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Study of Saccharomyces cerevisiae growth: screen the best conditions and evaluate the effects of wine and histamine.

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ABSTRACT

The rowanberries (Sorbus aucuparia, also called Tramazeira fruits in Portugal) are small orange-red "fruits" of a rowan tree, which are highly resistant to cold climates. They have been traditionally used in various processed foods due to their high relevance to human health. These berries have been described as an important source of phenolic compounds and a source of bacteria/yeasts, being suitable for the production of health-food products.

In this work, the microbiology quality of Tramazeira fruit samples was evaluated to select the one with higher microbial quality that could be used to isolate/select a yeast for further assays. After yeast identification as *Saccharomyces cerevisiae* (API method), an experimental design was applied to define optimal experimental conditions in a culture media for its growth. For this purpose, a fractional factorial design with 4 factors, 3 levels (including the center points) was applied varying the culture medium composition in glucose (10, 15 and 20 g/L), peptone (5, 7.5 and 10 g/L) and yeast extract (1, 3 and 5 g/L), as well, the inoculum quantity (10^5, 10^6 and 10^7 CFU/mL). The optimal culture media (standard media) had 10 g/L of glucose, 5 g/L of peptone, 1 g/L of extract yeast, and 10^7 CFU/mL of inoculum quantity, which allowed to obtain a growth value of 22.8 CFU, in logarithmic scale.

To test *Saccharomyces cerevisiae* growth, different matrices as grape juice or a mixture of grape juice and white wine (1:1), together with different concentrations of histamine (2, 5, 10, and 20 mg/L), were tested. It was shown that the *Saccharomyces cerevisiae* growth was delayed in the culture media with the presence of alcohol. Also, there was no evidence that histamine levels did affected the overall of *Saccharomyces cerevisiae* growth. Considering the overall results, there was a reduction in growth in the culture medium prepared with grape juice in comparison to the standard culture media (85.3% of the result obtained in the standard media), while for the culture medium with the mixture of grape juice and white wine, the growth was 67.0% of the standard media.

Keywords: Tramazeira fruit; culture media composition; *Saccharomyces cerevisiae* growth; grape juice; white wine; histamine.

RESUMO

A sorveira-brava (Sorbus aucuparia, também chamada de Tramazeira em Portugal), que produz uma pequena "fruta" laranja-avermelhada, é muito resistente aos climas frios. A Tramazeira têm sido tradicionalmente usada em vários alimentos processados devido à sua alta relevância para a saúde humana. As frutas têm sido descritas como uma importante fonte de compostos fenólicos e uma fonte de bactérias/leveduras, adequadas para a produção de produtos alimentares saudáveis.

Neste trabalho, a qualidade microbiológica do fruto da Tramazeira foi avaliada para selecionar a amostra com a maior qualidade microbiana que poderia ser usada para isolar/selecionar uma levedura para testes posteriores. Após a identificação da levedura como *Saccharomyces cerevisiae* (método API), um delineamento experimental foi aplicado para definir o meio de cultura ótimo para o seu crescimento. Para isso, foi aplicado um desenho fatorial fracionário com 4 fatores, 3 níveis (incluindo os pontos centrais), variando a composição do meio de cultura em glicose (10, 15 e 20 g/L), peptona (5, 7,5 e 10 g/L) e extrato de levedura (1, 3 e 5 g/L), bem como a quantidade de inóculo (10^5, 10^6 e 10^7 UFC/mL). O meio de cultura ótimo (meio padrão) continha 10 g/L de glicose, 5 g/L de peptona, 1 g/L de extrato de levedura e 10^7 UFC/mL de quantidade de inóculo, o que permitiu obter um valor de crescimento de 22,8 UFC, na escala logarítmica.

Para testar o crescimento de *Saccharomyces cerevisiae*, diferentes matrizes, como sumo de uva ou uma mistura de sumo de uva e vinho branco, com diferentes concentrações de histamina (2, 5, 10 e 20 mg/L), foram testados. Foi demonstrado que o crescimento de *Saccharomyces cerevisiae* foi retardado em meios de cultura com presença de álcool. Além disso, não houve evidências de que os níveis de histamina afetaram o crescimento geral da *Saccharomyces cerevisiae*. Considerando os resultados gerais, houve redução no crescimento do meio de cultura de sumo de uva (85,3% do resultado obtido com o meio de cultura padrão), enquanto para o meio de cultura de mistura de sumo de uva e vinho branco, o crescimento foi de 67,0% do obtido com o meio padrão.

Palavras-chave: Fruta da Tramazeira; composição de meios de cultura; crescimento de *Saccharomyces cerevisiae*; sumo de uva; vinho branco; histamina.

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1. GENERAL INTRODUCTION

During the past few decades, search, and development for novel functional microorganisms have become a topical issue for researchers, due to their economic value associated with their application. Plants may serve as an excellent platform for discovery of new microorganisms which is particularly interesting in the era of functional foods, fermentations, nutraceuticals, and personalized nutrition.

Overall, microorganisms are also important in various fermented foods such as wine, cheese, beer, vinegar, bread and in the production of industrially important acids, solvents, antibiotics, enzymes, etc. It should be noted that yeasts are the most widely used micro-organisms in the food industry due to their ability to ferment sugars to ethanol and carbon-dioxide.

In this work, the fruit of Rowanberries was used to isolate and select a yeast. This selected yeast was studied, under an experimental design, in order to establish an optimal culture media for its growth. It was intended to establish a standard growth rate for comparative purposes when the yeast growths in different culture media, as in the grape juice or a mixture of grape juice and white wine. And, as well, to verify the effect of the presence of a biogenic amine (histamine).

1.1. Rowanberries (*Sorbus aucuparia L.*)

The *Sorbus spp.* (common names rowans, whitebeams, and others) are deciduous shrubs or trees, which although being widely grown in the gardens and parks, are underutilized plants in terms of their applications as foods, nutraceuticals and/or cosmeceuticals. The rowans are the most widely studied Sorbus spp. Wild rowan trees are tolerant to harsh Nordic climate and poor growing environments such as rocky and windy slopes and even the mountains and may reach up to 15 m height.

The rowan berry s is the main product of the rowan's trees, but other parts also contain valuable phytochemicals; as bark, leaves, and inflorescences (Figure 1) which have been empirically used in folk medicines for centuries. The bark of the Sorbus trees is mostly smooth, lustrous, dark, with elongated horizontal lenticels; the leaves are pinnately compound, the leaflets toothed or rarely entire, while the inflorescences may be extra-large, convex panicles (Robertson et al., 1991).

The genus *Sorbus* belongs to the *Rosaceae* family and includes more than 250 species distributed mainly in temperate regions of the Northern Hemisphere. It is thought that it might have originated in East Asia and then migrated to other temperate areas such as Europe, North America, and North Africa. The main hubs for species diversity are the Himalayas and Western China, and secondarily the Caucasus. In the case of Europe, *Sorbus* species are mainly concentrated in the central region, comprising Czechoslovakia, Hungary, and Germany; as well, as in Greece and Portugal (Aldasoro et al., 1998), which can be observed in Figure 2.

Figure 1. Tree of rowan berries (*sorbus aucuparia L.*)

Its distribution is related to geographic regions with high summer temperatures, so that enough water is available. Rowan has a low tolerance to flooding, which results in reduced growth (Raspé et al .2000). It has been proven capable of enduring a high level of stress caused by mechanical damage, having fast wound regeneration, which helps protect compromised tissues from desiccation and pathogen invasion (Woodward et al., 1996). Also, it can stand the combination of stress caused by winter desiccation and a short growing season, since it can thrive and still produce viable seeds at higher altitudes than many other trees (Barclay et al., 1984).

The copious genus *Sorbus L.* (*Rosaceae, Maloideae*) covers up to 250 species, which in addition are divided into 6 subgenera, namely *Sorbus, Aria*, *Micromoles*, *Corms*, *Tomi aria*, and *Chama Mespilus*. According to Robertson et al (1991), the bitter fruits of wild rowan are round, and present several colors such as red, orange, yellow, pink, or white.

Usually, people consume rowanberries in small amounts as a mash to improve the appetite and stimulate production of gastric acid. In folk medicine these fruits have been used as a laxative, against rheumatism and kidney diseases, and gargle juice against hoarseness (Miletic et al., 2012). Rowan berries have been considered as diuretic, vasodilatory, anti-inflammatory, anti-diarrheal remedies, and a source of ascorbic acid (vitamin C); in some countries they also have been used for treating intestinal obstructions, various liver, and gallbladder diseases (Fomenko et al., 2016).

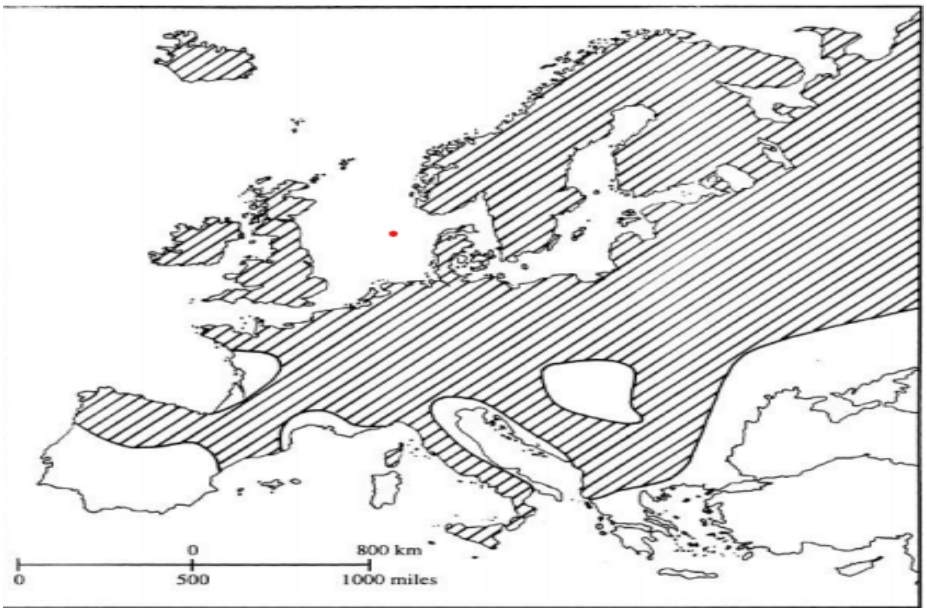

Figure 2. The European distribution of *Sorbus aucuparia* based on information in the literature (Taken from Raspé et al. 2000).

