PÂMELA MYNSEN MACHADO MARTINS

MICROENCAPSULATION BY SPRAY DRYING OF EPIPHYTIC COFFEE YEASTS FOR INOCULATION IN THE FERMENTATION PROCESS

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-graduação em Ciência dos Alimentos, área de concentração em Microbiologia de Alimentos e Processos fermentativos, para obtenção do título de Doutora.

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MICROENCAPSULAÇÃO POR SPRAY DRYING DE LEVEDURAS EPIFÍTICAS DO CAFÉ PARA INOCULAÇÃO NO PROCESSO FERMENTATIVO

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APROVADA em 13 de dezembro de 2022.
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LAVRAS – MG
2023

Aos meus queridos pais, Paulo e Daniela, por todo amor, incentivo, apoio e dedicação.
Dedico

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“Prefiro ter perguntas que não podem ser respondidas
do que respostas que não podem ser questionadas.”
(Richard Feynman)

RESUMO

A comercialização de leveduras na forma líquida não é indicada a longo prazo pois as células perdem a viabilidade. Além disso, há de risco de contaminação e maiores custos com transporte e armazenamento refrigerado. Assim, uma alternativa é a secagem por spray drying. Esta técnica pode ser utilizada de forma contínua e rápida, permitindo altas taxas de produção com baixos custos operacionais. Dito isto, o objetivo do trabalho foi avaliar a eficácia da microencapsulação por spray drying para promoção da secagem e revestimento de três leveduras epifíticas do café: *Saccharomyces cerevisiae* CCMA0543, *Torulaspora delbrueckii* CCMA0684 e *Meyerozyma caribbica* CCMA1738. O trabalho foi dividido em três etapas. A primeira etapa consistiu na avaliação da viabilidade celular das leveduras microencapsuladas em spray dryer de escala laboratorial. As leveduras (10^9 UFC mL^{-1}) foram microencapsuladas separadamente com maltodextrina (15%), maltodextrina (15%) com sacarose (2%) ou maltose (2%). Os resultados indicaram que a viabilidade celular variou entre 94,06 e 97,97%. Após 6 meses, ambas as leveduras armazenadas a 7 °C e 25 °C apresentaram 10^7 e 10^2 UFC mL^{-1}, respectivamente. Esta etapa mostrou que foi possível microencapsular as leveduras epifíticas do café por spray drying e que a maltodextrina foi eficiente como material de parede. A segunda etapa consistiu na avaliação do efeito dos parâmetros operacionais no processo de secagem para obtenção de leveduras microencapsuladas em spray dryer de maior escala. A concentração do material de parede e a temperatura de entrada do ar de secagem foram investigadas por meio do Delineamento do Composto Central Rotacional (DCCR). Em seguida, a performance fermentativa das leveduras microencapsuladas foram avaliadas em meio de casca e polpa de café. As leveduras atingiram viabilidade celular e rendimentos de secagem acima de 90 e 50%, respectivamente. Soro de leite em pó manteve a viabilidade celular das três leveduras ao longo de 90 dias de armazenamento à temperatura ambiente (25°C) e foi selecionado como material de parede para as três leveduras. *M. caribbica* mostrou-se mais sensível à secagem por spray drying e menos resistente ao armazenamento. Algumas diferenças foram encontradas na fermentação, mas as leveduras microencapsuladas mantiveram suas características biotecnológicas. Por fim, a terceira etapa consistiu em comparar os desempenhos fermentativos de leveduras líquidas e microencapsuladas em café fermentados por anaerobiose autoinduzida (SIAF). Após 180 h de fermentação no processo natural, *T. delbrueckii* microencapsulada (MT) ($7{,}97x10^7$ cel/g) apresentou uma população maior do que *T. delbrueckii* líquida (FT) ($1{,}76x10^7$ cel/g). O estado da levedura influenciou na concentração dos compostos orgânicos e voláteis. Os cafés inoculados com *S. cerevisiae* microencapsulada (MS) apresentaram notas dominantes de frutado, caramelo e nozes no processamento natural. Já no café descascado, os cafés inoculados com MT apresentaram caramelo, mel e nozes. Considerando os parâmetros analisados, as leveduras mais indicadas para processamento natural e descascado seriam MS e MT, respectivamente. Como conclusão, as leveduras microencapsuladas por spray drying foram metabolicamente ativas e podem ser consideradas com potencial comercial, principalmente para produtores de café interessados em utilizar culturas iniciadoras durante SIAF.

Palavras-chave: Otimização. Secagem. Culturas iniciadoras. SIAF.

ABSTRACT

The yeasts commercialization in the liquid form is not indicated in the long term as the cells lose viability. In addition, there is a risk of contamination and higher costs with transport and refrigerated storage. Thus, an alternative is spray drying. This technique can be used continuously and quickly, allowing high production rates with low operating costs. That said, the objective of this work was to evaluate the effectiveness of microencapsulation by spray drying to promote the drying and coating of three epiphytic coffee yeasts: *Saccharomyces cerevisiae* CCMA0543, *Torulaspora delbrueckii* CCMA0684 and *Meyerozyma caribbica* CCMA1738. The work was divided into three steps. The first step was evaluating of the cell viability of microencapsulated yeasts in a laboratory-scale spray dryer. The yeasts (10^9 CFU mL^{-1}) were microencapsulated separately with maltodextrin (15%), maltodextrin (15%) with sucrose (2%), or maltose (2%). The results showed that cell viability varied between 94.06 and 97.97%. After six months, both yeasts stored at 7 °C and 25 °C showed 10^7 and 10^2 CFU mL^{-1}, respectively. This step showed that it was possible to microencapsulate the epiphytic coffee yeasts by spray drying. Maltodextrin was efficient as a wall material. The second step evaluated the effect of operational parameters on the drying process to obtain microencapsulated yeasts in a larger-scale spray dryer. The wall material concentration and the drying air inlet temperature were investigated using the Central Rotational Composite Design (DCCR). Then, the fermentative performance of microencapsulated yeasts was evaluated in a coffee peel and pulp media. The yeasts reached cell viability and drying yields above 90 and 50%, respectively. Whey powder maintained the cell viability of the three yeasts over 90 days of storage at room temperature (25°C) and was selected as wall material for the three yeasts. *M. caribbica* was more sensitive to spray drying and less resistant to storage. Some differences were found in the fermentation, but the microencapsulated yeasts maintained their biotechnological characteristics. Finally, the third step compared the fermentative performances of liquid and microencapsulated yeasts in coffee fermented by self-induced anaerobiosis (SIAF). After 180 h of fermentation in the natural process, microencapsulated *T. delbrueckii* (MT) ($7.97x10^7$ cells/g) presented a larger population than liquid *T. delbrueckii* (FT) ($1.76x10^7$ cells/g). The state of the yeast influenced the concentration of organic and volatile compounds. Coffees inoculated with microencapsulated *S. cerevisiae* (MS) showed dominant notes of fruitiness, caramel, and nuts in natural processing. In the pulped coffee, the coffees inoculated with MT presented caramel, honey, and nuts. Considering the analyzed parameters, the most suitable yeasts for natural and pulped processing would be MS and MT, respectively. In conclusion, spray drying microencapsulated yeasts were metabolically active and may be considered with commercial potential, especially for coffee producers interested in using starter cultures during SIAF.

Keywords: Optimization. Drying. Starter cultures. SIAF.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

O armazenamento de microrganismos na forma líquida não é indicado a longo prazo, pois as células perdem a viabilidade, há maior dificuldade com o transporte e maior risco de contaminação. Dessa forma, diferentes tecnologias de secagem podem ser utilizadas para preservação de microrganismos, como por exemplo, liofilização (STEFANELLO *et al.*, 2019), spray drying (VANDEN BRABER *et al.*, 2020) e secagem em leito fluidizado (STRASSER *et al.*, 2009). O objetivo deste processo é permitir o armazenamento a longo prazo, preservando a viabilidade celular. Assim, a secagem tem sido uma ótima alternativa para o armazenamento de culturas (ELIZONDO; LABUZA, 1974; MORGAN *et al.*, 2006; SURYABHAN; LOHITH; ANU-APPAIAH, 2019).

O processo de secagem consiste na retirada da água presente no meio, e assim, as atividades biológicas dos microrganismos são retidas. Em comparação com uma cultura líquida, a cultura na forma seca oferece vantagens como facilidade de transporte, armazenamento, redução do odor e melhor controle de qualidade (FU; CHEN, 2011).

Embora exista diferentes métodos para preservação, alguns métodos podem ser mais adequados dependendo do objetivo (TAN; POH; CHIN, 2018). A liofilização tem sido utilizada para preservar microrganismos há décadas (CHEN; WANG, 2007). Este método proporciona produtos com alta qualidade e permite a manutenção da viabilidade da cultura por 10 a 20 anos, porém há limitações de volume, risco de contaminação cruzada, longo tempo e alto custo de processamento (FU; CHEN, 2011; SANTIVARANGKNA; KULOZIK; FOERST, 2007). Logo, a aplicabilidade em larga escala torna-se menos viável (MARQUES; SILVEIRA; FREIRE, 2006).

Dessa forma, outras abordagens de secagem foram estudadas com o objetivo de desenvolver um processo mais econômico para desidratação de microrganismos (OLIVEIRA *et al.*, 2007; SANTIVARANGKNA; KULOZIK; FOERST, 2006; STRASSER *et al.*, 2009; VANDEN BRABER *et al.*, 2020). O spray drying é o método mais utilizado na indústria alimentícia e pode ser utilizado para secagem de alimentos. Além disso, pode ser utilizado para microencapsular o material desejado, como por exemplo, as culturas iniciadoras. O objetivo é utilizar um material de parede com o intuito de proteger e preservar às células (FU; CHEN, 2011). Assim, este método fornece produtos de alta qualidade. Além disso, é econômico, flexível e pode ser realizado de forma contínua. Porém, a desvantagem é a alta

temperatura utilizada, o que pode afetar a viabilidade de determinados microrganismos (MARTÍN *et al.*, 2015).

Sendo assim, é importante entender os fatores intrínsecos e extrínsecos que influenciam a viabilidade celular durante a secagem térmica. Os fatores intrínsecos estão relacionados aos microrganismos que serão secos e, consequentemente, a tolerância das células ao estresse térmico e a desidratação. Estas tolerâncias são características específicas do microrganismo, sendo assim, estas podem variar entre as espécies. Por outro lado, os fatores extrínsecos estão relacionados às condições de secagem e ao material de parede utilizado. Vale ressaltar que ambos podem ser otimizados para atingir uma sobrevivência celular máxima (DOS SANTOS; FINKLER; FINKLER, 2014; FU; CHEN, 2011; LIEVENSE, L. C.; VAN'T RIET, 1993, 1994).

