salad dressings. *Biodegradation.* 30(4), 313-324

Daylin, R. R., Andrade, R. F. S. A., Goretti, S. S., Rodrigo, A. de H., Milagre, A. P., Patricia, N., Jose, C. V. J., Maria, A. de R. S., Campos-Takaki, G. M. (2017). Promising biosurfactant produced by a new *Candida tropicalis* UCP 1613 strain using substrates from renewable-resources. *African Journal of Microbiology Research*. 11 (23), 981–991.

Farias, C. B. B., Almeida, F. C., Silva, I. A., Souza, T. C., Meira, H. M., Rita de Cássia, F., Luna, J. M., Converti, A., Banat, I. M., & Sarubbo, L. A. (2021). Production of green surfactants: Market prospects. Electronic Journal of Biotechnology, 51, 28-39.

Geetha, S. J., Banat, I. M, Joshi, S. J. (2018). Biosurfactants: Production and potential applications in microbial enhanced oil recovery (MEOR). *Biocatalysis and Agricultural Biotechnology*. 14, 23–32.

Kishk, Y. F. M., Elsheshetawy, H. E. (2013). Effect of ginger powder on the mayonnaise oxidative stability, rheological measurements, and sensory characteristics. *Annals of Agricultural Sciences*, 58(2), 213–220.

Lee, B., Kim, J. K. (2001). Production of *Candida utilis* biomass on molasses in different culture types. *Aquacultural Engineering*. 25(2), 111–124.

Lira, I. R. A. S., Santos, E. M. S., Santos, J. C., Silva, R. R., Silva, Y. A., Durval, I. J. B., Jenyffer, M. C. G., Sarubbo, L. A., Luna, J. M. (2021). Production of Biosurfactant by *Candida Guillhermondii* and Application in a Mayonnaise Emulsion. *Chemical Engineering Science*. 87, 259-264.

Luna, J. M., Rufino, R. D., Sarubbo, L. A., Campos-Takaki, G. M. (2013). Characterization, surface properties and biological activity of a biosurfactant produced from industrial waste by *Candida sphaerica* UCP0995 for application in the petroleum industry. *Colloids and Surfaces B*, 102, 202–209.

Mallik, T., & Banerjee, D. (2022). Biosurfactants: The potential green surfactants in the 21st century. *Journal of Advanced Scientific Research*, 13(01), 97-106.

Marcelino, P. R. F., Gonçalves, F., Jimenez, I. M., Carneiro, B. C., Santos, B. B., Silva, S. S. (2020). Sustainable Production of Biosurfactants and Their Applications. *Lignocellulosic Biorefining Technologies*. 159–183.

McClements, D. J., Bai, L., Chung, C. (2017). Recent Advances in the Utilization of Natural Emulsifiers to Form and Stabilize Emulsions, Annual Review of Food Science and Technology, 8, 205–236.

Meyer, B. N., Ferrigni, N. A., Putnam, J. E., Jacobsen, L. B., Nichols, D. E., Mclaughlin, J. L. (1982). Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents. *Planta Medica*. 45(5), 31–34.

Meylheuc, T., Van Oss, C. J., Bellon-Fontaine, M. N. (2001). Adsorption of biosurfactant on solid surfaces and consequences regarding the bioadhesion of *Listeria monocytogenes* LO28. *Journal of Applied Microbiology*, 91(5), 822–832.

Mnif, I., Ghribi, D. (2016). Glycolipid biosurfactants: main properties and potential applications in agriculture and food industry. *Journal of the Science of Food and Agriculture*. 96(13), 4310-4320.

Nascimento, E. P., Campos, M. C. C., Alho, L. C. D., Weckner, M. P. F. C., Mantovanelli, B. C., Cunha, J. M. (2018). Growth of cupuaçu seedlings (*Theobroma grandiflorum*) under the effect of different biofertilizer compositions. *Revista Universidade Vale Rio Verde*. 15(2), 861-870.

Pacheco, M. V., Felix, F. C., Medeiros, J. A. D., Nunes, S. L., Nunes, M. L., Lima C., Silva-Lopes, A. L., Souza, W. M. A. T. (2017). Allelopathic potential of *Pityrocarpa moniliformis* leaf and fruit extracts on the germination of *mimosa caesalpiniifolia* seeds. *Revista Agroecossistemas*. 9(2), 250-262.