The leaves have sometimes been used to feed livestock while the fruits have been administered to domestic pigs and goats against bacterial infections. United States Department of Agriculture (USDA) database (2020) has a list showing the genus *Sorbus* that can be used in animal feeding.

1.2. Safety and quality analysis of fresh fruit

Since the fresh fruit is a favorable environment for the growth of microorganisms, it is important to verify its quality by verifying the presence of pathogenic and spoilage bacteria as: *Escherichia coli*, total mesophiles and coliform, *staphylococcus aureus* and yeast/molds.

Escherichia coli. The bacterium *E. coli* belongs to the family of Enterobacteriaceae. It is a Gram-negative rod-shaped bacterium, non-sporulating, nonmotile, or motile by peritrichous flagella, chemoorganotrophic, facultatively anaerobic, producing acid from glucose, catalase-positive, oxidase negative, and mesophilic. It is a well-known commensal bacterium that is among the first colonizing bacteria of the gut after birth. It is a highly successful competitor in the human gut and is the most abundant facultative anaerobe of the human intestinal microbiota (Kaper et al., 2004). As it is a facultative anaerobe, it survives when released to the environment and can be spread to new hosts. *E. coli* is thus an important component of the biosphere (Van et al 2010). Even though *E. coli* is a well-known commensal bacterium, many pathogenic strains of E. coli do exist. Several highly adapted *E. coli* clones have acquired specific virulence factors, which confer an increased ability to adapt to new niches and allow them to cause a broad spectrum of disease, and intestinal and extraintestinal infections (Kaper et al., 2004).

Total mesophiles. The mesophilic microorganism is one of the more general and extensively microbiological indicators of food quality, indicating the adequacy of temperature and sanitation control during processing, transport, and storage, and revealing sources of contamination during manufacture.

Total coliforms. Total Coliforms are a group of bacteria commonly found in animal waste, sewage, soil, and fruits. They are also found in the intestines of animals and humans. Total Coliforms are not likely to cause illness, but their presence indicates that the food may have been contaminated by more harmful microorganisms' total coliforms refers to a large group of Gram-negative, rod-shaped bacteria that share several characteristics. The group includes thermotolerant coliforms and bacteria of fecal origin, as well as some bacteria that may be isolated from environmental sources (Boehm, 2003).

Staphylococcus aureus. *S. aureus* cells are Gram-positive and appear in a spherical shape. They are often in clusters resembling a bunch of grapes when observed under a light microscope after Gram staining. The name 'Staphylococcus' was derived from Greek, meaning a bunch of grapes (staphyle) and berry (kokkos). It's a causative agent of a wide range of infectious diseases such as skin infections, bacteremia, endocarditis, pneumonia, and food poisoning. The organism was

originally a leading nosocomial pathogen and afterwards epidemiologically distinct (Tong et al., 2015).

Yeast. Yeasts are unicellular eukaryotic fungi with a particle size of 5 × 10 µm and play various roles in affecting the quality and safety of food products. They are ubiquitous, and commonly cause spoilage of fruits, vegetables, and other plant materials (Bouix et al., 1991). They reproduce mainly by budding or by binary fission (Kreger-Van, 1984) (Figure 3). The yeasts are clearly differentiating bacteria by their eukaryotic cell structure (Guiraud, 1998).

The yeast part of this work is the focus being expected to isolate one kind of yeast to study the best conditions of growth and verify if it can reduce/remove histamine contents in food products.

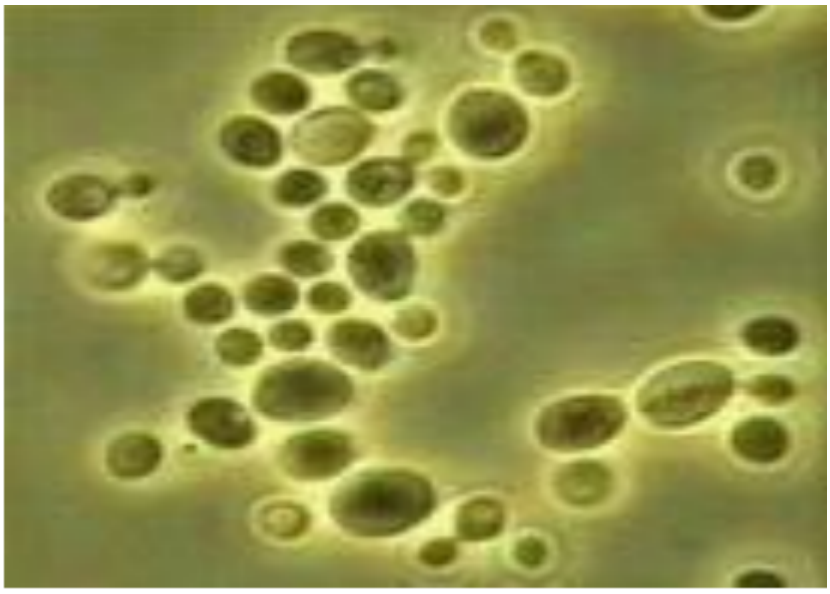

Figure 3. Division of yeast cells by budding (Kwon et al., 1992)

1.2.1. The yeast

Yeast are single celled microorganisms that can produce enzymes, carbon dioxide (CO_2), and other metabolites from carbohydrates, whose functional roles are frequently used in the processes of fermentation, baking, flavoring foods, adding nutritional value, and providing health benefits (Evans et al., 2000; Dubey et al., 2010).

1.2.1.1. Properties of yeast

As referred previously, yeasts are single celled eukaryotic microorganisms in the kingdom Fungi (Bennett, 1998; Ingraham et al, 2010), with a nuclear membrane and cell walls. Fungi lack chloroplasts, unlike plants, and are heterotrophs, a type of organism that consumes living and dead organic materials for energy and food (Bennett, 1998). Fungi accomplish this task by releasing proteolytic, glycolytic, or lipolytic enzymes to digest organic matter, or by absorbing small molecules such as amino acids and simple sugars through their cell wall (Baron, 1996). The Kingdom Fungi includes both macroscopic organisms such as mushrooms, and microscopic organisms such as yeast and mold (Ingraham et al, 2010). Yeasts exist as individual cells instead of forming filamentous vegetative cells called hyphae, which can be interconnected with other cells as a multicellular body as molds and mushroom fungi (Baron, 1996). Yeast species are generally grown under aerobic (presence of oxygen) conditions and fermented under anaerobic (lack of oxygen) conditions, which in the presence of sugar produces ethanol (Bekatorou et al., 2006).

1.2.1.2. Nutritional requirements

Yeast, like any other heterotrophic microorganism, requires the absorption of several organic (carbon and nitrogen compounds) and mineral compounds to survive. These substances are used for the development of cellular constituents or the maintenance of physiological activities. Also, the enzymatic systems implemented for the production and use of energy depend on the nature and the chemical composition of the assimilated substrate.

Carbon, one of the main components of organic matter, represents up to 50% of the dry weight of the yeast cell (Rivière, 1970). The most widely used precursors are hexoses, their dimers, and oligomers (Spencer et al., 1997). Certain groups can assimilate compounds of very varied chemical nature. Yeasts can assimilate organic acids (acetic, lactic, tartaric, malic, succinic, citric acids, etc.) as a carbon source. Polyhydric alcohols, alkanes, fatty acids and their esters, triglycerides and methanol can also be metabolized by some groups of yeast (Phaff et al., 1979; Tanaka et al., 1989).

Nitrogen is part of the structure of essential components of a living organism. However, unable to fix free nitrogen, yeast must assimilate it in its oxidized or reduced forms (Lammi,

2011). Many species exploit the nitrogen incorporated in organic matter such as amino acids, purines, and pyrimidines (Larue et al., 1968). Nitrites, nitrates, and urea can also be used.

Phosphorus, an essential element in the synthesis of nucleic acids, is taken up by yeast in its inorganic forms. Sulfur, entering the composition of certain amino acids, is assimilated in the form of sulfite and thiosulfate. Certain mineral elements acting at low doses are involved in many physiological activities (Boiron, 1996) and are therefore absorbed in tiny amounts. Walker (2000) mentions the effect of magnesium, manganese and zinc which are involved in membrane stability, the protection of the cell against negative factors and the synthesis of proteins, thiamine, and certain coenzymes. Among the essential elements are K^{+}, Na^{+}, Li^{+}, Cs^{+}, Rb^{+}, Co^{2+} (Pommier, 2003). However, these mineral elements are toxic to yeasts at high concentrations.