Neste processo de desidratação, as células podem entrar no estado conhecido como anidrobiose, utilizado para produção industrial de leveduras secas ativas. Dessa forma, levedura seca ativa é aquela no estado de anidrobiose em que seu metabolismo é temporariamente suspenso de forma reversível. Porém, é importante ressaltar que praticamente todos os componentes intracelulares das leveduras sofrem mudanças estruturais que podem ser adaptativas ou prejudiciais (RAPOPORT, 2017; RAPOPORT *et al.*, 2019).

Uma alternativa para reduzir esses impactos celulares é a utilização da microencapsulação. Estudos demonstram um aumento na proteção, e consequentemente, na viabilidade celular. A microencapsulação consiste em um processo em que partículas são revestidas ou incorporadas em uma matriz formando microcápsulas (GHARSALLAOUI *et al.*, 2007). Dentre outras vantagens encontram-se aumento da vida útil, proteção contra bacteriófagos e aumento da tolerância frente a agentes de intoxicação química. Além disso, facilita a manipulação e permite o aumento da distribuição homogênea em todo substrato a ser fermentado (MORTAZAVIAN *et al.*, 2007).

Grandes interesses são atribuídos à microencapsulação de compostos aromáticos, lipídeos, carotenoides e microrganismos (KANAKDANDE; BHOSALE; SINGHAL, 2007; SOOTTITANTAWAT *et al.*, 2005; VANDEN BRABER *et al.*, 2020). Em relação aos microrganismos, há estudos com bactérias probióticas (ALFARO-GALARZA *et al*., 2022; ANDRADE *et al*., 2019; LIN *et al*., 2019; ROSOLEN *et al*., 2019), fungos filamentosos (BRAGA *et al*., 2019) e leveduras (VANDEN BRABER *et al*., 2020; AGUIRRE-GUITRÓN *et al*., 2019; SURYABHAN *et al*., 2019). Após a microencapsulação, estudos mostraram que *Kluyveromyces marxianus* VM004 apresentou maior tolerância às condições gastrointestinais

simuladas (VANDEN BRABER *et al*., 2020) enquanto *Meyerozyma caribbica* foi eficaz no controle pós-colheita de *Colletotrichum gloeosporioides* em mangas.

Nesse contexto, *Saccharomyces cerevisiae* CCMA 0543, *Torulaspora delbrueckii* CCMA 0684 e *Meyerozyma caribbica* CCMA1738 foram isoladas do fruto do café e vêm sendo utilizadas na forma líquida como culturas iniciadoras durante a fermentação do café. Estas leveduras podem ser utilizados com o objetivo de produzir bebidas com sabores e aromas especiais (JIMENEZ *et al*., 2022; DA SILVA, *et al*., 2021; BRESSANI *et al.*, 2020; DA MOTA *et al.*, 2020; PEREIRA *et al.*, 2020). Porém, a utilização de culturas puras, na forma líquida, é inviável em relação à aplicação por parte dos produtores de café, devido a maior dificuldade e custos elevados com o transporte e armazenamento refrigerado, maior risco de contaminação e perda de viabilidade durante o armazenamento (TAN; POH; CHIN, 2018).

Há poucos trabalhos sobre microencapsulação de leveduras, sendo que a maioria estudou o comportamento de cepas de *Saccharomyces cerevisiae* (APONTE *et al.*, 2016; CHANDRALEKHA *et al.*, 2016; SURYABHAN; LOHITH; ANU-APPAIAH, 2019) e há apenas um estudo com *Meyerozyma caribbica*, isolada da manga (AGUIRRE-GÜITRÓN *et al.*, 2018). Até este momento, nenhum estudo com *Torulaspora delbrueckii*. Dito isto, faz-se necessário à busca por métodos economicamente viáveis e com maior grau de eficiência, como o spray drying, para microencapsulação das leveduras epifíticas do café: *Saccharomyces cerevisiae* CCMA 0543, *Torulaspora delbrueckii* CCMA 0684 e *Meyerozyma caribbica* CCMA 1738.

2 REFERENCIAL TEÓRICO

2.1 Preservação de microrganismos por secagem

A preservação de microrganismos por diferentes metodologias de secagem é utilizada a décadas e tem sido um ótimo método para armazenamento de culturas a longo prazo (ELIZONDO; LABUZA, 1974). Existem diversas coleções de culturas que dependem dos métodos de secagem para preservar uma vasta diversidade celular para propagações futuras. Em geral, o objetivo de qualquer tipo de secagem é permitir o armazenamento a longo prazo de microrganismos, preservando a viabilidade celular (MORGAN *et al.*, 2006). Assim, o microrganismo na forma seca oferece aplicações por um maior período de tempo em comparação com o microrganismo na forma líquida (TAN; POH; CHIN, 2018).

A preservação de microrganismos por secagem possibilita diversas aplicações na área de desenvolvimento científico e industrial como uso de culturas iniciadoras (DIMITRELLOU *et al.*, 2008) e agentes de biocontrole, como os biopesticidas (TEERA-ARUNSIRI; SUPHANTHARIKA; KETUNUTI, 2003). Além disso, a preservação também beneficia a melhoria de produtos relacionados à saúde, como comprimidos contendo probióticos (BANSAL; GARG, 2008) e suplementos funcionais em produtos alimentícios (PARVEZ *et al.*, 2006).

A secagem aborda o conceito da redução do teor de umidade dos bioprodutos, para aproximadamente 5-8%, no qual a biodegradação, causada pela atividade de microrganismos, enzimas ou reações químicas não enzimáticas é inibida (BÓRQUEZ *et al.*, 2013). Este conceito também pode ser aplicado para a preservação de microrganismos, no qual a água presente no meio de cultura é removida e as atividades microbianas são retidas. Além disso, culturas microbiológicas em estado seco, facilita o transporte, armazenamento, reduz o odor e permite melhor controle de qualidade (FU; CHEN, 2011). Há diversas metodologias de secagem para estes microrganismos e estas técnicas serão abordadas no próximo tópico.

2.2 Métodos de secagem de microrganismos

Embora exista alguns meios para preservar uma cultura desejada de microrganismos, alguns métodos podem ser mais adequados do que outros, dependendo do objetivo. Métodos de preservação limitados apenas à escala laboratorial podem ser diferentes dos aplicados à nível industrial, pois algumas técnicas pilotos podem ser simples e confiáveis, mas não escalonáveis (TAN; POH; CHIN, 2018). A liofilização tem sido utilizada para preservar microrganismos há décadas. Este é o principal método utilizado em coleções de culturas, incluindo a *American Type Collection* e a *National Collection of Type Cultures* (BOZOĞLU; ÖZILGEN; BAKIR, 1987).

A liofilização é realizada em três etapas: congelamento, secagem primária e secagem secundária. O congelamento é uma etapa importante no qual a solução aquosa ou a suspensão é passada para o estado sólido. Pode ser realizada a partir da imersão em nitrogênio líquido, em placa fria com temperatura regulada ou a partir da pulverização em um fluxo de gás frio. A secagem primária consiste na etapa de sublimação e a secagem secundária refere-se na eliminação da água ligada ou de parte do solvente que não estava congelado. Portanto é um método onde o material é desidratado por um processo onde a remoção de água ocorre sem submeter à amostra em altas temperaturas (CHEN; WANG, 2007).

A liofilização é capaz de fornecer manutenção da cultura por 10 a 20 anos. Além disso, proporciona produtos com alta qualidade porque diminui as mudanças relacionadas com a alta temperatura. Entretanto, há limitações no volume de secagem (FU; CHEN, 2011). Do ponto de vista econômico, o longo tempo e alto custo do processamento tornam a liofilização menos favorável para a produção em massa de células secas (SANTIVARANGKNA; KULOZIK; FOERST, 2007). Logo, a aplicabilidade comercial é prejudicada (MARQUES; SILVEIRA; FREIRE, 2006). Danos à membrana celular são causados durante o processo de congelamento devido à formação de cristais de gelo. Além disso, a alta pressão osmótica favorece condição de estresse (UZUNOVA-DONEVA; DONEV, 2002).

Outras abordagens de secagem foram investigadas com o objetivo de desenvolver um processo mais econômico para a desidratação de microrganismos. Estas vêm sendo investigadas quanto à sua viabilidade como alternativa ao processo de liofilização (FU; CHEN, 2011). Essas técnicas incluem: spray drying (VANDEN BRABER *et al.*, 2020), secagem em leito fluidizado (STRASSER *et al.*, 2009), secagem à vácuo (SANTIVARANGKNA; KULOZIK; FOERST, 2006), secagem por ar convectivo térmico em forno (DIMITRELLOU *et al.*, 2008) e secagem em leito de jorro (OLIVEIRA *et al.*, 2007). A secagem por spray drying será a técnica utilizada neste trabalho devido às suas vantagens e estas serão abordadas no próximo tópico.

2.2.1 Spray drying

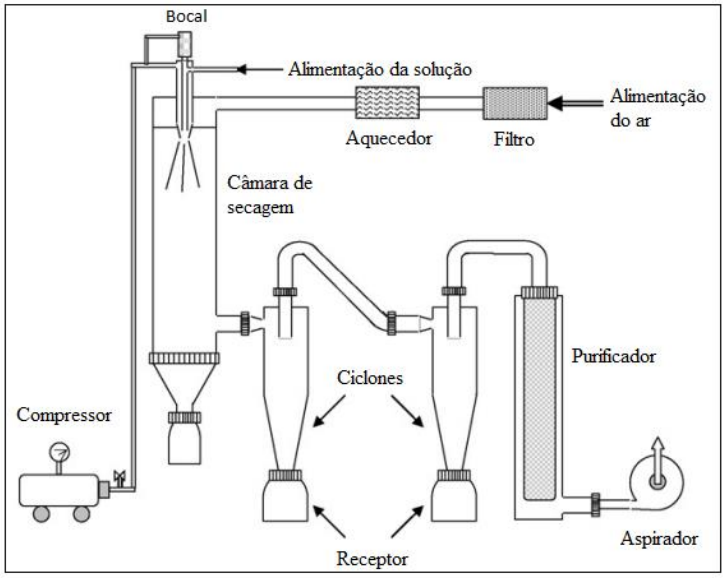
Na indústria alimentícia, o método de secagem mais utilizado é o spray drying. Este, é um método que produz um produto de boa qualidade, além de ser o mais econômico e flexível. Quando comparado à liofilização, o seu consumo de energia é de 6 a 10 vezes menor. Outra vantagem é que este processo pode ser realizado de forma contínua. Porém, a desvantagem é a alta temperatura utilizada durante o processo, o que pode afetar a viabilidade de certos microrganismos (MARTÍN *et al.*, 2015).

O spray drying é uma operação unitária que consiste na atomização de um produto líquido em uma corrente de gás quente para obter imediatamente um pó. O ar pode ser utilizado como gás ou menos frequentemente um gás inerte como, por exemplo, o nitrogênio. Dependendo do material de alimentação e das condições operacionais, pode-se produzir pós muito fino (10-50 µm) ou partículas maiores (2-3 mm) (GHARSALLAOUI *et al.*, 2007). Neste processo, alguns parâmetros são controlados, como condições da alimentação, o fluxo

de gás e a temperatura de entrada (MARTÍN *et al.*, 2015). Estas condições são otimizadas a partir das características desejáveis do produto e do pó (PEIGHAMBARDOUST; GOLSHAN TAFTI; HESARI, 2011).

