Benedito Rodrigues da Silva Neto
(Organizador)

Pesquisa Científica e Tecnológica em Microbiologia 2

Atena Editora
Ano 2020

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APRESENTAÇÃO

Temos o prazer de apresentar o segundo volume da obra "Pesquisa científica e tecnológica em microbiologia", contendo trabalhos e pesquisas desenvolvidas em diversos locais do país que apresentam análises de processos biológicos embasados em células microbianas ou estudos científicos na fundamentação de atividades microbianas com capacidade de interferir nos processos de saúde/doença.

Conforme destacamos no primeiro volume, a microbiologia é um vasto campo que inclui o estudo dos seres vivos microscópicos nos seus mais vaiados aspectos como morfologia, estrutura, fisiologia, reprodução, genética, taxonomia, interação com outros organismos e com o ambiente além de aplicações biotecnológicas. Como uma ciência básica a microbiologia utiliza células microbianas para analisar os processos fundamentais da vida, e como ciência aplicada ela é praticamente a linha de frente de avanços importantes na medicina, agricultura e na indústria. Os microrganismos são encontrados em praticamente todos os lugares, e hoje possuímos ferramentas cada vez mais eficientes e acuradas que nos permitem investigar e inferir as possíveis enfermidades relacionadas aos agentes como bactérias, vírus, fungos e protozoários.

O potencial desta obra é enorme para futuras novas discussões, haja vista que enfrentamos a questão da resistência dos microrganismos à drogas, identificação de viroses emergentes, ou reemergentes, desenvolvimento de vacinas e principalmente a potencialização do desenvolvimento tecnológico no estudo e aplicações de microrganismos de interesse.

Portanto apresentamos aqui temas ligados à pesquisa e tecnologia microbiana são com a proposta de fundamentar o conhecimento de acadêmicos, mestres e todos aqueles que de alguma forma se interessam pela saúde em seus aspectos microbiológicos. Parabenizamos à todos os envolvidos que de alguma forma contribuíram em cada capítulo e cada discussão, com destaque principal à Atena Editora que tem valorizado a disseminação do conhecimento obtido nas pesquisas microbiológicas.

Assim desejo a todos uma ótima leitura!

Benedito Rodrigues da Silva Neto

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STURDINESS OF BAKER'S YEAST STRAINS TO NATURAL BIOACTIVE COMPOUNDS

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ABSTRACT: Briefly, this chapter provides an overview about baker's yeast strains, more specifically about the sturdiness of different yeast strains in the face of essential oils. General aspects of plant-derived essential oils are discussed, and some applications of these substances are lightly addressed. The sturdiness of the baker's yeast strains is reported for 31 plant-derived essential oils extracted from Brazilian and exotic medicinal and aromatic plants.
KEYWORDS: *Saccharomyces cerevisiae;* Baker´s yeast strains; Essential Oils; Industrial applications

ROBUSTEZ DE LINHAGENS DE LEVEDURAS DE PANIFICAÇÃO A BIOATIVOS NATURAIS

RESUMO: Este capítulo fornece, resumidamente, uma visão geral sobre as linhagens de levedura de panificação, mais especificamente sobre a robustez das diferentes cepas destas levedura na presença de óleos essenciais. Aspectos gerais dos óleos essenciais derivados de plantas são discutidos e algumas aplicações dessas substâncias são levemente abordadas. A robustez das linhagens

de leveduras de panificação é relatada para 31 óleos essenciais derivados de plantas, extraídos de plantas medicinais e aromáticas brasileiras e exóticas.

1 I BAKER'S YEASTS

In fermentation processes involving yeasts for industrial purposes, whether for the production of beverages such as beer, wine or spirits, or for the production of bread and bakery products, or in the symbiotic matrices with bacteria as kombucha or kefir, one microorganism should be highlighted: *Saccharomyces cerevisiae*.

This microorganism has shown remarkable attributes since ancient times, and plays an important role due to its versatility and capacity to act in different kinds of substrates (STRATFORD, 2006).

In the food industry, *S. cerevisiae* represent a significant part of the microorganisms employed as baker´s yeast to produce bread and other bakery products in the fermentation processes. Inside this species, numerous strains are broadly used in industrial fermentation processes to produce not only bakery products, but also beer, wine, and bioethanol. Thus, it is necessary to know the characteristics of this microorganism and its behavior in different situations.