1.2.1.3. Yeast growth

Yeast population growth studies require inoculation of viable cells of a pure culture into a sterile medium and incubation of the culture under optimally controlled environmental conditions. The cells normally reproduce rapidly, and the dynamics of the microbial growth is charted by means of a population growth curve, which is constructed by plotting increase in cell number, metabolic activity, or absorbance against time of incubation. The curve can be used to delineate the stages of the growth cycle and facilitate measurement of cell numbers and growth rate of yeast as expressed by its generation time (Figure 4), thus the time required for a microbial population to double.

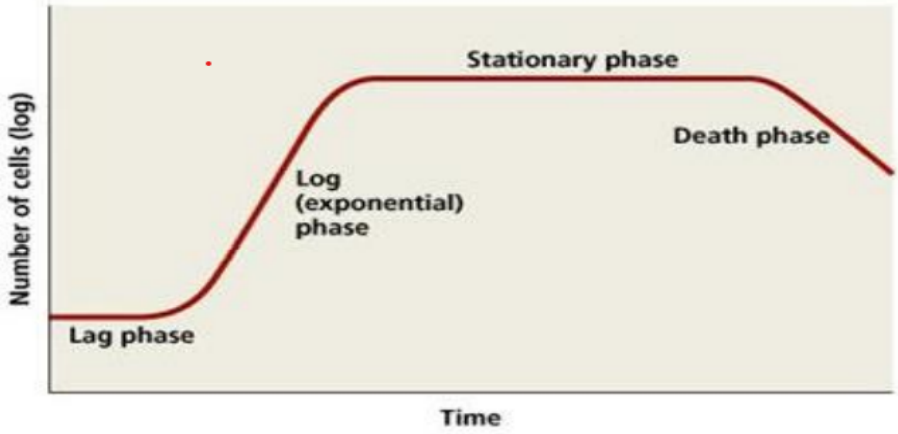

Figure 4. Yeast growth curve characterization (Yates et al., 2007).

The stages of a typical growth curve comprise lag phase (1), log (exponential) phase (2), stationary phase (3) and death (decline) phase (4). Each of these phases represents a distinct period of growth that is associated with a typical physiological change in the cell culture (Yates et al., 2007).

Phase 1. During lag phase yeast adapt to the media and prepare cellular machinery to grow. In this stage cells are not able to divide yet.

Phase 2. Exponential phase (log phase) is characterized by cell duplication. This phase may be characterized mathematically by doubling time (time necessary to double population) or by growth rate (which is a measure of the number of divisions per cell per unit time).

Phase 3. During the stationary phase the population remains stable and happens due to a limiting nutrient or an inhibition factor. Stationary phase results from a situation in which growth rate and death rate are equal.

Phase 4. In dead phase growth media nutrients become exhausted or toxic compounds reach critical concentration and cells die.

1.2.1.4. Identification of yeasts

The identification of yeast can be carried out by morphological and molecular identification. For the morphological, the observations of colony morphology constitute an important identification criterion for fungi and yeasts, where the main characteristics evaluated are shape (circular, irregular, scattered), height (flat, slightly raised, or markedly raised), pigmentation (red, white, pink, colorless), size (pinpoint, small, medium, large), and texture (Odutayo et al., 2004). The molecular characteristics of yeasts include examining the genetic material of the organisms. Molecular methods used to identify organisms from organisms include polymerase chain reaction (PCR), nucleic acid hybridization, and fingerprinting of plasmids (Leggat et al., 1994). These methods have revolutionized the identification and classification of yeasts due to their speed and accuracy.

1.2.1.5. Fermentation process

Microorganisms used in fermentation convert various substrates to precious products (such as industrial chemicals and fuels). The success of these microbes lies in their genetic nature. They possess specialized enzymes that are capable of metabolizing substrates. They break down a variety of substrates into different metabolites with concomitant production of heat and gases. Presently, bacteria and fungi are widely used in industries to produce useful chemicals and gases such as ethanol, acetic acid, methane and H_2 (Huang et al., 2007). Bacteria are used mostly because of their potential to be genetically engineered and their high growth rate (Wackett, 2008).

Fungi have been used industrially to produce value-added products such as steroid hormones and several antibiotics. Huang et al. (2007) study referred several practical applications of fungi yeast, which are representative of its present economic impact (Table 1). Also, it shows that yeast have a wide range of applications, giving reason to isolate and study new microorganisms that can bring added economic value. One example of an important yeast used in food area is *Saccharomyces cerevisiae*.

Table 1. Industrial applications of fungi/yeast (Huang et al., 2007)

Applications	**Examples**
Baking and brewing	Bread, beer, wine, spirits
Bio-based fuels	Bioethanol from sucrose, glucose, and xylose
Bioremediation	Heavy metal removal, wastewater treatment
Chemicals	Glycerol, bio-surfactants, enzymes, organic acids
Healthcare	Human therapeutic proteins, steroid hormones
Nutrition and animal feed	Biomass, polysaccharides, vitamins, single cell proteins

Saccharomyces cerevisiae is a unicellular organism, with two full sets of chromosomes completely sequenced (Landry et al., 2006; Huang et al., 2007), which is found in soils and fruits (Naumov et al., 2003). It has been used for baking, brewing and wine making (Landry et al., 2006; Huang et al., 2007). *S. cerevisiae* is unable to use pentose sugars as its sole energy

source (Gray et al., 2006; Katahira et al., 2008). This fungus has been used in industrial-scale production of ethanol (Huang et al., 2007), however, with low tolerance to high temperatures (above 35 °C). In ethanol presence, *S. cerevisiae* has been reported to be more tolerant to inhibition than utmost bacterial species (Edgardo et al., 2008). Selection of new species that grows between 40 to 45 °C (selection of survivors after the heat shock process), have been lately researched (Rikhvanov et al., 2001; Edgardo et al., 2008). Fermentation study require the evaluation of the best conditions for the microorganism's growth, which generally implies the evaluation of several parameters such as, glucose, peptone and extract yeast concentrations, as well as, the inoculum quantity.

1.2.1.6. Optimal yeast growth

Various studies have been carried out in order to know the effect of the compositions of the culture media on the growth of yeasts. For instance, the study of Jonathan et al. (2001) was to verify the impact of effect of carbon, nitrogen and mineral sources on growth of *Psathyerella atroumbonata*, showing that growth was enhanced by glucose and yeast extract compared to the other source of carbon and nitrogen used. Krahulec et al. (2021) studied the effect of the different suppliers of the components of the medium in the *Pichia pastoris* growth. It was shown that peptone had the highest impact on *Pichia pastoris* production where the difference between best and worst results was triple. The results demonstrated that media components from different vendors have a high impact on the growth of Pichia pastoris.

The optimization on the sources of culture media components for a microorganism growth, one of the aims to study in this work, usually use an experimental design to select the variables that contribute to increase the growth of a given microorganism. For example, Beigbeder et al. (2021) used an experimental design to better understand the effect of three process parameters (concentration of nutrient, yeast, and initial sugar) on the ethanol productivity using diluted sugar beet molasses and *Saccharomyces cerevisiae* yeast. Also, Sejong et al (1995) studied the yeast growth in order to find the optimum conditions of tryptone, yeast extract, glucose, Tween 80, and incubation temperature for the growth of *Lactobacillus casei YIT 9018* and to assess the effects of these factors. Effects involving incubation temperature, yeast extract, glucose, and tryptone were significant, whereas the only significant effect involving Tween 80 was the interaction effect between temperature and Tween 80. It

turned out that growth of *L. casei* YIT 9018 was most strongly affected by the incubation temperature.

The study of Chan et al (2002) optimized the composition of the medium for bacteriocin production by *Lactococcus lactis* ATCC 11454 by studying six factors (sucrose, soy peptone, yeast extract, KH_2PO_4, NaCl and $MgSO_4 \cdot 7H_2O$). Sucrose, soy peptone, yeast extract, KH_2PO_4 se were found to be important factors and had positive effects on cell growth, however, only soy peptone and KH_2PO_4 were found to be the two important factors for bacteriocin production and had negative and positive effects, respectively.

Considering the above mentioned, the parameters glucose, peptone, extract yeast and the inoculum quantities are important to establish a model that could explain how they influence a yeast growth. So, in this work, the first task was to apply an experimental design to establish the yeast behavior when changes are introduced in the culture media. Also, as a preliminary analysis of how yeast behaves in different culture media and for comparison purposes, it was studied the yeast growth in grape juice and in a mixture of grape juice and white wine. The histamine (toxic substance) was also introduced in the assays to evaluate its effects.

Histamine is a biogenic amine that is also present in grape juice and wine. Figure 5 shows its molecular structure:

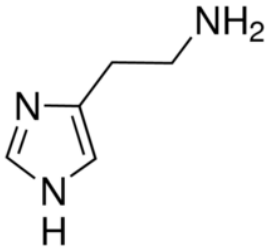

Figure 5. Histamine molecular structure

Histamine is a chemical created in the human body that is released by white blood cells into the bloodstream when the immune system is defending against a potential allergen and it can result in an allergic reaction from allergy triggers such as pollen, mold, and certain foods. Foods that are fermented, aged, or overly processed likely contain more histamine than fresh foods. So, it is important to discover microorganisms that can reduce the number of histamines in processed food products (Vidal-Carou et al., 1990; Lee et al., 2016). Some symptoms of histamine intolerance are, for instance, headaches or migraines, nasal congestion or sinus issues,

fatigue, digestive issues, irregular menstrual cycle, nausea and vomiting (Comas-Basté et al., 2020).

The evaluation of the yeast growth in time (0, 24, 48, 72 and 120 hours) was done by using, as response, the concentrations of glucose and optical density (measured at 640 nm). Figure 19 shows the changes in the optical density of *Saccharomyces cerevisiae*, as well as the glucose concentration in the two different culture mediums with added histamine.