De acordo com Devakate *et al*. (2009), o spray dryer consiste em uma bomba de alimentação, atomizador, aquecedor e dispersor de ar, câmara de secagem e equipamentos destinados à descarga do produto, transporte, embalagem e remoção do ar (FIGURA 1).

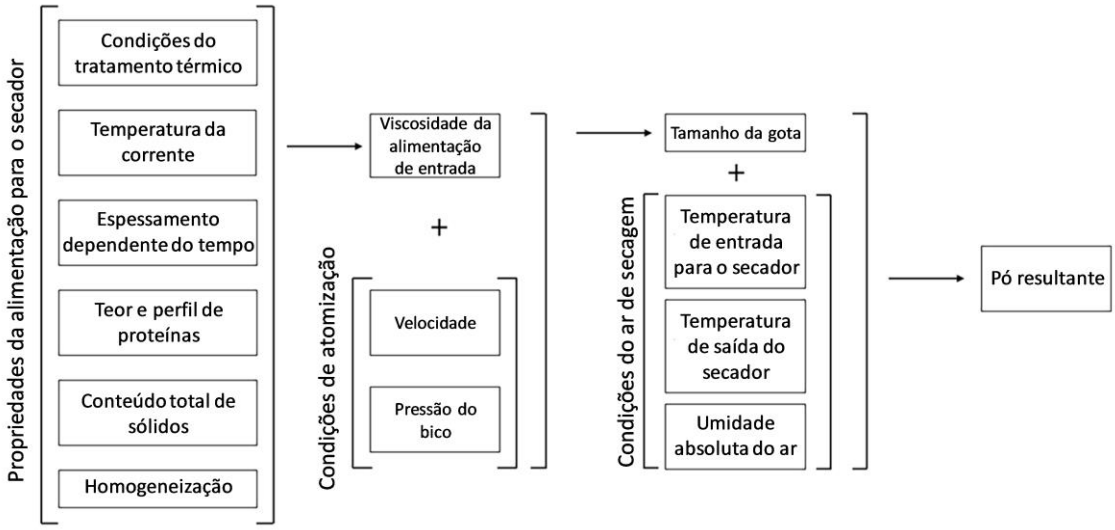
Figura 1 – Esquema do spray dryer.



Fonte: Adaptado de Devakate et al. (2009).

A atomização é uma etapa relevante, pois é neste ponto que ocorre a conversão do fluido em gotículas com o objetivo de aumentar a área superficial do material a ser secado. Isto é importante, porque a taxa de evaporação é diretamente proporcional à área superficial da gota (PATEL; PATEL; SUTHAR, 2009). A atomização ocorre geralmente no topo da câmara principal, através de um dispositivo conhecido como atomizador (FILKOVÁ; MUJUMDAR, 1995). Há diferentes tipos: rotativos, bicos de pressão, pneumáticos e ultrassônicos. Sobretudo, outros fatores também afetam a obtenção do pó (FIGURA 2) (O'SULLIVAN *et al*., 2019).

Figura 2 – Fatores que afetam a secagem por spray drying.



Fonte: Adaptado de Sullivan (2019).

Dessa forma, vários fatores influenciam o pó obtido, como as propriedades da alimentação e as condições de atomização e do ar de secagem (O'SULLIVAN *et al*., 2019).

Devido à diminuição do teor e da atividade de água, o spray drying é uma técnica utilizada para garantir uma maior estabilidade do produto, evitar degradações químicas e/ou biológicas, reduzir os custos em relação ao armazenamento e transporte e para obter produtos com alguma propriedade específica (GHARSALLAOUI *et al*., 2007). Porém, há diversos fatores importantes que afetam a viabilidade celular durante o processo de secagem.

2.3 Fatores que afetam a sobrevivência celular em um processo de secagem

A sobrevivência celular é o parâmetro mais importante uma vez que a técnica de secagem só será útil se os microrganismos mantiverem sua função e reprodutibilidade (TAN; POH; CHIN, 2018). O requisito fundamental para manter a viabilidade celular é manter as estruturas celulares essenciais intactas após a secagem e totalmente funcionais após a reidratação. Os fatores que influenciam a viabilidade celular durante a secagem térmica são divididos em dois grupos: intrínsecos, relacionados aos microrganismos que serão secos e extrínsecos, relacionados às condições do processo (FU; CHEN, 2011).

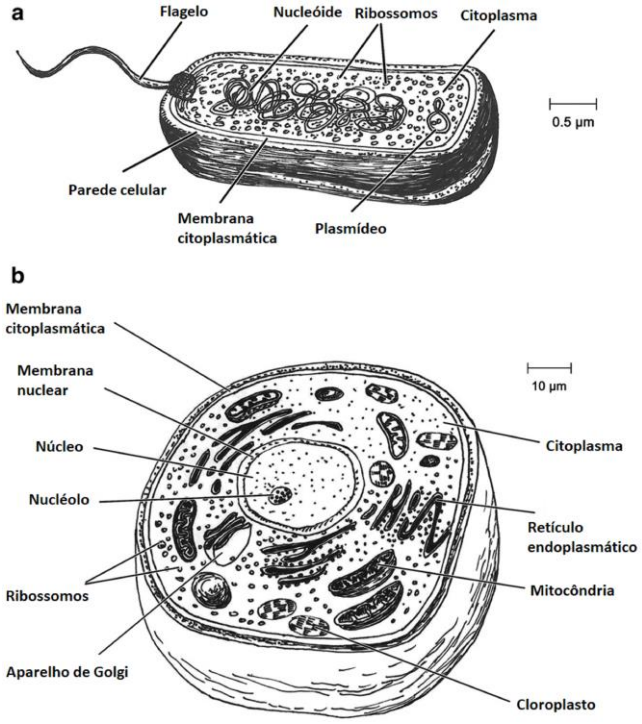
2.3.1 Fatores intrínsecos

A sobrevivência intrínseca refere-se à capacidade da própria célula resistir a inativação. O requisito básico de manutenção da sobrevivência celular é a capacidade de

impedir que a secagem danifique as estruturas celulares vitais. Esta, varia muito entre os diferentes microrganismos (ALONSO, 2016). Foi sugerido que a tolerância a tensões térmicas e mecânicas é maior em bactérias Gram-positivas, seguida de leveduras e por último, bactérias Gram-negativas. Possivelmente devido à fina estrutura da parede celular das mesmas, o que as torna mais susceptíveis à alterações nas condições ao redor das células (DONSÌ *et al*., 2009).

Os conídios produzidos por fungos proporcionam uma taxa de sobrevivência melhor após a secagem térmica quando comparado com microrganismos na forma vegetativa. As principais razões é a alta resistência dos conídios à secagem e outros estresses ambientes que estão relacionados à sua estrutura única (HORACZEK; VIERNSTEIN, 2004). Em relação às células vegetativas, as estruturas celulares de microrganismos procariótico e eucarióticos são diferentes (FIGURA 3).

Figura 3 – Estruturas celulares de (a) uma célula procariótica e (b) uma célula eucariótica.



Fonte: Adaptado de MADIGAN et al. (2016).

Entretanto, a tolerância ao estresse de microrganismos varia de espécie para espécie bem como de tensão para tensão. Por exemplo, para uma mesma cepa, a tolerância ao estresse será diferente para células cultivadas em diferentes meios de cultura e/ou utilizadas em diferentes estágios de crescimentos. Por isso é difícil comparar os resultados relatados na literatura (FU; CHEN, 2011). Apesar das variações de tolerâncias ao estresse entre os

microrganismos, acredita-se que os mecanismos relacionados ao dano celular sejam os mesmos para todos os organismos em nível molecular (CROWE; HOEKSTRA; CROWE, 1992).

Durante o processo de secagem térmica há duas tensões significativas para as células microbianas: calor e desidratação. Os mecanismos de danos induzidos por essas tensões à nível celular são diferentes (SANTIVARANGKNA; KULOZIK; FOERST, 2008). Alguns componentes celulares sensíveis ao calor incluem DNA / RNA, proteínas e enzimas (VAN DE GUCHTE *et al*., 2002). Além disso, esta tensão pode atuar na degradação de estruturas celulares essenciais, como por exemplo, no ribossomo (OBUCHI *et al*., 2000).

O estresse por desidratação causa tensões osmóticas e oxidativas. Deste modo, afeta principalmente a membrana citoplasmática, alterando sua fluidez ou estado físico, além de causar peroxidação lipídica (GARRE *et al*., 2010). A peroxidação lipídica refere-se à quebra da fração lipídica na membrana celular através de uma reação em cadeia da oxidação movida a radicais (MYLONAS; KOURETAS, 1999). Como resultado, ocorre o vazamento seguido pela perda de substâncias celulares essenciais a vida, tornando-se fatal a célula (GARRE *et al*., 2010).

Dessa forma, ao longo do processo de evolução, os microrganismos desenvolveram a capacidade de se adaptarem aos fatores extremos do meio ambiente, tais como congelamento, descongelamento e seca. Essas condições ambientais promovem à suspensão reversível temporária do metabolismo dos organismos vivos e esse estado é chamado de anabiose ou criptobiose. No caso de desidratação, como ocorre no processo de secagem, as células podem entrar no estado conhecido como anidrobiose (RAPOPORT *et al*., 2019).