As mentioned above, *Saccharomyces cerevisiae* is widely used in different industrial segments, mainly in the food industry, in which the use of natural products free of synthetic additives has been a trend.

Recently, because of the higher demand for natural products and the development of green consumerism, plant-derived essential oils have been gaining popularity.

According to several authors, essential oils have long acted as flavoring agents in beverages and food. Besides that, they could be used as a natural source of antimicrobial compounds, and due to their versatility, they could have a great potential to replace synthetic additives as natural agents for food preservation (DEANS; RITCHIE, 1987; CONNER, 1993; BURT, 2004; RAUT; KARUPPAYIL, 2014).

More recently, natural products are in the spotlight, and they have been highly valued by several industrial segments. In the food industry, their visibility has led to many studies using plant-derived essential oils focusing on antimicrobial and antioxidant properties (KUORWEL et al., 2011; KALEMBA, KUNICKA, 2003; NEGI, 2012).

Despite the amount of studies and articles concerning the application of essential oils as potential antimicrobials and as food preservatives, in the industrial segments, very few reports have demonstrated their direct application in processes which use *Saccharomyces cerevisiae*.

As referred to previously, this important microorganism is used as baker´s yeast in order to produce bread and other bakery products. In addition, there are several strains with different characteristics responsible for their wide application in several industrial segments as a platform for fermentation processes.

In this study, we took a step toward exploring this question by conducting an initial baker's yeast characterization in order to determine the sturdiness profile from different yeast strains in the face of several essential oils extracted from Brazilian native plants and also exotic plants.

For this reason, baker's yeasts were previously evaluated considering their genetic identification in a sub specific level using molecular fingerprint tools.

2 I STRAINS DIFFERENTIATION

Our study involved six microorganisms belonging to three different commercial baker's yeasts.

The baker's yeasts strains were isolated from baker's yeast samples commercially used in Brazil and all of them are identified as *Saccharomyces cerevisiae*. This yeast species has an important and strong impact in the food and beverage industries.

Firstly, all commercial baker's yeasts were grown using the spread-plating technique, in which a small sample is spread over the surface of an agar plate in order to evaluate the biotypes, i.e., we evaluated the morphology of discrete colonies formed across the surface of the WL Nutrient medium agar (WLN, DIFCO 242420).

After that, we used a streak-plating technique to check the purity of the culture of yeast. Then, single colonies, which are comprised of millions of cells growing in a cluster on an agar plate, were transferred to another plate using the spread-plate technique. Thus, the pure microorganisms were grown in petri dishes with Potato Dextrose Agar medium (PDA, DIFCO 213400) to be submitted to DNA extraction protocol (BIDENNE et al., 1992 modified; OAKLEY-GUTOWSKI et al., 1992 modified; ARGUESO, et al. 2008).

All isolates were distinguished by an electrophoretic karyotyping profile obtained from pulsed field gel electrophoresis (PFGE). This technique consists of the separation of intact chromosomal DNA according to its size on a gel matrix of agarose. According to the number and size of the chromosomes present in each strain, specific banding patterns were obtained.

In a different way to conventional DNA electrophoresis, which is able to separate molecules of up to 50 kilobases, PFGE is able to do the separation of large DNA molecules, as yeast chromosomes, which range from several hundred to several thousand kilobases (ZIMMERMAN; FOURNIER, 1996). It is possible because this technique uses an electric field that periodically changes direction in a gel matrix of agarose.

Unquestionably, nowadays there is an immense variety of molecular techniques for the identification of microorganisms. Nonetheless, not all of them are able to have sufficient sensitivity to distinguish some microorganisms at the sub-specific level, i.e., by distinguishing among different strains.

According to VILANOVA et al. (2007), PFGE has a greater discriminatory power when compared to mtDNA restriction analysis for *Saccharomyces cerevisiae* yeast clones. Therefore, this greater resolution in the power of differentiation among strains makes it better suited for the detection of genetic diversity in yeasts.

For this reason, we used PFGE to do the differentiation of baker's yeast strains. Below we present the DNA electrophoretic profile for all baker's yeast strains evaluated (Figure 1). The terms used to name the baker's yeast groups were stablished based on the commercial description: (ADY Active dry baker's yeast; SDY Sweet dough baker's yeast; SY strong baker's yeast).