The results from the different concentrations of histamine in the culture mediums were consistent. However, the growth inhibition of *Saccharomyces cerevisiae* in culture mediums which contain white wine was verified. The sample that contains 20 mg of histamine in grape juice was the first that achieved the stationary phase in less than 24 hours followed, in descending order, by the sample that contain 10 mg of histamine in grape juice, 5 mg of histamine in grape juice and 2 mg of histamine in grape juice

1.3. Experimental design for variable screening

The works for establishing the best growth conditions usually use experimental designs for variable selection in order to establish medium composition (Chan et al., 2002; Beigbeder et al., 2021). Several experimental designs were used as the central composite design (CCD) or fractional factorial design (FFD). The purpose is to reduce the number of experiments but with the purpose to cross the effects of the variables under study and obtain general information on the influences of the variables on the analyzed response. This procedure allows to identify the variables that are a significant influence on the desired response and to determine the appropriate ranges within which the variables change.

As example, Beigbeder et al. (2021) studied the production of ethanol with the *Saccharomyces cerevisiae* yeast from non-treated sugar beet molasses using a CCD to establish 16 assays to investigate the effect of three fermentation process parameters (initial sugar, yeast, and nutrient concentrations) on ethanol productivity while considering several operating parameters such as ethanol yield and sugar utilization rate. The experimental design used 3 levels (minimum, center and maximum) for sugar concentrations (125, 225 and 325 g/L), yeast concentrations (0.2, 0.6 and 1.0 g/L) and nutrient concentration (0, 2 and 4 g/L). By applying response surface methodology (RSM), a second-order mathematical model was obtained to evaluate its ability to make accurate predictions based on specific desired process outputs.

Also, Sejong et al. (1995) optimized the conditions for the growth of *Lactobacillus casei* YIT 9018 in tryptone-yeast extract-glucose medium using a CCD with 50 assays (2 blocks with center points) with the factors tryptone, yeast extract, glucose, Tween 80, and temperature. Five levels (design with 2 blocks) were considered for each factor: tryptone (0, 1.155, 2, 2.845 and 4 g/L), yeast extract (0, 0.404, 0.7, 0.996 and 1.4 g/L), glucose (0, 1.443, 2.5, 3.557 and 5 g/L), Tween 80 (0, 0.058, 0.1, 0.142 and 0.2 g/L) and temperature (25, 31.9, 37, 42.1 and 49 ºC). The response variable was the logarithmic number of viable cells.

As an example of FFD application, the work of Chan et al. (2002) identified which ingredient(s) of the medium has a significant effect on nisin production (bacteriocin product) using the *Lactococus lactis*. Since fractional factorial designs are very useful in identifying the important nutrients and interactions between two or more nutrients in relatively few experiments as compared to the CCD. Six ingredients (sucrose, soybean peptone, yeast extract, KH_2PO_4, NaCl and $MgSO_4$) with 2 levels in culture medium established a number of 16 assays to be used (with center points). The 2 levels (minimum and maximum) used were: 5 and 10 g/L for sucrose, soybean peptone, yeast extract, KH_2PO_4 and NaCl; 1 and 3 g/L of $MgSO_4 \cdot 7H_2O$.

The mathematical models obtained by fitting the response variable to the factors under the study can be a linear model (Chan et al., 2002) with only the main factors or can consider some interaction effects or a polynomial model (Sejong et al., 1995; Beigbeder et al., 2021), depending on the number of degrees of freedom.

OBJECTIVES

Overall, this study pretends to isolate a yeast from the fruit of Tramazeira tree to apply an experimental design to establish an optimal culture media for its growth and compare its standard growth behavior with other culture medium as, grape juice or a mixture of grape juice and white wine, both with different levels of added histamine. The purpose was to test a new isolated microorganism in order to give an added value to Tramazeira (*Sorbus aucuparia L.*) fruits, since it could be used in several scientific areas, as the food area.

The present study had the following specific objectives:

1. Quality microbiological characterization of Tramazeira (*Sorbus aucuparia L.*) fruits located in Bragança (Northeast of Portugal): mesophilic aerobic, mold, yeast, coliform, fecal and salmonella detection/count;
2. Isolation and growth of yeasts;
3. Selection of a yeast;
4. Application of an experimental design for variable selection and to establish the optimal experimental conditions for the selected yeast growth;
5. Verification of the influence of grape juice, white wine and histamine in the yeast growth.

2. MATERIAL AND METHODS

2.1. Preparation of samples

The fruits of Tramazeira tree were collected in September in Bragança (Polis park, Portugal) in 5 different trees, having obtained five samples (Figure 6). In the laboratory of IPB, the separation of the fruit from the leaves were done immediately in a fume hood to avoid the contamination of fruit, while doing the microbiology analysis. The 5 samples were used directly for the preparation of the samples for the quality microbiological characterization.

Figure 6. Tramazeira fruit on the tree and samples taken from 5 different trees

2.2. Microbiological control

The objective of the microbiological control was to verify the quality and safety of the Tramazeira fruits that were collected. For this, the fruits were analysed in order to detect the presence of microorganisms: total mesophilic, yeast and molds, total coliforms, *Escherichia coli* and *staphylococcus aureus*.

Sample preparation. The mass of 10 g of each Tramazeira sample (each sample was collected from 5 different trees) was aseptically taken and homogenized using a Stomacher for 2 min with 90 mL of sterile peptone water (Figure 7 and 8). Decimal serial dilutions were prepared from this homogeneous in the same sterile diluents (1:10, w/w). The purpose of this step is also

used for the pre-enrichment prior to the selective enrichment phases and to revive the microorganisms also used to perform the dilutions for the microbiological examination.

Figure 7. Stomacher machine

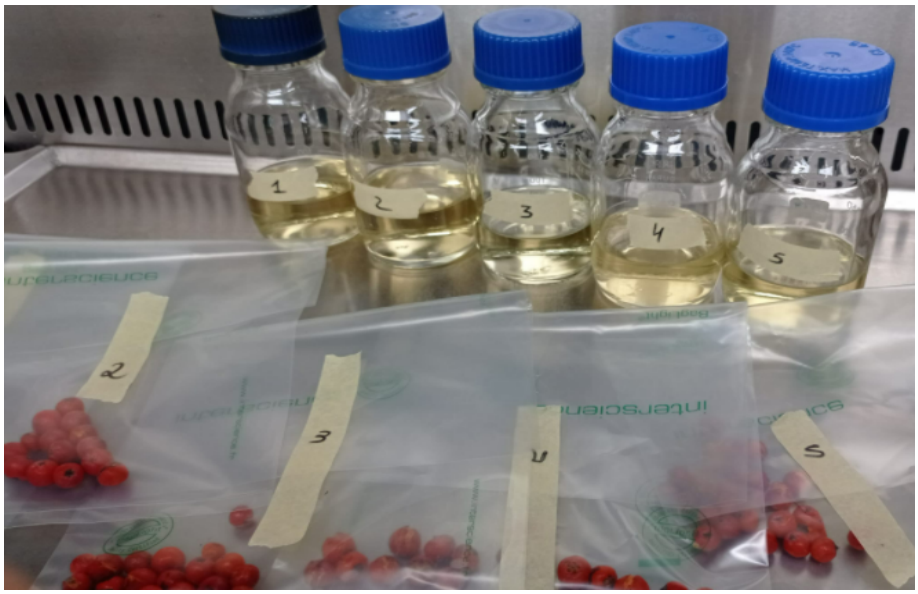

Figure 8. Samples diluted with peptone water

Total mesophilic microorganisms. The aerobic mesophilic microorganisms were counted by incorporation of 1 mL of each dilution into standard Plate Count Agar (PCA-DIFCO) as shown in Figure 9, as recommended in NP-3788 (2002) – Portuguese Legislation. The incubation was carried out in an oven at 30°C and 72h. Microbial counts were expressed as colony-forming units per gram of sample (CFU/g).

$$\frac{CFU}{g} = \frac{\sum C}{V * (n_1 + 0{,}1n_2) * d}$$

Where:

Σc is the sum of the colonies on all counted plates;

V is the volume of the inoculum sown in each plate;

n1 is the number of plates from the first counted dilution;

n2 is the number of plates from the second counted dilution;

d is the dilution from which they were eliminated as first counts.

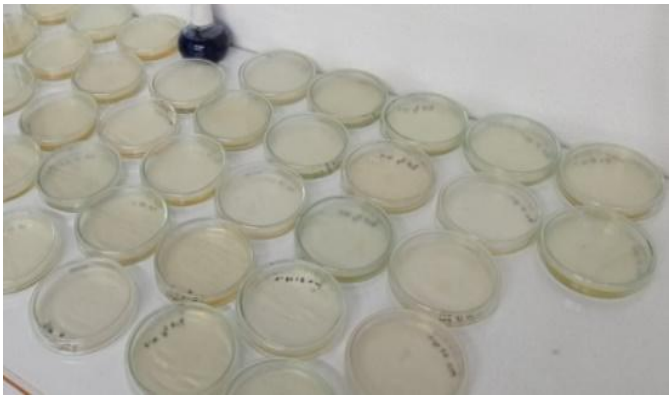

Figure 9. Examples of plates contain PCA

Yeast and molds. Molds and yeasts enumeration were made in Rose Bengal medium (VWR Chemicals) and incubated at 25ºC for 5 days as shown in Figure 10 Dilutions were inoculated by surface spreading, using a sterilized glass spreader. Microbial counts were expressed as colony forming units per gram of sample (CFU/g).