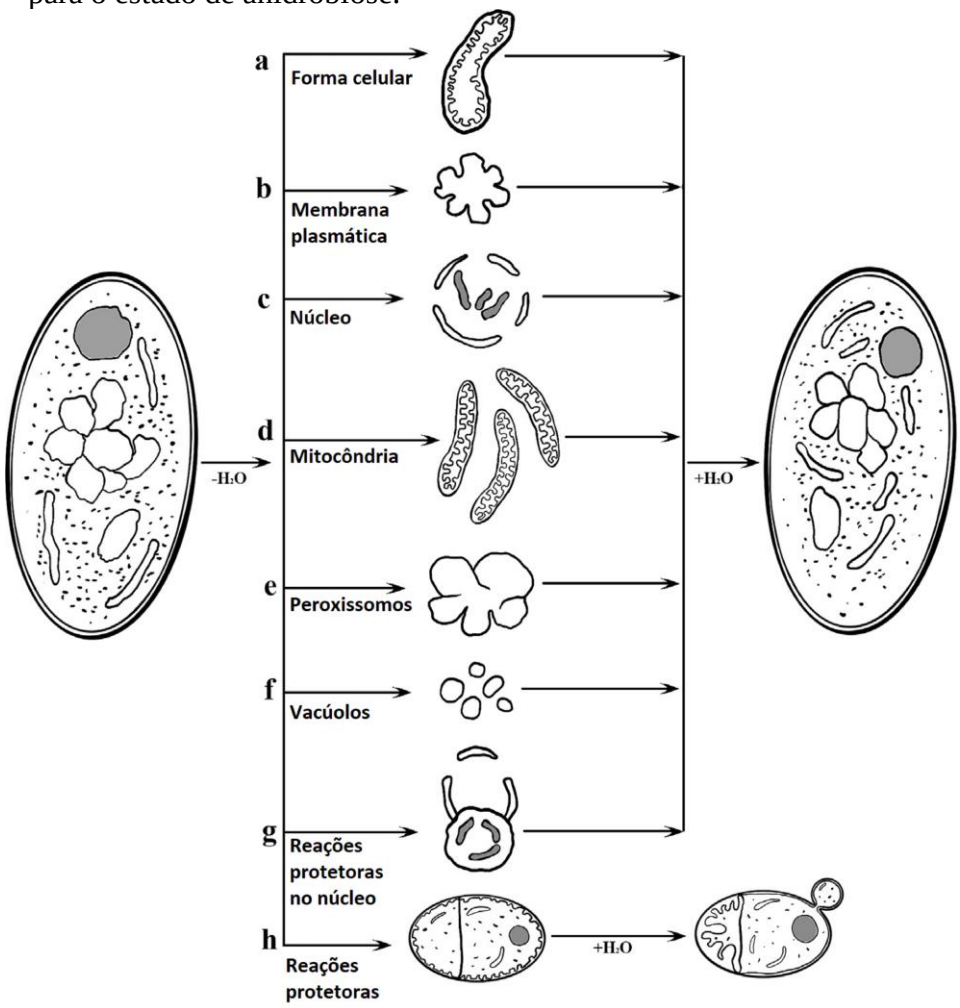
2.3.1.1 Anidrobiose em células de leveduras

O estado de anidrobiose é utilizado para a produção industrial de leveduras secas ativas. Dessa forma, levedura seca ativa é aquela no estado de anidrobiose em que seu metabolismo é temporariamente suspenso de forma reversível, caso haja manutenção da viabilidade celular, como resultado de sua forte desidratação (RAPOPORT, 2017).

A desidratação de microrganismos permite manter as principais características fisiológicas de diferentes culturas, porém a desidratação e reidratação induzem restrições mecânicas, estruturais e oxidativas que podem causar a morte celular (DUPONT *et al*., 2014). Assim, é preciso entender como essas restrições afetam as diferentes estruturas celulares. Durante a desidratação de leveduras, mudanças estruturais e funcionais essenciais ocorrem em

praticamente todas as organelas e componentes celulares (FIGURA 4) (RAPOPORT *et al*., 2019).

Figura 4 – Importantes mudanças estruturais nas células de levedura durante sua transição para o estado de anidrobiose.



Fonte: Adaptado de RAPOPORT et al., (2019).

Os principais componentes da parede celular das leveduras são manoproteínas, β-glucanos e quitina (MORENO *et al*., 2008). Dito isto, as mudanças mais expressivas que ocorrem na parede celular durante a secagem são modificações na forma. Isto ocorre devido ao alongamento celular provocado pelas alterações de glucano e quitina (FIGURA 4a). Além disso, foi relatado o aparecimento de fibrilas e aumento das cargas positivas e negativas da superfície da célula devido às alterações das manoproteínas. Essas alterações são reversíveis, porém possíveis danos às proteínas podem ser desfavorável para resistência e viabilidade da célula (BOROVIKOVA *et al*., 2016; RAPOPORT *et al*., 2019).

Em relação à membrana plasmática, há formação de dobras para manter a sua integridade devido à vasta diminuição do volume celular (FIGURA 4b). O processo de

desidratação causa a diminuição do espaçamento entre os fosfolipídeos da membrana e o ordenamento das cadeias de hidrocarbonetos. Alguns carboidratos, como a trealose, interagem com os grupos polares dos fosfolipídeos quando a água é removida e consequentemente permite um maior espaçamento entre os lipídeos, diminuindo a desordem das cadeias dos hidrocarbonetos (CROWE; HOEKSTRA; CROWE, 1992; LESLIE *et al*., 1994). Maiores detalhes serão discutidos no tópico 4.4.1.

Danos nas proteínas da membrana também podem ser perigosos para a sobrevivência celular durante a desidratação e reidratação, como aquelas transportadoras de potássio e glicerol (BOROVIKOVA *et al*., 2014; DUSKOVA *et al*., 2015). Além disso, o ergosterol, componente bioquímico principal da membrana plasmática de levedura, desempenha papel essencial durante a desidratação. Este confere estabilidade mecânica e estrutural, bem como proteção à danos de oxidação causados pela transição do meio aquoso para o ar (DUPONT *et al*., 2011).

Em relação às mitocôndrias há ausência de alterações estruturais visíveis, porém como mecanismo de proteção foi relatado à condensação do DNA mitocondrial (FIGURA 4d) (RAPOPORT *et al*., 2019). As principais características do mtDNA não são alterados após a desidratação, porém o sistema de transporte de elétrons da mitocôndria pode ser interrompido durante a desidratação (SHIMA; ANDO; TAKAGI, 2008). Há pouca informação sobre a reação da mitocôndria no processo de desidratação-reidratação, mas sabe-se que estas são primeiras estruturas que restauram sua atividade (RAPOPORT, 2017).

A desidratação acarreta a diminuição do tamanho de grandes vacúolos e/ou na fragmentação, bem como mudanças em sua forma (FIGURA 4f). Além disso, há a redistribuição de proteínas em algumas regiões das membranas vacuolares (RAPOPORT, 2017; RAPOPORT *et al*., 2019). Já nos peroxissomos, a desidratação leva a mudanças essenciais no estado dos lipídeos de suas membranas (FIGURA 4e) (SIBIRNY, 2016).

Em relação às gotículas lipídicas, um estudo mostrou que a tolerância de algumas leveduras a desidratação aumentava se as condições de cultivo levassem ao acúmulo de maiores quantidades de fosfatidilcolina pelas células. Logo, concluiu-se a correlação positiva entre a resistência das células de levedura à desidratação-reidratação e a soma de éster de esterol e triacilgliceróis armazenados por essas células. Dessa forma, foi sugerido que esses lipídios neutros poderiam contribuir para diferentes processos metabólicos e de reparo, que ocorrem nas células de levedura durante a reativação (RODRÍGUEZ-PORRATA *et al*., 2011).

Por fim, no núcleo ocorre a condensação da cromatina (FIGURA 4c). Esta é uma das principais reações protetoras intracelulares, pois protege o genoma de possíveis danos durante os processos de desidratação e reidratação. Em alguns casos, a cromatina condensada se encontra em um dos polos. Em seguida, ocorre a formação de novas membranas nucleares para separar a parte que contém a cromatina do restante. No momento de reidratação da levedura, a parte do núcleo sem a cromatina sofre autólise gradual. Assim, sugere-se que esta reação diminui a quantidade de água ligada necessária para manter a viabilidade celular no estado de anidrobiose (RAPOPORT, 2017; RAPOPORT *et al*., 2019).

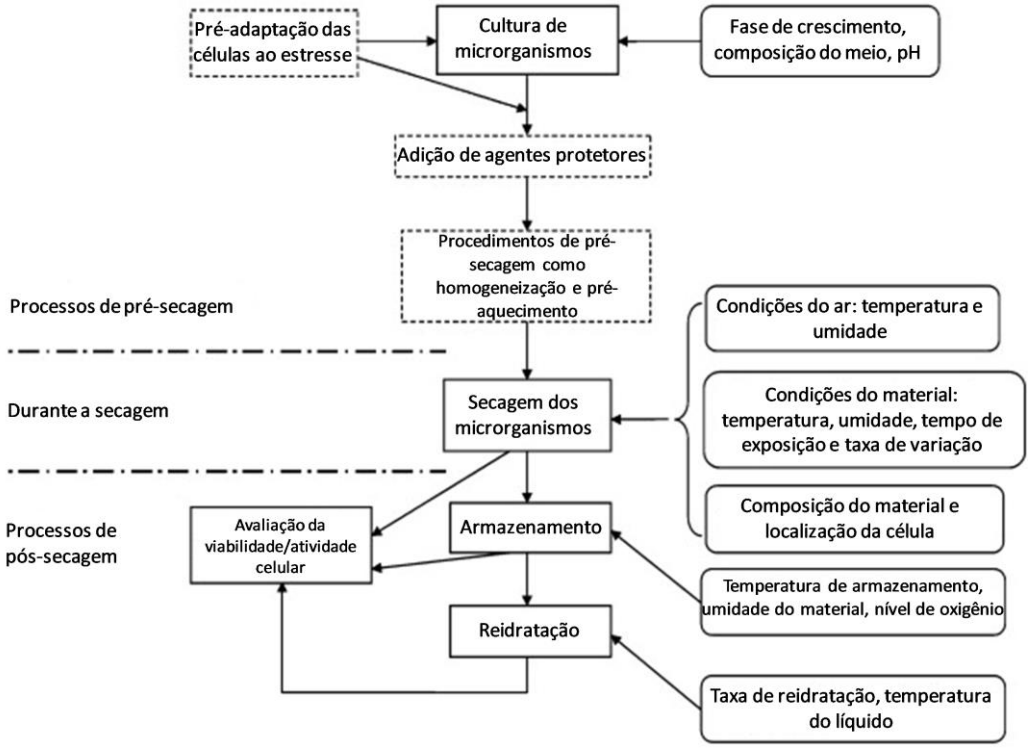
O processo de secagem também pode levar a mudanças essenciais no estado dos lipídeos nas membranas nucleares. Porém, a desidratação não leva a mudanças de sequência no DNA. Genes importantes para a tolerância à dessecação são transcritos e traduzidos antes e durante a desidratação (CALAHAN *et al*., 2011). Dessa forma, a transição das células de levedura não leva a nenhum dano grave ao genoma e ao núcleo celular (JENKINS; POWELL; SMART, 2010).

Portanto, praticamente todos os componentes intracelulares das leveduras sofrem mudanças estruturais que podem ser adaptativas ou prejudiciais. Os mecanismos mais importantes para sobrevivência celular na anidrobiose são: estabilidade dos ácidos nucleicos, condensação da cromatina, preservação da integridade da membrana, acúmulo de substâncias protetoras intracelulares e capacidade das leveduras de iniciar os mecanismos de proteção (RAPOPORT *et al*., 2019).

2.3.2 Fatores extrínsecos

Como citado anteriormente, os fatores extrínsecos estão relacionados às condições do processo de secagem que afetam a viabilidade celular. O processo pode ser dividido em três fases: pré-secagem, secagem e pós-secagem (FIGURA 5).

Figura 5 – Principais etapas do processamento durante a secagem de microrganismos e fatores extrínsecos importantes em cada etapa.



Fonte: Adaptado de FU; CHEN (2011).