Pacwa-Płociniczak, M., Płaza, G. A., Piotrowska-Seget, Z., Cameotra, S. S. (2011). Environmental applications of biosurfactants: Recent advances. *International Journal of Molecular Sciences*. 12(1), 633–654.

Pinto, M. I. S., Campos Guerra, J. M., Meira, H. M., Sarubbo, L. A., & de Luna, J. M. (2022). A Biosurfactant from Candida bombicola: Its Synthesis, Characterization, and its Application as a Food Emulsions. *Foods*, 11(4), 561.

Ribeiro, B. G., Veras, B. O., Santos A. J., Guerra, J. M. C., Sarubbo, L. A. (2020a). Biosurfactant produced by *Candida utilis* UFPEDA1009 with potential application in cookie formulation. *Electronic Journal of Biotechnology*. 46, 14 – 21.

Ribeiro, B. G., Santos, M. M., Silva, I. A., Meira, H. M., Oliveira, A. M., Guerra, J. M. C., Sarubbo, L. A. (2020b). Study of the biosurfactant production by *Saccharomyces cerevisiae* URM 6670 using agroindustrial waste. *Chemical Engineering Transactions*. 79, 61–66.

Santos, D. K. F., Meira, H. M., Rufino, R. D., Luna, J. M., Sarubbo, L. A. (2017). Biosurfactant production from *Candida lipolytica* in bioreactor and evaluation of its toxicity for application as a bioremediation agent. *Process Biochemistry*. 54, 20–27.

Santos, D. K. F., Rufino, R. D., Luna, J. M., Santos, V. A., Sarubbo, L. A. (2016). Biosurfactants: Multifunctional biomolecules of the 21st century. *International Journal of Molecular Sciences*, 17(3), 401.

Sato, A. C. K. (2005). Influência do tamanho de partículas no comportamento reológico da polpa de jabuticaba, Campinas – SP, *Dissertação Mestrado*. 1, 86.

Shepherd, R., Rockey, J., Sutherland, I. W., Roller, S. (1995). Novel bioemulsifiers from microorganisms for use in foods. *Journal of Biotechnology*. 40(3), 207–217.

Silva, I. A., Veras, B. O., Ribeiro, B. G., Aguiar, J. S., Campos, J. M. G., Luna, J. M., Sarubbo, L. A. (2020). Production of cupcake-like dessert containing microbial biosurfactant as an emulsifier. *PeerJ*. 8, 9064.

Silva, R. R., da Silva, Y. A., e Silva, T. A. D. L., Sarubbo, L. A., & de Luna, J. M. (2022). Recent advances in environmental biotechnology: role of biosurfactants in remediation of heavy metals. *Research, Society and Development*, 11(5), e4411527453-e4411527453.

Singh, V. P. (2018). Recent approaches in food bio-preservation-a review. *Open Veterinary Journal*. 8(1), 104-111.

Szczurek, A., Fierro, V., Pizzi, A., Celzard, A. (2013). Mayonnaise, whipped cream and meringue, a new carbon cuisine, *Carbon - Journals*., 58, 245–248.

Tiquia, S. M., Tama, N. F. Y., Hodgkis, I. J. (1996). Effects pig-manure on phytotoxicity sawdust litter of spent. *Environmental Pollution*. 93(3), 249–256.

Wang, H., Roelants, S. L. K. W., To, M. H., Patria, R. D., Kaur, G., Lau, N. S., Lau, C. Y., Van Bogaert, I. N. A., Soetaert, W., Lin, C. S. K. (2019). *Starmerella bombicola*: recent advances on sophorolipid production and prospects of waste stream utilization. *Journal of Chemical Technology & Biotechnology*. 94(4), 999–1007.

Zouari, R., Besbes, S., Ellouze-Chaabouni, S., Ghribi-Aydi, D. (2016). Cookies from composite wheat-sesame peels flours: Dough quality and effect of *Bacillus subtilis* SPB1 biosurfactant addition. *Food Chemistry*, 194, 758–769.