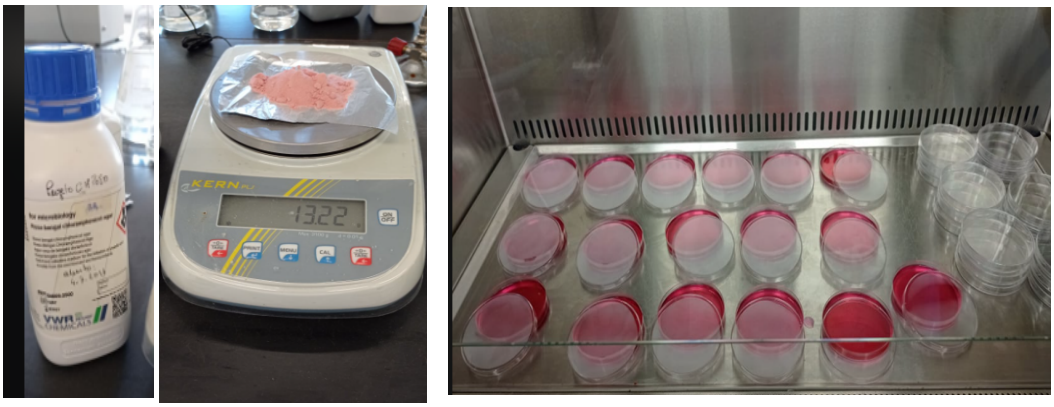

Figure 10. Preparation of Rosa Bengal

Total coliforms and *Escherichia coli*. The counting of Coliforms and *Escherichia coli* was done using the SimPlate CEc-CI method (AOAC® Official Method 2005.03) with multiple test medium (BioControl System), 1 ml from the basic dilution was placed in the center of the SimPlate plating device, and 9 ml of a mixed nutrient agar with blue color was added at the same spot. The SimPlate was rotated to disperse the sample and remove air-bubbles. The SimPlates were stacked and stored at 37±1 ºC for 24–28 h. Wells were counted positive for total coliforms based on the color change and counted positive for *E. coli* based on color change and fluorescence under UV light (365 nm). The coliform and *E. coli* populations were determined based on the number of positive wells correlated with the SimPlate conversion table. The results are expressed in colony forming units per gram for sample (CFU/g).

Figure 11. Examples of Simplate

Staphylococcus aureus. The detection was performed according to the protocol of NP 4400-1 (2002). Serial dilutions of the sample were inoculated in Baird-Parker Broth with Egg Yolk Tellurite (Himedia) during 24 h at 37ºC. After, it was selected 3-5 characteristic colonies, to testify the presence of coagulase and catalase with rabbit plasma. Microbial counts were expressed as colony-forming units per gram (CFU/g).

2.3. Yeast isolation and identification

2.3.1. Isolation of endophytic fungi

The fungi were obtained by imprinting the intact tramazeira fruit samples gently on Potato Dextrose Agar (PDA, Difco) plates supplemented with 0.01% (w/v) chloramphenicol (Oxoid, Basingstoke, Hampshire, UK). After surface sterilization of fruit tissues. Surface sterilization was performed through sequential immersion of fruits in 70% (v/v) ethanol (1 min), 3% (v/v) sodium hypochlorite (2 min), 70% (v/v) ethanol (1 min), and further rinsed three times with sterile distilled water (one min each). After being dried, each fruit was cut into five segments (ca. 5 × 5 mm) and transferred into the same medium used to isolate epiphytes (PDA). Therefore, the samples were inoculated. Validation of the surface sterilization procedure was done by imprinting the surface sterilized fruit tissues onto PDA media. Plates were incubated at 25 ± 2 °C in the dark and were daily observed for microbial growth and colonies counting.

2.3.2. Identification of yeasts

API 20 C AUX Yeast is the identification system consists of 20 wells containing 19 different carbon source substrates and negative control. The system has been used in accordance with the manufacturer's instructions, except for modifications made to suit the slower, more filamentous growth of some of the dematerialized fungi. The modifications included an extended culture incubation time 8-10 days, unlike 2-3 days required for yeasts, and indirect (versus direct for yeasts) technique for preparing the inoculum, as described below. However, only the 3-day readings were used for the final analysis. To prepare the incubation box, 5ml of distillate water was put into the honey combed wells to create a humid atmosphere. After that, the strip from each individual packaging was removed and placed in the incubation tray.

Using an inoculation loop, a portion of yeast colony from a young culture (18-24 hours) was picked up into a test tube with 2 ml of NaCl at 85%. The suspension with a turbidity equal to 2 McFarland must be used immediately after preparation. Take 100 µl of the previous suspension and mix with the API C ampoule, and homogenization using a pipette to avoid bubble air formation. From the inoculation of the strip, the couples were filled with the

suspension obtained in the ampoule API C medium. Place the incubation box at 30°C for 48-72 hours.

2.4. Optimization of the growth yeast conditions

After isolation and identification of the yeasts from Tramazeira fruit, a yeast was selected based on its abundance, having been identified as the yeast *Saccharomyces cerevisiae*. The next step was to apply an experimental design, using a software R, in order to know the best conditions of culture medium for its growth.

A fractional factorial design with 4 factors, 3 levels and 3 center points was applied to establish 11 assays to assess yeast growth, where the composition of the culture mediums varied in glucose (10; 15 and 20 g/L), peptone (5; 7,5 and 10 g/L) and yeast extract (1; 3 and 5 g/L), as well, the inoculum quantity (10^5; 10^6 and 10^7 CFU/mL) as shown in the Table 2. The yeast selected was put grown in the standard liquid medium 24 hours before using.

Table 2. Different conditions for pre-inoculum preparation established by a fractional factorial design.

Assay	Glucose, g/l	Peptone, g/l	Extract Yeats, g	Inoculum, g/l
1	15	7,5	3	10^6
2	20	5	1	10^7
3	20	10	1	10^5
4	10	10	5	10^5
5	20	10	5	10^7
6	15	7,5	3	10^6
7	10	10	1	10^7
8	20	5	5	10^5
9	10	5	1	10^5
10	10	5	5	10^7
11	15	7,5	3	10^6

The preparation of the different solutions was done in Erlenmeyer flasks (250 ml). After measuring the different compositions of the pre-inoculum, 150 ml of distilled water was added, and the flasks were covered. Posteriorly, the flasks were put in the autoclave to sterilize at 121 °C for 15 min. The Erlenmeyer flasks properly identified need to be cold (room temperature), to inoculate the yeast. The final step was putting the flasks in the incubator at 25°C with agitation (Figure 12).

Figure 12. Synthetic liquid medium.

The follow up of the yeast growth was monitored daily, until its decline phase, by measuring the change of the Erlenmeyer's weight, colony forming units (CFUs) and optical density (UV-3100PC Spectrophotometer) at 640 nm.

The objective of this task was to verify the optimal conditions for the yeast growth. The results will allow us to carry out the next work, which is to compare its behavior in culture media of grape juice and in a mixture of grape juice and white wine, with the presence of the histamine compound at different concentrations.

2.5. Fermentation study

Preparation of the solutions. Different solutions were made in sterilized Erlenmeyer flasks, with the variation of the concentration of histamine (2 mg/L, 5 mg/L, 10 mg/L and 20 mg/L) in the grape juice (100 mL) and in mixture of grape juice and white wine, in proportion 1:1 (v/v). Sulfur dioxide (SO_2) was added to stop the growth of bacteria and thus prevent fermentation.

The yeast was inoculated in the two culture medium with the concentration 10^6 CFU/mL. After that, all solutions were incubated at 25°C. In order to verify the *Saccharomyces cerevisiae* growth behavior, samples were taken every day todetermine the CFUs, optical density (OD) and the sugar content.

Reducing Sugar content. The dinitro salicylic acid (DNS) method was used for the estimation of reducing sugars, it detects the presence of a free carbonyl group (C=O) of reducing sugars. This involves the oxidation of the aldehyde functional group (in glucose) and the ketone functional group (in fructose).

In an assay tube, mix 500 µL of sample with 500 µL with DNS reagent, then incubate in a water bath for 5 minutes at 100 °C. Before adding 5 ml of distilled water, the solution needs to be cooled down to room temperature. The absorbance was read in the spectrophotometer (UV-3100PC Spectrophotometer) at 540 nm. To calculate the presence of sugars, the calibration curve was prepared using standard solutions of glucose (G) with concentrations between 0.05 g/L and 1,00 g/L. The purpose of the DNS was to detect the consumption of glucose by the yeast.

2.6. Statistical analysis

The results were treated in the software Excel (version 2020) and R (version x64 4.0.5). The mean and standard deviation values were used to describe experimental results. The results from the experimental design were analysed with the response surface methodology and the model's validation was performed by checking (Maroco, 2007):

- randomness and normality of the residuals;
- cook's distance (values greater than 1 are indicative that these are excessively influential in the model);
- leverage values (values below 0.2 are acceptable, values between 0.2 and 0.5 are risky and values higher than 0.5 indicate the presence of an influential value or outlier);
- model's p-value (to evaluate the significance of the model obtained using the significance level of 0.05).

3. Results and discussion

3.1. Tramazeira fruit microbiology and yeast isolation

To verify the microbiological quality of Tramazeira fruits, the samples were analyzed microbiologically checking the contents in total mesophiles, yeast and molds, total coliforms, *E. coli* and *S. aureus* positive coagulase. The results were obtained as estimates of the yeasts colony-forming unit per gram of sample (CFU/g) that represents the counts of the viable cells, measured in the Petri dish where the sample was applied into a medium. Table 3 shows the mean and standard deviation values for the CFU per gram of the analysed microorganisms.