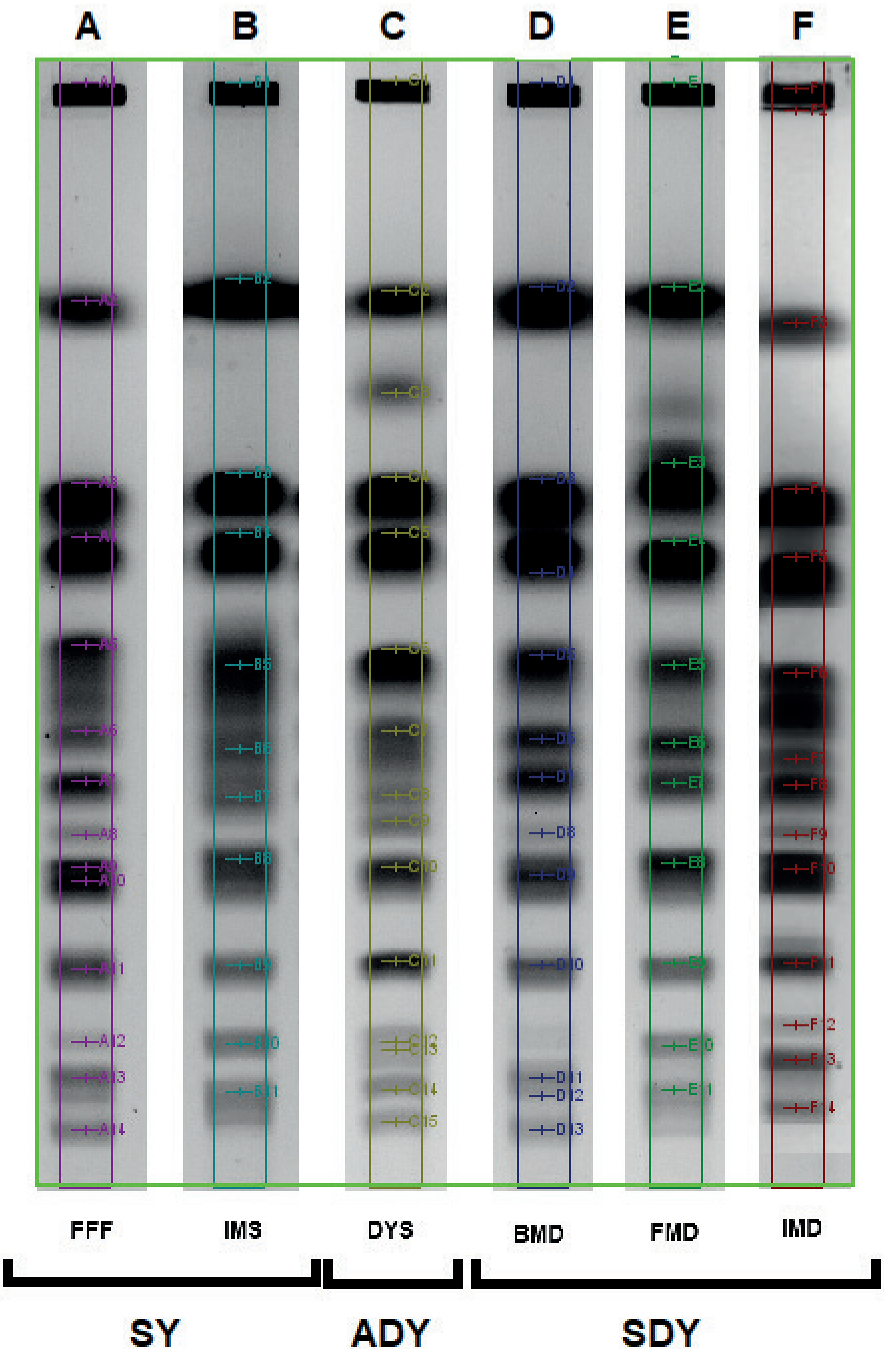

Figure 1: Genomic DNA electrophoretic profile of baker's yeast strains (*Saccharomyces cerevisiae*) obtained using PFGE (*Pulsed Field Gel Electrophoresis*) in agarose gel 0.8%. Image acquisition by UVP Vision Works LS system. (A) Strain FFF; (B) Strain IMS; (C) Strain DYS; (D) Strain BMD; (E) Strain FMD; (F) Strain IMD.