Em relação à fase de pré-secagem, as condições do microrganismo que influenciam na viabilidade celular durante o processo incluem: fase de crescimento, composição e pH do meio. De acordo com os estudos, as células colhidas na fase estacionária apresentaram maior tolerância ao estresse em relação à fase lag e exponencial (LINDERS *et al*., 1997; MENG *et al*., 2008). Essa resistência é considerada um resultado da resposta ao estresse devido à falta de nutrientes e o acúmulo de metabólitos tóxicos que ocorrem durante a fase estacionária.

A composição do meio de cultura atua sobre a influência do fluxo metabólico das células microbianas (FU; CHEN, 2011). Este, ao ser manipulado ou ao fornecer ambientes estressantes, podem acumular solutos compatíveis como produtos finais do metabolismo. Alguns exemplos são glicerol, betaína, dissacarídeos não redutores e aminoácidos (KETS; TEUNISSEN; DE BONT, 1996; KETS; BONT, 1994; WELSH, 2000). Este acúmulo dentro de uma célula protege-a do estresse osmótico durante a desidratação. Isto é alcançado equilibrando a diferença osmótica entre o interior e o exterior da membrana celular (DESMOND *et al*., 2001). O melaço é um meio adequado para o crescimento de cepas de leveduras durante os processos de fermentação. Durante a fase inicial, o alto teor de açúcar presente no melaço aumenta significativamente a pressão osmótica a que as células de leveduras são submetidas, permitindo assim um potencial acúmulo de solutos compatíveis (GÓMEZ-PASTOR *et al*., 2011).

Outro fator importante é o pH, porém há resultados contraditórios relatados na literatura (LINDERS *et al*., 1997; SILVA *et al*., 2005). Isto pode ser atribuído às diferentes cepas e condições de secagem utilizadas (FU; CHEN, 2011).

Após serem coletadas do meio de crescimento, as células microbianas são geralmente submetidas a tratamentos adicionais antes de serem secadas. Em uma escala laboratorial, estas etapas incluem centrifugação e ressuspensão em veículos de proteção. Em um processo industrial, varia de acordo com o equipamento utilizado, como por exemplo, homogeneização e atomização para o spray dryer. Os portadores de proteção podem ser agrupados em três categorias: portador no estado líquido, no estado sólido e utilizado para encapsular as células (FU; CHEN, 2011). Estes serão abordados com maiores detalhes no tópico 4.4.1.

Durante o processo de secagem térmica há vários fatores importante, mas a temperatura de saída na secagem por spray drying foi reconhecida como a de maior impacto na viabilidade celular. Estudos mostram que uma temperatura de saída mais alta acarreta em um aumento da morte celular (BOZA; BARBIN; SCAMPARINI, 2004; WANG; YU; CHOU, 2004). A temperatura de saída é uma indicação da alta temperatura que as células podem ter sido expostas dentro do secador. Alguns estudos mostraram que a variação da temperatura de saída entre 40 a 60°C produziu uma taxa de sobrevivência satisfatória após a secagem por spray drying (HORACZEK; VIERNSTEIN, 2004; REDDY; MADHU; PRAPULLA, 2009).

Mesmo que seja obtido um produto seco contendo microrganismos viáveis após a secagem, estes precisam ser mantidos durante o período de armazenamento e após a reidratação (FU; CHEN, 2011). Além disso, os microrganismos na forma seca podem ser utilizados para diferentes aplicações. Por exemplo, para as leveduras ou culturas iniciadoras secas, a atividade de fermentação representa o critério mais importante (DIMITRELLOU *et al*., 2009; TSAOUSI; DIMITRELLOU; KOUTINAS, 2008).

Durante o armazenamento a taxa de diminuição da viabilidade celular está relacionada à temperatura de armazenamento. Foram observadas maiores perdas em temperaturas de armazenamento mais elevadas (KEARNEY *et al*., 2009), entretanto temperatura mais baixa (4 °C) pode prolongar o tempo de armazenamento sem perda significativa da viabilidade celular (DIMITRELLOU *et al*., 2008). Este mecanismo de perda provavelmente ocorre devido à degradação natural das macromoléculas essenciais à vida, uma vez que lipídeos e proteínas sofrem oxidação e desnaturação durante um longo período de armazenamento (FU; CHEN, 2011).

Outro fator importante é a umidade do produto seco. Um baixo nível de umidade é mais vantajoso (YING *et al*., 2010). Em relação ao oxigênio, o armazenamento sob vácuo

fornece melhor retenção na atividade celular do que sem vácuo (CHÁVEZ; LEDEBOER, 2007). Portanto, pode-se observar que fatores que permitem o livre movimento de moléculas e favorecem as reações bioquímicas tendem a diminuir a viabilidade celular durante o armazenamento, como a alta temperatura, umidade e exposição ao ar.

Por fim, a reidratação também afeta o número de células viáveis porque cria outro desafio osmótico. Esta etapa pode levar a ruptura da membrana citoplasmática e/ou vazamento do conteúdo celular (MULLER *et al*., 2010). Por isso recomenda-se uma taxa lenta de reidratação para obter maior recuperação celular (POIRIER *et al*., 1999).

Todos esses parâmetros também podem variar de acordo com a técnica utilizada. Apesar de ser considerado um processo de desidratação, o spray drying pode ser utilizado para microencapsular o material desejado, como as culturas iniciadoras. O objetivo é utilizar um material de parede com o intuito de proteger às células (FU; CHEN, 2011).

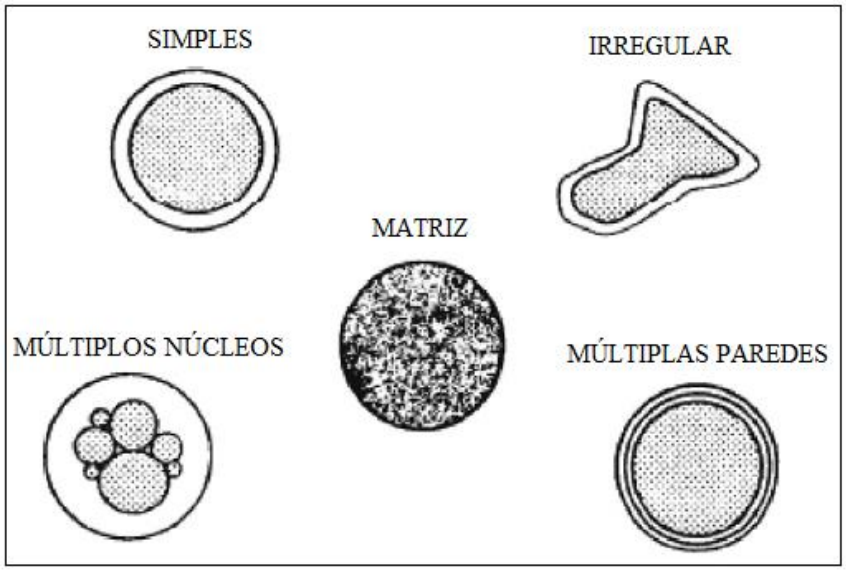
2.4 Microencapsulação

A microencapsulação consiste em um processo em que partículas são revestidas ou incorporadas em uma matriz homogênea ou heterogênea. Esta, é uma tecnologia de embalagem de materiais no estado sólido, gasoso ou líquido a partir de finos revestimentos poliméricos onde se formam partículas denominadas de microcápsulas (GHARSALLAOUI *et al*., 2007).

As microcápsulas possuem formato esférico e diâmetros entre alguns micrômetros e milímetros. A classificação das cápsulas geralmente é feita de acordo com o seu tamanho podendo ser classificadas em macrocápsulas (> 5 μm), microcápsulas (0,2 a 5 μm) ou em nanocápsulas (<0,2 μm) (SILVA *et al*., 2014).

O material dentro da microcápsula é chamado de núcleo, fase interna ou preenchimento e a parede é denominada de revestimento, material de parede ou membrana. A forma e o tamanho das microcápsulas dependem dos materiais e dos métodos utilizados para encapsular, podendo ser como um revestimento em membrana simples, parede ou membrana irregular, estrutura de múltiplas paredes ou múltiplos núcleos ou de composições variáveis (FIGURA 6) (GIBBS *et al*., 1999).

Figura 6 – Diferentes tipos de formas das cápsulas.



Fonte: Adaptado de GIBBS et al. (1999).

Portanto, podem ser obtidos diversos tipos de partículas de acordo com as propriedades físico-químicas do núcleo, composição da parede e técnica de microencapsulação escolhida (GIBBS *et al*., 1999). Como já mencionado anteriormente, a secagem por spray drying é a técnica mais utilizada na indústria de alimentos por ser econômico, flexível, adaptável ao equipamento e por produzir partículas de boa qualidade (DESAI; PARK, 2005).

No setor alimentício, o uso desta técnica aumentou, pois, os materiais microencapsulados podem ser protegidos de condições extremas como umidade e calor, aumentando a estabilidade e mantendo a viabilidade (GIBBS *et al*., 1999). Além disso, também pode ser utilizado para impedir perdas nutricionais; incorporar mecanismos de liberação; mascarar ou preservar aromas e sabores; utilizar ingredientes sensíveis e transformar líquidos em sólidos de fácil manuseio (DESAI; PARK, 2005).

Em relação à microencapsulação de células, há vários benefícios, uma vez que ela pode ser utilizada de forma eficiente para preparar culturas iniciadoras com maior viabilidade. Além disso, proporciona a proteção contra bacteriófagos e aumento da tolerância frente a agentes de intoxicação química. Consequentemente, protege as células contra mudanças indesejadas como, por exemplo, as mutações genéticas. Logo, a microencapsulação permite o aumento da vida útil, convertendo-os em forma de pó. Isto facilita a manipulação e permite o aumento da distribuição homogênea em todo o produto (MORTAZAVIAN *et al*., 2007).

Porém, a escolha do material de parede da microcápsula tem grande influência nas propriedades da substância encapsulada, especialmente na eficiência do processo e por isso deve ser escolhida com cuidado (DIAS *et al*., 2017). Além disso, pode-se utilizar portadores

de proteção para uma melhor sobrevivência microbiana durante o processo de secagem (FU; CHEN, 2011).

2.4.1 Portadores de proteção

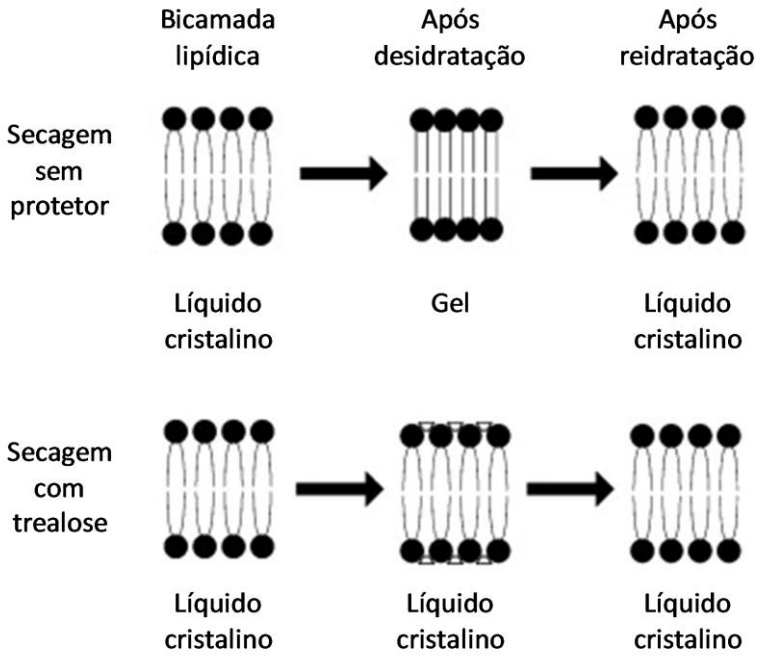
Os portadores de proteção comumente utilizados podem ser agrupados em: transportador no estado líquido, transportador no estado sólido e transportador utilizado para encapsular células (FU; CHEN, 2011).