Table 3. Results (mean and standard deviation) of the microbiological analysis performed to the Tramazeira fruit samples (CFU/g of sample)

Samples	Total mesophiles	Yeast and molds	Total coliforms	*E. coli*	*S. aureus*
S1	9,12±0,05	13,10±0,60	7,82±0,17	<1	absent
S2	13,7±0,16	13,89±0,03	6,89±0,13	<1	absent
S3	21,77±0,07	20,80±0,11	10,58±0,08	<1	absent
S4	20,76±0,06	16,72±0,19	8,29±0,07	<1	absent
S5	18,61±0,30	13,78±0,10	8,82±0,13	<1	Absent

The evaluation of certain microbial groups can be a good indicator of the microbiological quality of the fruit. These microorganisms can be used as indicators of inadequate product manufacturing and/or handling. The mean level of contamination for mesophilic aerobic microorganisms in Tramazeira fruit samples varied between 9,12±0,05 CFU/g (lowest value in sample S1) and 21,77±0,07 (highest value in sample S3) CFU/g. The molds and yeasts are widely distributed in the environment and can easily reach the fruit through contaminated equipment or air. As the Table 3 show, the highest contents in molds and yeasts was also in the sample 3 (20,80±0,11 CFU/g). All samples had coliforms and, maintaining consistency in the results, sample 3 had the highest values (10,58±0,08 CFU/g). The *E. coli* and *S. aureurs* were absent in all samples analyzed. These results strongly suggested to reject the use of sample 3 in this experience in order to avoid contaminations in the next step,

which consists of isolating a yeast to be used in the next study, screening variables to optimize the yeast growth.

Sample 1 was selected for the following studies as it presented, in general, low levels of microorganisms, mainly of mesophilic aerobic microorganisms. Due to several treatments for the isolation of yeasts (sections 2.3.1), a yeast was isolated from culture media based on its morphological characterization and abundance. The API results (section 2.3.2) confirmed that the selected and isolated yeast was a *saccharomyces cerevisiae*.

In this work, the *S. cerevisiae* growth was studied with the purpose to establish a time assay, in the log step, to carry out the multiple experiments for the purpose of making comparisons. A fractional factorial design with 4 factors, 3 levels and 3 center points was applied varying the culture medium composition in glucose, peptone and yeast extract, as well, the Inoculum quantity. Table 4 presents the levels applied to each variable studied. Data treatment was performed for screening the best conditions of the four factors under study (glucose, peptone, yeast extract, and inoculum quantity) using the growth rate measurement (μc; h^{-1}) to evaluate the *S. cerevisiae* growth. As referred in section 2.4 (Table 2), 11 assays (varying culture medium) were prepared to evaluate the best conditions for maximize the cell growth of *S. cerevisiae*.

Table 4. Levels of independent variables (responses)

Variables	Level -1	Level +1
Glucose, g/l	10	20
Peptone, g/l	5	10
Yeast extract, g/l	1	5
Quantity of inoculum	10^5	10^7

A mathematical model was fitted to the results obtained considering the 4 main factors and the interaction terms between them, at the significant level of 0.05. The obtained model explained 94.6% of the data variability, being significant the main factor inoculum quantity (p value = 0.0004 at 0.05 significant level) and the interaction term between glucose and peptone was significant (p value = 0.0324). The significant model (p value = 0.0034) is represented by the following equation

Response = 0,143(±0,006) + 0,057(±0,007) * [Quantity of inoculum] – 0,020(±0,007) * [glucose]:[peptone]

As can be seen, the errors of the terms present in the model are smaller. The Figure 13 presents the Lenth Plot (shows the factor effects with significance levels based on robust estimation of contrast standard errors) and Figure 14, the Daniel plot (shows the signed effects absolute values of the effects displayed) allowing to verify the terms importance in the final model. Both figures confirmed that the levels of glucose, peptone and yeast extract used in the experimental design were not significant to the yeast growth, meaning that the medium composition for the *S. cerevisiae* growth studies can have the lowest levels of these three parameters. This was also in accordance when analyzing the effects of the interaction term between the glucose and peptone factors, allowing to infer that the best growth results occurred when both parameters had the lowest levels.

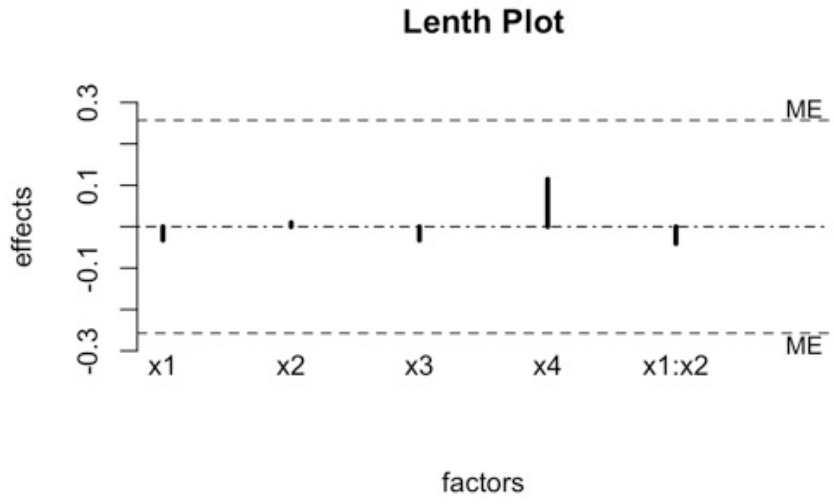

Figure 13. Lenth plot showing the term´s importance in the final model.

X1 – glucose ; X2 – peptone; X3 - yeast extract; X4 - inoculum quantity.

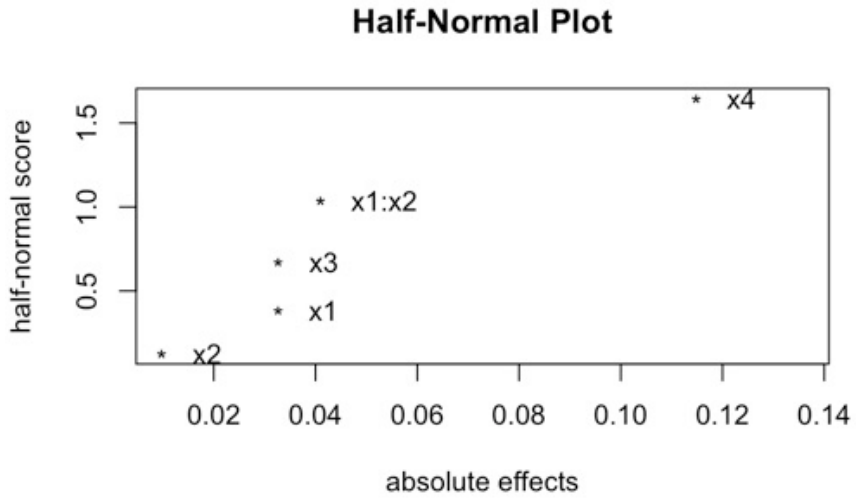

Figure 14. Daniel plot showing the term´s importance in the final model.

X1 – glucose-X2 – peptone; X3 - yeast extract; X4 - inoculum quantity.

The model's quality was also evaluated by considering its residues (predicted performance). Figure 15 shows several plots that allows to verify that the model obtained was satisfactory since it meets the regression assumptions validation. It shows that residuals have a random behavior, are approximately normally distributed since the vast majority of residuals follow a straight line. However, there were some significant influential cases, since some cases had Cook's distance values greater than 1, indicative that these are excessively influential in the model, and leverage values higher than 0.6, revealing the presence of influential values.