The differentiation among the yeast strains was stablished by the DNA electrophoretic profile comparison. Figure 2 illustrates the analysis results using PFGE and UVP Vison Works L.S. systems.

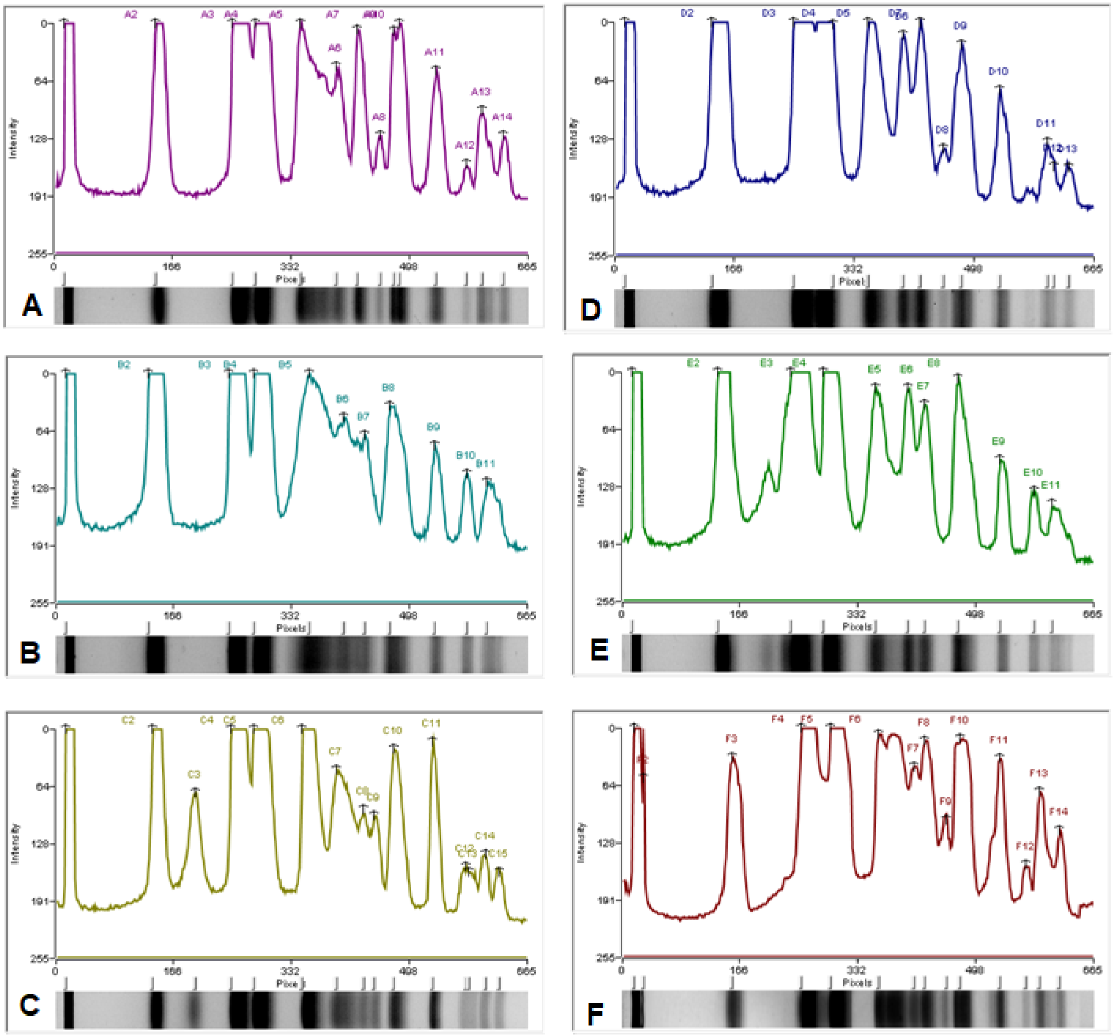

Figure 2: Differentiation among baker's yeast strains (*Saccharomyces cerevisiae*). Comparative of genomic DNA electrophoretic profile. Image acquisition and differentiation analysis system by UVP Vision Works LS. (A) Strain FFF; (B) Strain IMS; (C) Strain DYS; (D) Strain BMD; (E) Strain FMD; (F) Strain IMD.