Os transportadores na forma líquida têm dois mecanismos de proteção às células (FU; CHEN, 2011). Primeiramente, alguns compostos como a trealose, podem reagir e estabilizar estruturas celulares durante os processos de secagem e reidratação. Além disso, a matriz pode atuar como uma blindagem física, aliviando o estresse térmico e osmótico durante a secagem, bem como o estresse osmótico durante a reidratação (CROWE; HOEKSTRA; CROWE, 1992).

Geralmente, preferem-se os açúcares devido ao seu baixo custo, natureza química inócua e uso comum na indústria de alimentos (SANTIVARANGKNA; HIGL; FOERST, 2008). Além disso, a presença de diferentes açúcares fermentáveis no meio de crescimento leva à formação de metabólitos, como o manitol, que podem aumentar a viabilidade das culturas durante a secagem (SANTIVARANGKNA; KULOZIK; FOERST, 2008).

Os açúcares não fermentáveis exercem um estresse hiperosmótico nas células. Consequentemente induz o acúmulo de solutos compatíveis, tornando-as mais resistentes ao estresse osmótico durante a secagem (SANTIVARANGKNA; KULOZIK; FOERST, 2008). Os dissacarídeos podem atuar como alternativa às moléculas de água, sustentando a conformação original da bicamada lipídica da membrana celular (FIGURA 7) (CROWE; HOEKSTRA; CROWE, 1992; CROWE, 2002).

Figura 7 – Esquema da potencial transição de fase da membrana celular na presença e ausência de trealose, após a desidratação e reidratação.



Fonte: Adaptado de CROWE; HOEKSTRA; CROWE (1992).

Além disso, os dissacarídeos podem retardar a desnaturação das proteínas através de ligações de hidrogênio com as proteínas, preservando a estrutura celular (PEIGHAMBARDOUST; GOLSHAN TAFTI; HESARI, 2011). Assim, a trealose é um dos melhores protetores contra estresses térmicos e osmóticos e apresenta um efeito protetor universal nas células anidrobióticas (CROWE; HOEKSTRA; CROWE, 1992). Porém, a desvantagem é o custo elevado.

Os transportadores na forma sólida são misturados sem solução, ou seja, diretamente aos péletes celulares obtidos após a centrifugação. Porém, o efeito ainda é pouco conhecido. Finalmente, os transportadores por encapsulamento, conhecidos como material de parede, formam microcápsulas que contém células durante a secagem por spray drying (FU; CHEN, 2011).

Dito isto, a escolha adequada do material de parede é muito importante porque influencia na eficiência e estabilidade da microcápsula durante o armazenamento. Esta, baseia-se nas propriedades físico-químicas, como a solubilidade, peso molecular, propriedades formadoras de filmes e capacidade de formar emulsões (GHARSALLAOUI *et al.*, 2007). Além disso deve ter características como não ser reativo com o núcleo, viabilidade econômica e ter a capacidade de fornecer a máxima proteção contra condições ambientais tais como oxigênio, calor, luz e umidade (DESAI; PARK, 2005).

A escolha do material de parede na microencapsulação por spray drying envolve procedimentos de tentativa e erro. As microcápsulas formadas são avaliadas quanto à

eficiência de encapsulamento, estabilidade sob diferentes condições de armazenamento, grau de proteção fornecido ao material do núcleo, observações por microscopia, entre outras avaliações (GHARSALLAOUI *et al*., 2007).

Grande parte dos materiais de parede não possuem todas as propriedades desejadas, portanto uma prática comum é a mistura de dois ou mais materiais. Estes materiais podem ser selecionados a partir de polímeros naturais e sintéticos como, por exemplo, os carboidratos, incluindo o amido, amidos modificados, sacarose, lactose, maltose e maltodextrina; as gomas como a goma arábica, alginato e carragena; lipídeos como as ceras, parafinas, óleos e gorduras hidrogenadas; proteínas como proteínas do soro ou soro do leite, glúten, caseína, gelatina e albumina, entre outros (SILVA et al., 2014).

Dessa forma, a busca por protetores econômicos e eficientes faz-se necessário. Materiais de parede mostraram-se efetivos para secagem de microrganismos como sacarose (SURYABHAN; LOHITH; ANU-APPAIAH, 2019), sorbitol e maltose (APONTE *et al*., 2016; LINDERS *et al*., 1997) e maltodextrina (REDDY; MADHU; PRAPULLA, 2009). Maltodextrina são carboidratos formados pela hidrólise parcial de amidos e são quimicamente compostas por unidades de D-glicose conectadas em cadeias de comprimento variável. Estas têm sido amplamente utilizadas como material de parede em secagem por spray drying devido a sua eficácia, baixo custo, boa solubilidade, controle de umidade, entre outros (ARSLAN *et al*., 2015; CHANDRALEKHA *et al*., 2016; SURYABHAN; LOHITH; ANU-APPAIAH, 2019).

As proteínas também são excelentes materiais para microencapsulação de microrganismos (ARSLAN *et al*., 2015; BRAGA et al., 2019; CHANDRALEKHA *et al*., 2016; VANDEN BRABER *et al*., 2020). Muitas proteínas têm sido amplamente utilizadas como agentes de revestimento devido às suas propriedades que atuam como uma boa barreira contra a permeabilidade ao oxigênio e ao gás carbônico. Cada proteína possui um conjunto único de propriedades físico-químicas. Dessa forma, sua sequência particular de aminoácidos permite uma ampla variedade de interações intra e intermoleculares (PECH-CANUL *et al*., 2020).

Em relação às leveduras, Arslan *et al*. (2015) avaliaram diferentes materiais encapsulantes (gelatina, concentrado proteico do soro de leite, amido modificado, maltodextrina, proteína isolada da ervilha e goma arábica) na secagem de células de leveduras (*Saccharomyces cerevisiae* var. *boulardii*), por spray drying. O maior rendimento foi obtido com o uso do concentrado proteico do soro de leite e goma arábica.

Em outro estudo, Chandralekha *et al*. (2016) avaliaram a viabilidade das células de leveduras (*Saccharomyces cerevisiae*) após a secagem por spray drying utilizando diferentes materiais de parede: maltodextrina, amido de milho, goma arábica, goma acácia, polietilenoglicol 8000, betaciclodextrina e leite em pó. Dentre esses materiais o amido de milho e a maltodextrina apresentaram maior rendimento e sobrevivência celular.

Dito isto, a microencapsulação fornece vários benefícios aos materiais que serão encapsulados (DESAI; PARK, 2005). A aplicação na indústria de alimentos é vasta e o estudo da preservação de microrganismos por spray drying tem sido amplamente explorada como um processo industrial alternativo para preservação de culturas iniciadoras (PEIGHAMBARDOUST; GOLSHAN TAFTI; HESARI, 2011).

2.5 Aplicações da microencapsulação

A microencapsulação proporcionou um grande impacto na indústria alimentícia. Tanto na ciência dos alimentos como na biotecnologia, esta técnica envolve a integração de ingredientes naturais, aditivos voláteis, enzimas, microrganismos e poli fenóis em microcápsulas atribuindo maiores estabilidade, deixando-os protegidos e preservados (NAZZARO *et al*., 2012).

Os compostos aromatizantes contribuem para o aroma característico dos alimentos. Estes são altamente voláteis e assim facilmente perdidos e degradados. Por isso, a microencapsulação de aromas é muito importante para as indústrias aromatizantes já que os sabores de alimentos microencapsulados apresentam uma maior estabilidade química (GHARSALLAOUI *et al*., 2007).

Em alguns alimentos, os microrganismos probióticos devem sobreviver em um número significativo de 10^6 a 10^8 UFC/g. O crescimento, a sobrevivência e a morte desses microrganismos dependem das condições de armazenamento como temperatura e umidade, e das propriedades dos alimentos como o pH, disponibilidade de água, entre outros (BURGAIN *et al*., 2011). A microencapsulação revelou ser um dos métodos mais eficientes para manter a estabilidade e viabilidade dos microrganismos uma vez que fornece proteção durante o processamento e armazenamento dos alimentos, além de proteger contra as condições gástricas (MARTÍN *et al*., 2015).

Diversos estudos sobre secagem de bactérias probióticos a partir do spray drying foram realizados (ALFARO-GALARZA *et al*., 2020; ANDRADE et al., 2019; LIN *et al*., 2019; ROSOLEN *et al*., 2019).

Em relação aos fungos filamentosos, Braga *et al*., (2019) avaliaram o processo de microencapsulação de *Trichoderma asperellum* por spray drying para uso na agricultura como controlador de fitopatógenos. Entre os materiais de parede utilizados, a maltodextrina foi responsável pela maior sobrevivência de conídios com temperatura do ar de secagem a 90°C.

Em relação as leveduras, Vanden Braber *et al*. (2020) avaliaram o efeito da microencapsulação de *Kluyveromyces marxianus* VM004 por spray drying na viabilidade durante a secagem e armazenamento e em condições gastrointestinais simuladas. A eficiência de encapsulamento foi de 91% para uma suspensão de 10% de concentrado de proteina de soro de leite. Além disso, a levedura microencapsulada apresentou tolerância significativamente maior às condições gastrointestinais simuladas.

Outro estudo avaliou a aplicação de *Meyerozyma caribbica* seca por spray drying no controle pós-colheita de *Colletotrichum gloeosporioides* em mangas. O uso da levedura foi eficaz no controle pós-colheita do patógeno fúngico em frutas armazenadas à 25 °C, porém foi menos eficiente à 13 °C. Além disso, a aplicação da levedura não afetou os parâmetros de qualidade das mangas durante o armazenamento (AGUIRRE-GÜITRÓN *et al*., 2019).

Duas potenciais leveduras probióticas *Saccharomyces cerevisiae* (cepa KTP) e *Issatchenkia occidentalis* (ApC) foram microencapsuladas por spray drying. A *Saccharomyces cerevisiae* var. *boulardii* (NCDC 363) foi utilizada como referência. As microcápsulas de maltodextrina incorporadas com sacarose ou sorbitol obtiveram um incremento de 35 a 45% na viabilidade celular das leveduras. A microencapsulação também melhorou o crescimento das leveduras na condição gastrointestinal simulada (SURYABHAN; LOHITH; ANU-APPAIAH, 2019).