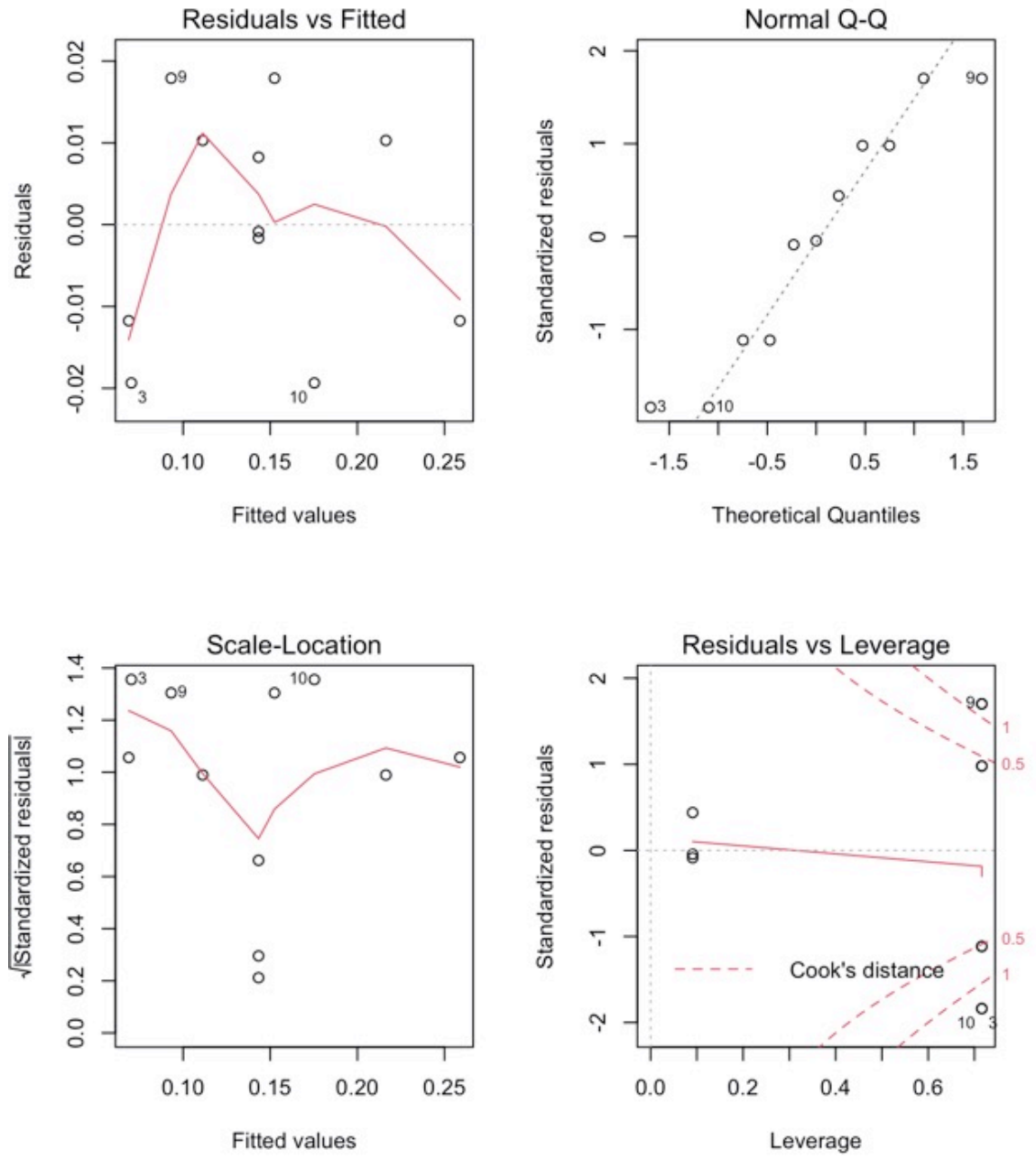

Figure 15. Model residuals' plots of: residuals vs fitted; normal Q-Q; standardize residuals vs fitted values; standardized residual vs leverage, including cook's distance limits.

Regarding the inoculum quantity main effect, Figure 16 presents the relation between growth value in function of the logarithmic inoculum quantity. As can be seen, the highest growth value occurred at the highest inoculum quantity (growth of 0.21 GY, h^{-1} at quantity of 10^7 CFU).

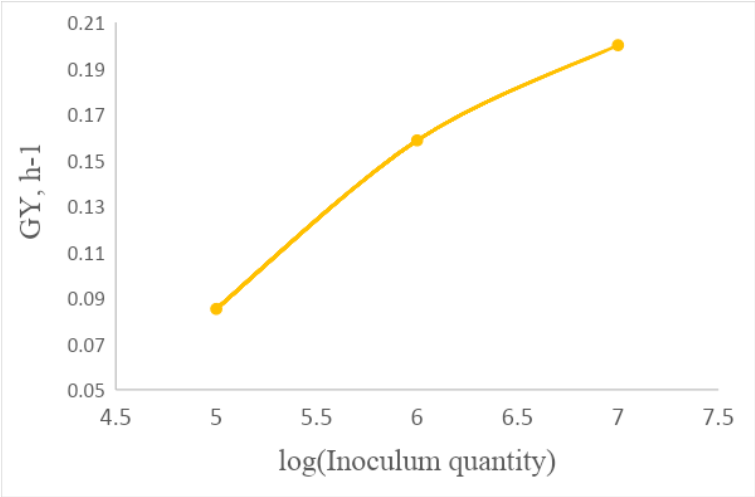

Figure 16. Yeast growth in function of inoculum quantity.

The Figure 17 shows the overall CFU results (logarithmic scale) from the 11 assays carried out (experimental design) measured at three different times: 0, 24 and 48 hours. It is possible to infer that at 0 hours the yeast growth was in the lag step, at 24 hours in the log step and at 48 hours at the stationary phase. As can be seen, in general, at 24 hours the *S. cerevisiae* growth was the highest, showing that this yeast can give results in a 24-hour period under the experimental conditions used in this study.

So, considering the objective of establishing a yeast growth best experimental conditions and then apply these conditions to study the effects after adding new matrices to the growth medium, a test of 24 hours will be used considering the optimum medium composition defined by: 10 g/L of glucose; 5 g/L for peptone; 1 g/L for yeast extract; and yeast quantity of 10^7 CFU. For this synthetic culture medium, the CFU values (logarithmic scale) at 24 hours of *Saccharomyces cerevisiae* growth was about 22.8.

The study of Jungyeon et al. (2017) used metabolomics to investigate the effect of minimal (M9 media) and complex media (LB and YP-glucose medium) on the intracellular metabolic profiles of *Escherichia coli* and *Saccharomyces cerevisiae.* It was referred that the cellular metabolic activity for growth is one of the most representative phenotype differences

between microorganisms, and in the exponential phase, the cellular metabolic activity for growth is at the highest among different growth phases and is the most distinct between microorganisms (Matthew et al., 2012; Jungyeon et al., 2017). Therefore, yeast studies should use growth times in their mid-exponential phase.

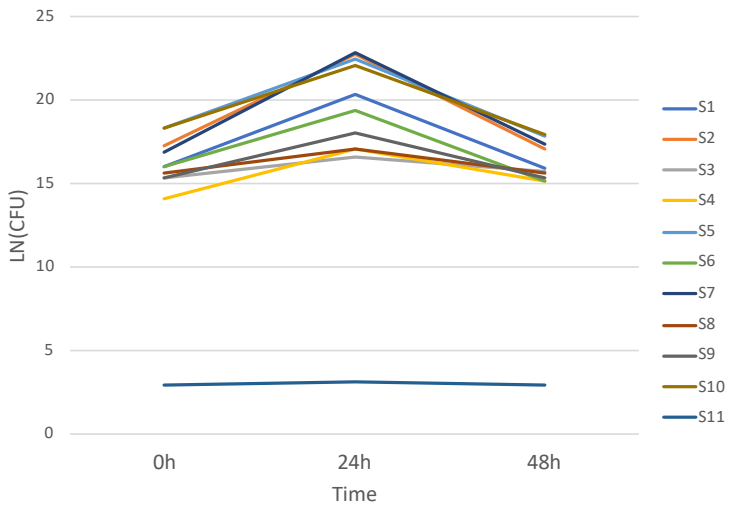

Figure 17. *Saccharomyces cerevisiae* growth: yeasts colony-forming unit (logarithmic scale) in medium culture prepared with composition defined by an experimental design, measured at three different times.

The obtained optimal experimental conditions allowed to establish the best conditions of the isolated *S. cerevisiae* growth. The next work, for comparative purposes, involves using two new culture medium compositions: grape juice or mixture of grape juice and white wine (1:1). Also, the introduction of different histamine concentrations in the medium was to evaluate if the isolated yeast had any influence due to the presence of this compound.

3.3. Grape juice, wine and histamine effects in the isolated yeast growth

To test the *Saccharomyces cerevisiae* growth in different cultures media, several assays were carried out in medium culture prepared with grape juice (must) or with a mixture of grape juice and white wine (proportion 1:1, v/v), both with histamine concentrations (2, 5, 10 and 20 mg/L).

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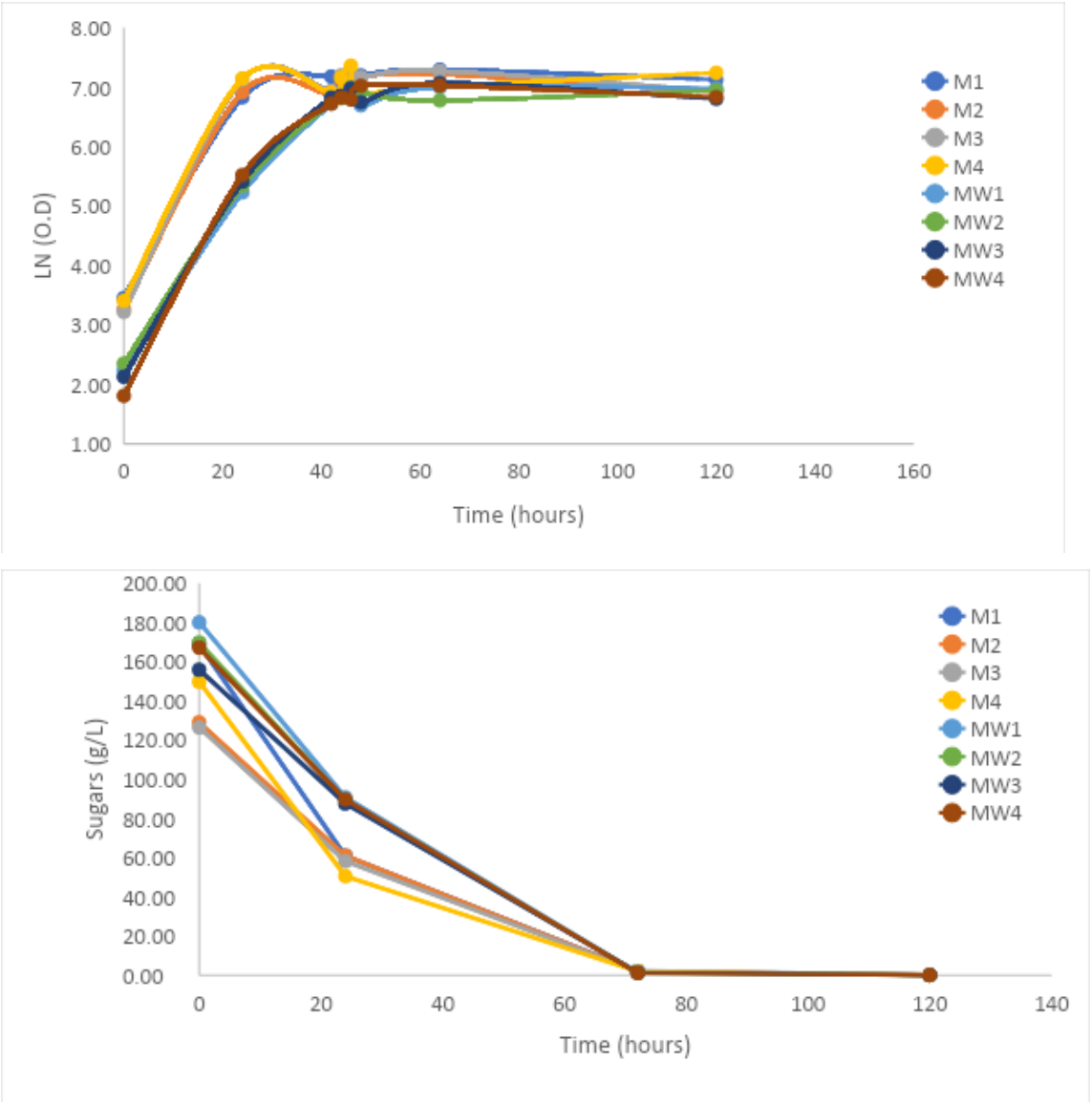