After the PFGE analysis, six strains were identified as different yeast strains. Each one was tested with 31 plant-derived essential oils.

The next session presents some concepts about plant-derived essential oils, reveals the essential oils studied, and summarizes some findings from our studies with baker's yeast strains' sturdiness to these compounds.

3 I ESSENTIAL OILS

Recently, synthetic additives have been accused of causing toxic and carcinogenic effects, and the consumer concerns regarding healthier food options have increased. As a result, the popularity of natural products has been blasting and the demand for alternatives to synthetic food additives has gained significant market share.

Given the fact that plant-derived essential oils (EOs) contain volatile components with antimicrobial and antioxidant activities and have been used for centuries for medicinal and cosmetic purposes, as well as flavor and fragrance markets, they would be strong candidates to this replacement (HAMMER et al., 1999; HALBERSTEIN, 2005; Gurib-Fakim, 2006; MARÍN et al., 2016; Jamshidi-Kia et al., 2018).

Plant-derived essential oils are a mixture of several compounds, with different chemical origins. Their composition vary and could include terpenes, alcohols, acids, esters, epoxides, aldehydes, ketones, amines, and sulfides. These complex aromatic and volatile mixtures could be obtained from different plant materials such as leaves, flowers, buds, roots, and barks (GUENTHER, 1948; BURT, 2004). The production of these phytochemicals is mentioned by several authors as a stress response of the plants, which possess a wide range of tools to combat pathogenic infections (THEIS; LERDAU, 2003).

In addition, these complex mixtures extracted from plants have high potential to scale down the use of synthetic compounds, which are widely used by the food industry to control undesirable contaminants, since they have important properties, like antimicrobial and antioxidant actions (DEANS; RITCHIE, 1987; HAMMER et al., 1999; BURT, 2004; SARTORATTO et al., 2004; SENDRA, 2016).

In this study, 31 plant-derived essential oils were used. These essential oils were extracted from plants belonging to the Medicinal and Aromatic Plant Collection (CPMA) of the Chemical, Biological and Agricultural Multidisciplinary Research Center (CPQBA) at University of Campinas (UNICAMP), in Brazil.

The sturdiness of 6 different strains of *S. cerevisiae*, previously identified and differentiated, was established considering their capability to grow in the face of different essential oils, in all concentrations evaluated.

The sturdiness was inferred from the growing ability considering the inhibitory effect of the plant-derived essential oils (EOs) using the microdilution test and determining Minimal Inhibitory Concentration - MIC (NCCLS, 2002) for each of the 31 essential oils. Figure 3 summarizes the results from the experiments with the 31 EOs in different concentrations, in which we catalogue the resistance profiles of all baker's yeast strains studied.