Como visto anteriormente há diversos fatores que afetam a microencapsulação de microrganismos, como a cepa utilizada, o cultivo em determinado meio de cultura e os diferentes estágios de crescimentos em que às células são colhidas. Isto dificulta criar um determinado padrão para os microrganismos e como consequência torna-se difícil comparar os resultados relatados por diferentes grupos de pesquisa (FU; CHEN, 2011). Portanto, para cada cepa é necessário um estudo em relação às condições de processo e material de parede a ser utilizado.

Dito isto, a microencapsulação por spray drying das leveduras epifíticas do café, utilizadas como culturas iniciadoras durante o processo fermentativo, têm demonstrado ser uma alternativa viável para que estas possam chegar até o produtor de café de forma segura.

2.6 Fermentação do café

A fermentação do café é um processo natural que ocorre nos grãos para retirada da mucilagem e consequentemente para a diminuição do teor de água presente no mesmo (SILVA *et al*., 2013).

Este processo é caracterizado por ser uma fermentação semissólida, batelada simples, com ou sem agitação. Uma alternativa é a utilização de fermentações conduzidas em biorreatores com indução de anaerobiose pela ação dos microrganismos naturalmente presente. O objetivo é produzir bebidas com aromas e sabores especiais, como doces, cítricos e frutados no qual agregam valor ao produto. Para isso é preciso realizar o controle da temperatura, qualidade da água, fatores físico-químicos como pH e grau Brix, tempo de processamento, dentre outros (LEE *et al*., 2015).

A etapa de fermentação ocorre naturalmente a partir da degradação da mucilagem presente ao redor dos grãos uma vez que este apresenta elevada quantidade de substâncias pécticas (AGATE; BHAT, 1966). No início das pesquisas, a literatura sugeriu que a degradação dessas substâncias pécticas ocorria a partir de enzimas pectolíticas produzidas pelo próprio fruto, mas hoje se conclui que essas enzimas não são suficientes e que a ação microbiana também se faz necessária (FRANK; LUM; DELACRUZ, 1965). Essas substâncias são degradadas pelas pectinases, enzimas produzidas por bactérias e leveduras. Sua degradação produz ácido galacturônico, ramnose, galactose, arabinose e metanol (PUERTA, 2013).

Diferentes processos bioquímicos ocorrem durante a fermentação espontânea do café no qual as enzimas produzidas pelas bactérias e leveduras presentes no mesocarpo degradam os açúcares, proteínas e lipídeos e os convertem em álcoois, ácidos, ésteres e cetonas (FRANK; LUM; DELACRUZ, 1965; PUERTA, 2013). Este processo permite a formação de diferentes compostos orgânicos, no qual podem agregar valor ao produto (EVANGELISTA *et al*., 2014).

A ação dos microrganismos varia de acordo com as espécies presentes, além das diferentes propriedades químicas e físicas do mesocarpo e fatores externos como temperatura e disponibilidade de oxigênio (SCHWAN; WHEALS, 2003).

Assim, os frutos e grãos do café possuem uma grande diversidade microbiana como bactérias, leveduras e fungos filamentosos (SILVA *et al*., 2000). Esta biodiversidade depende de diversos fatores, como a variedade do café (RIBEIRO *et al*., 2018), método de processamento utilizado (EVANGELISTA *et al*., 2015; SILVA *et al*., 2000; VILELA *et al*., 2010) e os fatores ambientais de onde estão sendo cultivados (MARTINS *et al*., 2020a).

Dessa forma, microrganismos isolados do café, podem ser utilizados como culturas iniciadoras durante a fermentação com o objetivo de produzir bebidas com sabores e aromas especiais (BRESSANI *et al*., 2020; MARTINEZ *et al*., 2019).

2.6.1 Uso de culturas iniciadoras no café

De modo geral, a cultura iniciadora é descrita como um material que contém um grande número de microrganismos viáveis, que podem ser adicionados com o objetivo de causar alterações desejáveis no alimento. Ao se adaptar ao substrato, elas aceleram o processo de fermentação e facilitam o controle sobre a fase inicial (HOLZAPFEL, 1997). A sua utilização pode melhorar significativamente a qualidade, segurança e aceitabilidade dos alimentos fermentados (HOLZAPFEL, 2002). Além disso, espera-se que esses microrganismos tenham algumas características como não ser patógeno, ser de fácil adaptação à matéria prima e ao processo e que possam criar um ambiente que iniba o crescimento de microrganismos indesejáveis (CORSETTI *et al*., 2012).

Durante o processamento de pós colheita do café, a fermentação induzida com a utilização de culturas iniciadoras pode melhorar a qualidade sensorial da bebida (BRESSANI *et al*., 2020). Isto ocorre devido à produção de enzimas extracelulares e ácidos orgânicos produzidos, por exemplo, pelas leveduras. O seu metabolismo pode acarretar na hidrólise de macromoléculas produzindo assim, importantes precursores de aroma, como açúcares redutores, aminoácidos e ácido clorogênico (LEE *et al*., 2015).

Espécies de microrganismos que apresentaram capacidade de serem utilizadas como cultura iniciadora foram estudados. Estes foram submetidas a testes de atividades de poligalacturonase, pectina liase e pectina metilesterase. Assim, algumas leveduras foram identificadas como potenciais culturas iniciadoras no café, como *Saccharomyces* sp., *Pichia* sp., *Candida* sp. e *Torulaspora* sp., pois apresentaram maior eficiência em relação à atividade pectinolítica para a degradação da mucilagem durante a fermentação do café (SILVA *et al*., 2013).

Em um primeiro estudo, Evangelista *et al*. (2014) avaliaram o potencial de cepas de leveduras - *Saccharomyces cerevisiae* UFLA YCN727, *S. cerevisiae* UFLA YCN724, *Candida parapsilosis* UFLA YCN448 e *Pichia guilliermondii* UFLA YCN731 como culturas iniciadoras utilizando o processamento natural. Em todos os testes houve a presença das leveduras até o final da fermentação. O café inoculado com levedura apresentou atributos diferenciados quando comparado ao café sem inoculação, o que indica uma maior qualidade

sensorial. O café inoculado com as culturas *Candida parapsilosis* UFLA YCN448 e *Saccharomyces cerevisiae* UFLA YCN727 apresentaram um aroma especial de caramelo, ervas e frutas.

Ribeiro *et al*. (2017) avaliaram o potencial de cepas de leveduras, *Saccharomyces cerevisiae* (CCMA 0200 e CCMA 0543) como culturas iniciadoras em duas variedades de café utilizando o processamento semi-seco. O café inoculado com *Saccharomyces cerevisiae* CCMA 0543 apresentou um melhor desempenho devido à sua maior persistência durante o processo de fermentação e secagem do café e ao maior número de compostos voláteis produzidos.

Em outra pesquisa, Martins *et al*. (2019) avaliaram o desempenho de *Saccharomyces cerevisiae* CCMA 0200 e *Torulaspora delbrueckii* CCMA 0684 em duas variedades de café: Mundo Novo e Catuaí Vermelho. Foram utilizados o método úmido e o impacto na qualidade sensorial e no perfil dos compostos foram analisados. *Torulaspora delbrueckii* CCMA 0684 adaptou-se melhor ao processo. Propanoato de 2-furanmetanol e 2-etil-3,5-dimetilpirazina foram identificados apenas nos tratamentos inoculados. Estes compostos são importantes contribuintes para o aroma do café.

Em um estudo mais recente, os autores demonstraram como os ácidos orgânicos e os perfis voláteis foram impactados pela fermentação do café. Neste trabalho foram utilizadas quatro culturas iniciadoras: *Meyerozyma caribbica* (CCMA 0198), S*accharomyces cerevisiae* (CCMA 0543), *Candida parapsilosis* (CCMA0544) e *Torulaspora delbrueckii* (CCMA 0684); duas variedades de café: Bourbon Amarelo e Canário Amarelo e dois processamentos: natural e descascado. As leveduras produziram diferentes compostos e atribuíram características sensoriais distintas à bebida (BRESSANI *et al*., 2020).

Por fim, o método de utilização dessas culturas iniciadoras na fermentação do café é uma etapa importante e será abordada no próximo tópico.

2.6.2 Métodos de utilização das culturas iniciadoras no café

O método utilizado para inoculação de microrganismos no café consiste no crescimento dos mesmos em meio de cultura específico até atingirem a concentração de células desejadas para serem inoculadas. As células precisam ser lavadas e diluídas em água para inoculação (EVANGELISTA *et al*., 2014).

Em um estudo foram testados diferentes métodos de inoculação no café cereja descascado utilizando leveduras como culturas iniciadoras. Cada levedura foi cultivada em

meio de cultura YEPG, próprio para o seu crescimento, até atingirem concentração de 10^9 células/ml para inoculação. As células foram lavadas e diluídas em 500 ml de água para inoculação em dois diferentes métodos. O primeiro consiste na inoculação direta a partir da pulverização da solução com a levedura sobre os grãos, em estrutura de madeira e o segundo método ocorre a partir da inoculação no café cereja descascado colocado em baldes de poliestireno. Ambos os métodos de inoculação obtiveram bons resultados em relação à qualidade do café. Contudo, o método do balde favoreceu a permanência dos microrganismos durante o processamento do café (MARTINEZ *et al*., 2017).

Este método de utilização das culturas puras, na forma líquida, é inviável em relação à indústria e aos produtores de café, principalmente devido à maior dificuldade e altos custos com transporte e armazenamento refrigerado. Dito isto, faz-se necessário à busca por métodos economicamente viáveis e eficientes para secagem de microrganismos, como o spray drying, facilitando o acesso para os produtores e garantindo a viabilidade, segurança e armazenamento das culturas iniciadoras visando à melhoria da qualidade do café.

3 CONSIDERAÇÕES GERAIS

Saccharomyces cerevisiae, *Torulaspora delbrueckii*, *Pichia kluyveri*, *Pichia kudriavzevii, Candida parapsilosis*, *Meyerozyma caribbica* e *Hanseniaspora uvarum* são utilizadas como culturas iniciadoras na fermentação do café e têm mostrado resultados promissores. As leveduras são cultivadas em meio de cultura específico até atingir a concentração celular adequada. Entretanto, o armazenamento de microrganismos na forma líquida não é economicamente vantajoso. O armazenamento a longo prazo reduz a viabilidade celular e a estabilidade genética das cepas, o que pode estar associado à perda de robustez celular em processos de fermentação industrial. Além disso, o transporte e armazenamento refrigerado bem como possíveis contaminações dificultam a sua utilização.