Figure 18. *Saccharomyces cerevisiae* growth: optical density (logarithmic scale) and glucose consumption in optimal medium culture prepared in grape juice (must) or in a mixture of grape juice and white and with and with different levels of histamine. M1 - grape juice with 2 mg/L of histamine; M2 - grape juice with 5 mg/L of histamine; M3 - grape juice with 10 mg/L of histamine; M4 - grape juice with 20 mg/L of histamine; MW1 - grape juice and white wine with 2 mg/L of histamine; MW2 - grape juice and white wine with 5 mg/L of histamine; MW3 - grape juice and white wine with 10 mg/L of histamine; MW4 - grape juice and white wine with 20 mg/L of histamine.

However, samples that contain same quantity of histamine in the culture medium containing grape juice reached stationary phases after 48 hours. Also, as showed in the Figure 18, the consumption of sugar for the samples without the presence of white wine was higher

than the samples with white wine in the medium. This may be the effect of alcohol content that delay *Saccharomyces cerevisiae* growth and assimilation of nutriments (Walker et al., 2003).

The yeast growth was also evaluated by measuring the yeasts colony-forming unit (Figure 19). As can be seen, the samples with culture medium prepared with grape juice reached, in less than 24 hours, high values of CFU (up to 20.00 CFUs/mL). The remaining culture media with the presence of alcohol (added white wine) were delayed. This delay could be explained that the presence of alcohol in the culture media could be a stress for the yeast to growth that it needs more time to adapt in these stressful conditions which can stop the degradation of histamine.

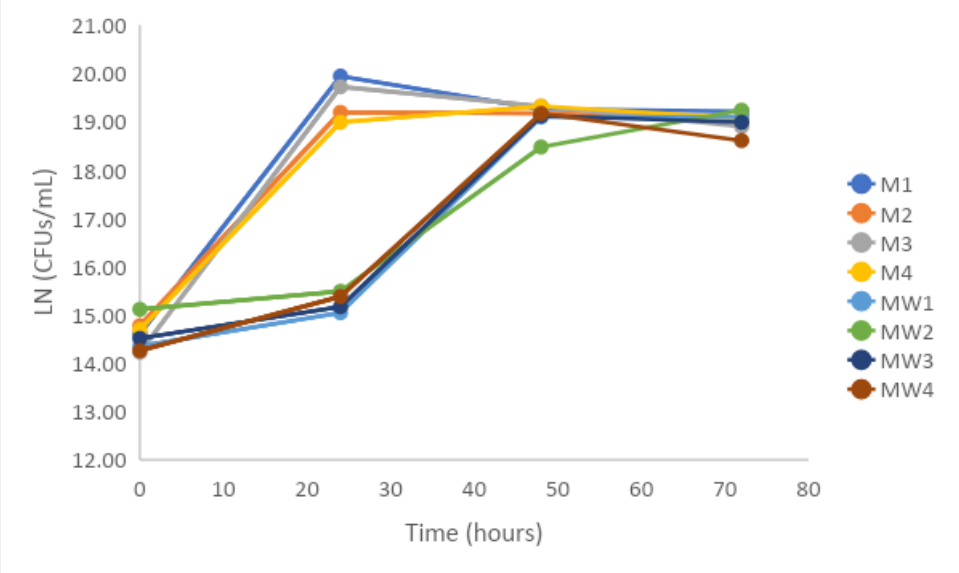

Figure 19. *Saccharomyces cerevisiae* growth: yeasts colony-forming unit (logarithmic scale) in optimal medium culture prepared in grape juice (must) or in a mixture of grape juice and white and with and with different levels of histamine. M1 - grape juice with 2 mg/L of histamine; M2 - grape juice with 5 mg/L of histamine; M3 - grape juice with 10 mg/L of histamine; M4 - grape juice with 20 mg/L of histamine; MW1 - grape juice and white wine with 2 mg/L of histamine; MW2 - grape juice and white wine with 5 mg/L of histamine; MW3 - grape juice and white wine with 10 mg/L of histamine; MW4 - grape juice and white wine with 20 mg/L of histamine.

In the standard culture medium, the CFU values (logarithmic scale) at 24 hours of *Saccharomyces cerevisiae* growth was about 22.8. The CFU values (logarithmic scale) obtained in the culture medium prepared in grape juice (must) varied between 19.0 and 19.9, while for the culture medium with the mixture of grape juice and white wine varied between 15.0 and 15.5. The variability within the results are not explained by the different levels of added

histamine since, no trend was found with its increasing levels. Considering that the histamine concentrations varied between 2 to 20 mg/L and considering the variation within the results, it was inferred that these histamine levels did not affected the overall of *Saccharomyces cerevisiae* growth.

However, it was shown that *Saccharomyces cerevisiae* growth was the highest in the standard culture medium, followed by the one prepared with grape juice, which means that its composition affected its growth, but not with as much impact as when white wine was added. These results agree with several studies of the growth of *Saccharomyces cerevisiae* yeast on alcohol-containing media. Walker et al. (2003) about production of fermented beverages showed that ethanol reduces the *S. cerevisiae* growth. Although, they are referred as yeasts that generally stand higher ethanol levels. The *Saccharomyces*' high ethanol tolerance is one of the key factors for its use in fermentation when ethanol exceeds 9 e10%. The study of Antoce et al. (1997) about *saccharomyces* growth in the presence of added ethanol referred that the addition of ethanol and methanol up to 7.65% had clear effects of inhibition on growth of the yeast studied, reducing the growth rate constant, and delaying growth. The study of Kubota et al. (2014) explained the effect of ethanol on cell growth of *Saccharomyces cerevisiae*, for the examination of the effect of various concentrations of ethanol on the growth of wild-type cell culture. A 6% concentration of ethanol decreased the growth rate of the cells by 50%. Birch et al. (2000) referred that ethanol is an inhibitor of yeast growth at relatively low concentrations, inhibiting cell division, decreasing cell volume and specific growth rate, while at high ethanol concentrations reduce cell vitality and increase cell death.

The *Saccharomyces cerevisiae* is considered a model organism, a valuable tool for all aspects of basic research, with multiple applications. For instance, *Saccharomyces cerevisiae* has been studied as a biosorbent of organic compounds such as dyes (El-Gendy et al., 2015), pesticides and herbicides (MacRae, 1985), inorganic compounds such as metals (Zheng et al., 2018), as well as bioactive compounds of plants and tea (Jilani et al., 2015; Rubio et al., 2018).

CONCLUSION

In this study, *Saccharomyces cerevisiae* was isolated from a Tramazeira fruit, a yeast very well-known and used in the food industry (for instance, in baking). It is a eukaryotic model organism in biological studies since it can easily be cultured. The results showed that the selected optimal culture media from the results obtained using the fractional factorial design has the composition: 10 g/L of glucose, 5 g/L of peptone, 1 g/L of extract yeast and 10^7 CFU/mL of inoculum quantity. The lowest concentrations of these parameters (except the quantity of inoculum) were selected considering that they allowed a good growth of the yeast and with the possibility of having a good distinction between the several tests. This standard culture media will be used in future works for studying the isolated *Saccharomyces cerevisiae* performance in bioremediation, biodegradation and bioproduction assays.

To test the *Saccharomyces cerevisiae* growth, different culture matrices as grape juice or a mixture of grape juice and white wine, together with different concentrations of histamine (2, 5, 10, and 20 mg/L), were tested. It was shown that the *Saccharomyces cerevisiae* growth was delayed in the culture media with the presence of alcohol. Alcohol can be a stress for the yeast to grow, needing more time to adapt to these stressful conditions. Also, there was evidence that histamine levels did not affected the overall of *Saccharomyces cerevisiae* growth. Considering the overall results, the standard culture media should be considered as a standard media for the isolated *Saccharomyces cerevisiae* growth, showing a value of 22.8 CFU values, in logarithmic scale, followed by a reduction in growth in the culture media prepared in grape juice (85.3% of the result obtained in the standard media), while for the culture medium with the mixture of grape juice and white wine, the growth was 67.0% of the standard media.

The objectives of the work were achieved, that is, to define a procedure to establish a suitable culture media to obtain a growth pattern for an isolated yeast that can later be applied in the study of yeast behavior by introducing different matrices into the medium of culture.

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