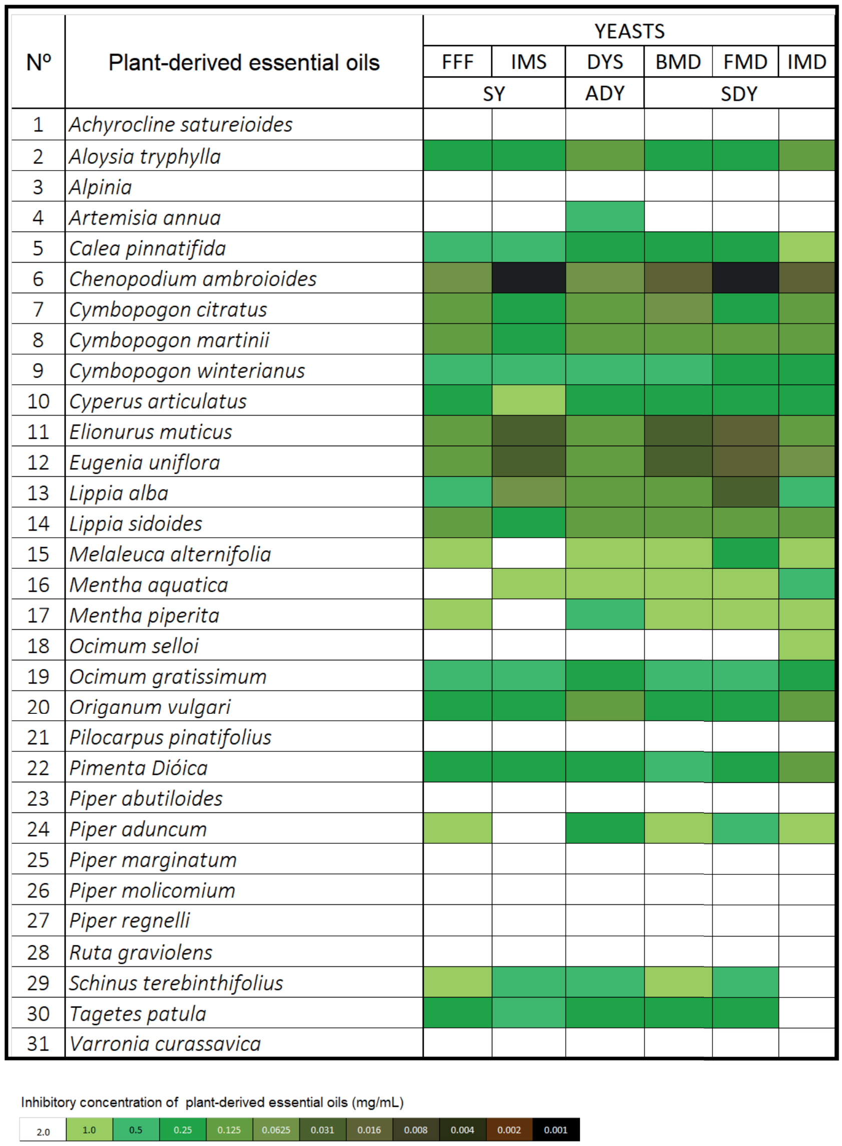

FIGURE 3 Minimal Inhibitory Concentration (MIC) - Effect of plant-derived essential oils on baker's yeast strains growth.

4 I RESULTS

The convergence among resistance or susceptibility profile comparing all strains demonstrated a similarity of 75%, considering all OE evaluated. In other words, the robustness profile differed among the 3 groups of yeasts studied for only 8 essential oils. (*Artemisia annua*; *Melaleuca aternifolia*; *Mentha aquatica*; *Mentha piperita*; *Ocimum gratissimum*; *Schinus terebinthifolius*; *Piper aduncum* and *Tagetes patula* essential oils).

Considering all EOs, the strains presented an average robustness of 35%. The strains commercially referred as Strong Yeast revealed greater sturdiness, achieving 45% for one strain and an average of 42%, comparatively (Figure 4).

Contrastingly, DYS yeast strain, belonging to Active dry yeast (ADY) group, showed the worst sturdiness pattern to all essential oils evaluated (Figure 4).

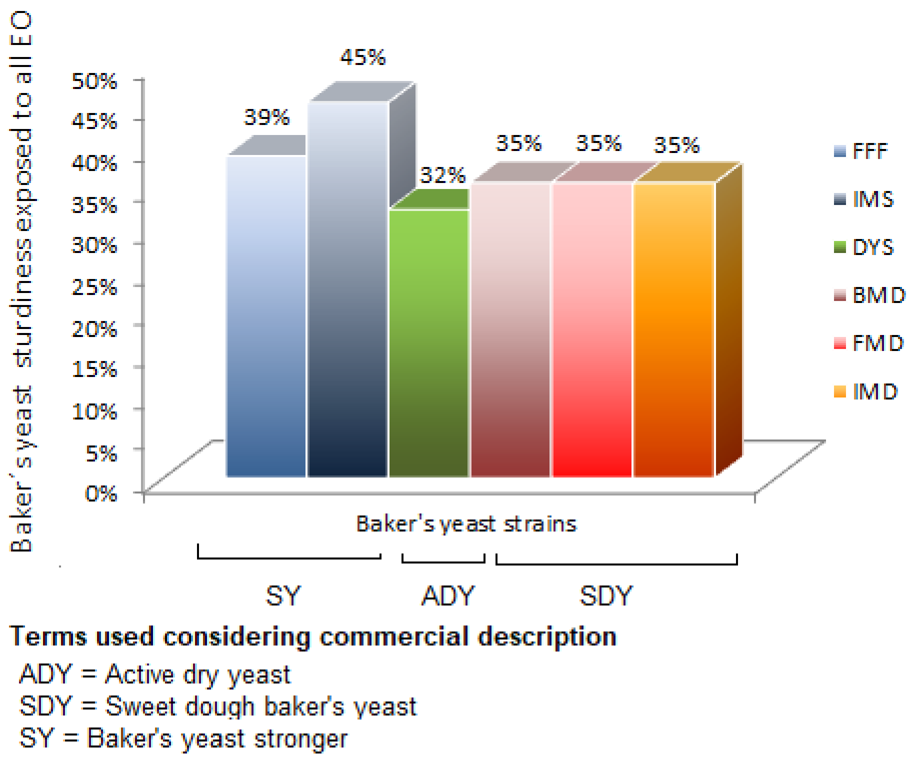

FIGURE 4 Baker's yeast sturdiness considering all essential oils evaluated.

For the groups in which two or more strains were tested, the same robustness pattern was observed in the different strains inside the same group. Interestingly, although the strains were isolated from yeasts of distinct brands/suppliers, the same standard of robustness was observed for different lineages of the same group (Figure 4).

All strains were resilient to EO of *Varronia curassavica (sin = Cordia verbenaceae DC.); Achyrocline; satureioides; Pilocarpus pinatifolius; Ruta graviolens*; and several species of Piper sp.,in all tested concentrations.

Distinctly, most of the baker's yeast strains have presented slight inhibitory growth pattern in the face of *Artemisia annua, Melaleuca alternifolia, Mentha aquatica* and *Mentha piperita* essential oils, in which concentrations can reach 0,5mg/mL.

Conversely, the essential oils extracted from: *Cymbopogon citratus; Cymbopogon martini; Cyperus articulatus, Elionurus muticus; Chenopodium ambrosioides; Lippia alba; Pimenta dióica* and *Tagetes patula*, were very harmful for all the yeasts assessed in much lower concentrations of EOs.

5 I FINAL CONSIDERATIONS

This chapter provided the characterization of several baker's yeast strains, including the genetic differentiation among strains and the establishment of the sturdiness profile using essential oils obtained from native Brazilian plants and exotic ones.

Despite the significant number of research studies of plant-derived essential oils as potential food preservatives, antioxidants and antimicrobials, extremely few show their application in processes using *Saccharomyces cerevisiae* as fermentation platforms.

In this chapter, both the sturdiness of different baker's yeast strains in the face of several EOs and the inhibitory effect of them, were discussed.

Generally, the yeast strains' robustness presented results convergence, i.e., the same EO inhibitory effect was observed for different strains for one specific plant-derived essential oil.

Although there is a general convergence of results regarding the robustness of the strains against one specific OE, especially in the case of lineages of the same group or commercial classification, the results related to the concentrations of EO highlighted the need to evaluate sturdiness of the strains with different essential oils, even if they belong to plants of the same genus.

On the one hand, the results demonstrate that the sturdiness of baker's yeast strains distinctly vary when submitted to essential oils, but on the other hand, it is not possible to predict the effect of EO extracted from plants belonging to the same plant family or genus.

For some groups (family or genus), the results of yeast robustness point toward the same pattern. One example is the yeast strains' sturdiness presented with EOs extracted from the vast majority of plants belonging to the genus Piper sp. and the genus *Mentha* sp. By contrast, in other cases, the sturdiness to EOs extracted from plants belonging to the same plant genus is not necessarily similar, as EOs extracted from *Ocimum* spp plants demonstrated significant difference in the baker's yeast sturdiness profile.

Furthermore, the sensitivity profile of baker's yeast strains in the face of essential oils with a high harmful effect exhibited a more convergent outcome. One example is the inhibitory effect of EOs extracted from plants belonging to family Verbenacea and Poaceae. EOs extracted from *Lippia* spp. (family Verbenaceae) and *Cymbopogon* spp. (family Poaceae), were very toxic for all the baker's yeast strains, presenting similar harmful effects against all of them.

In short, not only the baker's yeast sturdiness results, but also the essential oils inhibitory effect results were significant and have implications on technological applications for both the food industry and other industrial segments that use *Saccharomyces cerevisiae* strains.

Finally, these findings open new perspectives for the industrial application of bioactives as plant-derived essential oils.

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