Assim, o processo de secagem torna-se uma alternativa para a preservação a longo prazo das leveduras epífitas do café, contribuindo para a manutenção da viabilidade celular, transporte, armazenamento, redução do odor e contaminações. O spray drying permite a produção de preparações microbianas secas ativas em escala industrial. Esta técnica permite altas taxas de produção com baixos custos operacionais. Algumas de suas vantagens incluem boa qualidade do produto final, baixo custo, maior controle de variáveis do processo e operação contínua. Dessa forma, a utilização de métodos economicamente viáveis e com maior grau de eficiência para microencapsulação de leveduras epifíticas do café, facilita o

acesso para os produtores. Consequentemente, garante a viabilidade celular, segurança e armazenamento das culturas iniciadoras, visando a melhoria da qualidade da bebida.

REFERÊNCIAS

AGATE, A. D.; BHAT, J. V. Role of pectinolytic yeasts in the degradation of mucilage layer of *Coffea robusta* cherries. **Applied microbiology**, v. 14, n. 2, p. 256–60, mar. 1966. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/5959859>. Acesso em: 10 ago. 2021.

AGUIRRE-GÜITRÓN, L. *et al*. Application of powder formulation of *Meyerozyma caribbica* for postharvest control of *Colletotrichum gloeosporioides* in mango (*Mangifera indica* L.). **LWT - Food Science and Technology**, v. 113, p. 108271, out. 2019. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0023643819306115>. Acesso em: 12 ago. 2021.

AGUIRRE-GÜITRÓN, L. *et al*. Optimisation of the spray drying process of formulating the post-harvest biocontrol agent *Meyerozyma caribbica*. **Biocontrol Science and Technology**, v. 28, n. 6, p. 574–590, 3 jun. 2018. Disponível em: <https://www.tandfonline.com/doi/full/10.1080/09583157.2018.1468997>. Acesso em: 15 set. 2021.

ALFARO-GALARZA, O. *et al*. Protective effects of the use of taro and rice starch as wall material on the viability of encapsulated *Lactobacillus paracasei* subsp. *Paracasei*. **LWT - Food Science and Technology**, v. 117, p. 108686, jan. 2020. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S002364381931028X>. Acesso em: 16 set. 2021.

ALONSO, S. Novel preservation techniques for microbial cultures. *In*: **Novel food fermentation technologies**. [s.l.] Springer, Cham, 2016. p. 7–33.

ANDRADE, D. P. *et al*. Stability of microencapsulated lactic acid bacteria under acidic and bile juice conditions. **International Journal of Food Science & Technology**, v. 54, n. 7, p. 2355–2362, 28 jul. 2019. Disponível em: <https://onlinelibrary.wiley.com/doi/abs/10.1111/ijfs.14114>. Acesso em: 17 jun. 2021.

APONTE, M. *et al*. Impact of different spray-drying conditions on the viability of wine *Saccharomyces cerevisiae* strains. **World Journal of Microbiology and Biotechnology**, v. 32, n. 1, p. 13, 28 jan. 2016. Disponível em: <http://link.springer.com/10.1007/s11274-015-1956-5>. Acesso em: 27 ago. 2021.

ARSLAN, S. *et al*. Microencapsulation of probiotic *Saccharomyces cerevisiae* var. *boulardii* with different wall materials by spray drying. **LWT - Food Science and Technology**, v. 63, n. 1, p. 685–690, set. 2015. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0023643815001875>. Acesso em: 14 jul. 2021.

BANSAL, T.; GARG, S. Probiotics: From Functional Foods to Pharmaceutical Products. **Current Pharmaceutical Biotechnology**, v. 9, n. 4, p. 267–287, 1 ago. 2008. Disponível em: <http://www.eurekaselect.com/openurl/content.php?genre=article&issn=1389-2010&volume=9&issue=4&spage=267>. Acesso em: 12 ago. 2021.

BOROVIKOVA, D. *et al*. Potassium uptake system Trk2 is crucial for yeast cell viability during anhydrobiosis. **FEMS Microbiology Letters**, v. 350, n. 1, p. 28–33, jan. 2014. Disponível em: <https://academic.oup.com/femsle/article-lookup/doi/10.1111/1574-6968.12344>. Acesso em: 20 jul. 2021.

BOROVIKOVA, D. *et al*. Anhydrobiosis in yeast: cell wall mannoproteins are important for yeast *Saccharomyces cerevisiae* resistance to dehydration. **Yeast**, v. 33, n. 8, p. 347–353, ago. 2016. Disponível em: <http://doi.wiley.com/10.1002/yea.3164>. Acesso em: 20 jul. 2021.

BÓRQUEZ, R. *et al*. Atmospheric Freeze-Impingement Drying of an Autochthonous Microencapsulated Probiotic Strain. **Drying Technology**, v. 31, n. 5, p. 535–548, 4 abr. 2013. Disponível em: <http://www.tandfonline.com/doi/abs/10.1080/07373937.2012.745091>. Acesso em: 28 out. 2021.

BOZA, Y.; BARBIN, D.; SCAMPARINI, A. R. . Effect of spray-drying on the quality of encapsulated cells of *Beijerinckia* sp. **Process Biochemistry**, v. 39, n. 10, p. 1275–1284, jun. 2004. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0032959203002577>. Acesso em: 05 jul. 2021.

BOZOĞLU, T. F.; ÖZILGEN, M.; BAKIR, U. Survival kinetics of lactic acid starter cultures during and after freeze drying. **Enzyme and Microbial Technology**, v. 9, n. 9, p. 531–537, set. 1987. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/0141022987900822>. Acesso em: 18 mar. 2021.

BRAGA, A. B. A. C. *et al*. Evaluation of lethality temperature and use of different wall materials in the microencapsulation process of *Trichoderma asperellum* conidias by spray drying. **Powder Technology**, v. 347, p. 199–206, abr. 2019. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0032591019301469>. Acesso em: 14 jul. 2021.

BRESSANI, A. P. P. *et al*. Organic acids produced during fermentation and sensory perception in specialty coffee using yeast starter culture. **Food Research International**, v. 128, p. 108773, fev. 2020. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0963996919306593>. Acesso em: 18 mar. 2021.

BURGAIN, J. *et al*. Encapsulation of probiotic living cells: From laboratory scale to industrial applications. **Journal of Food Engineering**, v. 104, n. 4, p. 467–483, jun. 2011. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S026087741000631X>. Acesso em: 25 maio 2021.

CALAHAN, D. *et al*. Genetic Analysis of Desiccation Tolerance in *Saccharomyces cerevisiae*. **Genetics**, v. 189, n. 2, p. 507–519, out. 2011. Disponível em: <http://www.genetics.org/lookup/doi/10.1534/genetics.111.130369>. Acesso em: 10 ago. 2021.

CHANDRALEKHA, A. *et al*. Encapsulation of yeast (*Saccharomyces cerevisiae*) by spray drying for extension of shelf life. **Drying Technology**, v. 34, n. 11, p. 1307–1318, 17 ago. 2016. Disponível em: <https://www.tandfonline.com/doi/full/10.1080/07373937.2015.1112808>. Acesso em: 27

ago. 2021.

CHÁVEZ, B. E.; LEDEBOER, A. M. Drying of Probiotics: Optimization of Formulation and Process to Enhance Storage Survival. **Drying Technology**, v. 25, n. 7–8, p. 1193–1201, ago. 2007. Disponível em: <http://www.tandfonline.com/doi/abs/10.1080/07373930701438576>. Acesso em: 18 mar. 2021.

CHEN, G.; WANG, W. Role of Freeze Drying in Nanotechnology. **Drying Technology**, v. 25, n. 1, p. 29–35, 12 fev. 2007. Disponível em: <http://www.tandfonline.com/doi/abs/10.1080/07373930601161179>. Acesso em: 20 jul. 2021.

CORSETTI, A. *et al*. Application of starter cultures to table olive fermentation: an overview on the experimental studies. **Frontiers in Microbiology**, v. 3, 2012. Disponível em: <http://journal.frontiersin.org/article/10.3389/fmicb.2012.00248/abstract>. Acesso em: 28 out. 2021.

CROWE, J. H.; HOEKSTRA, F. A.; CROWE, L. M. Anhydrobiosis. **Annual Review of Physiology**, v. 54, n. 1, p. 579–599, out. 1992. Disponível em: <http://www.annualreviews.org/doi/10.1146/annurev.ph.54.030192.003051>. Acesso em: 15 set. 2021.

CROWE, L. M. Lessons from nature: the role of sugars in anhydrobiosis. **Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology**, v. 131, n. 3, p. 505–513, mar. 2002. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S1095643301005037>. Acesso em: 17 jun. 2021.

DA MOTA, M. C. B. *et al*. Influence of fermentation conditions on the sensorial quality of coffee inoculated with yeast. **Food Research International**, v. 136, p. 109482, out. 2020. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S096399692030507X>. Acesso em: 19 mar. 2021.

DESAI, K. G. H.; PARK, H. J. Recent Developments in Microencapsulation of Food Ingredients. **Drying Technology**, v. 23, n. 7, p. 1361–1394, jul. 2005. Disponível em: <http://www.tandfonline.com/doi/abs/10.1081/DRT-200063478>. Acesso em: 14 jul. 2021.

DESMOND, C. *et al*. Environmental adaptation of probiotic lactobacilli towards improvement of performance during spray drying. **International Dairy Journal**, v. 11, n. 10, p. 801–808, jan. 2001. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0958694601001212>. Acesso em: 20 jul. 2021.

DEVAKATE, R. V. *et al*. Purification and drying of bromelain. **Separation and Purification Technology**, v. 64, n. 3, p. 259–264, jan. 2009. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S1383586608003596>. Acesso em: 07 set. 2021.

DIAS, D. R. *et al*. Encapsulation as a tool for bioprocessing of functional foods. **Current Opinion in Food Science**, v. 13, p. 31–37, fev. 2017. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S221479931730019X>. Acesso em: 19 mar. 2021.

DIMITRELLOU, D. *et al*. Evaluation of thermally-dried *Kluyveromyces marxianus* as baker's yeast. **Food Chemistry**, v. 115, n. 2, p. 691–696, jul. 2009. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0308814608014969>. Acesso em: 12 ago. 2021.

DIMITRELLOU, D. *et al*. Fermentation efficiency of thermally dried immobilized kefir on casein as starter culture. **Process Biochemistry**, v. 43, n. 12, p. 1323–1329, dez. 2008. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S1359511308002432>. Acesso em: 16 set. 2021.

DONSÌ, F. *et al*. Main factors regulating microbial inactivation by high-pressure homogenization: Operating parameters and scale of operation. **Chemical Engineering Science**, v. 64, n. 3, p. 520–532, fev. 2009. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0009250908005198>. Acesso em: 13 ago. 2021.

DOS SANTOS, R. C. S.; FINKLER, L.; FINKLER, C. L. L. Microencapsulation of *Lactobacillus casei* by spray drying. **Journal of Microencapsulation**, v. 31, n. 8, p. 759–767, 4 dez. 2014. Disponível em: <http://www.tandfonline.com/doi/full/10.3109/02652048.2014.932026>. Acesso em: 17 jun. 2021.

DUPONT, S. *et al*. Nature of sterols affects plasma membrane behavior and yeast survival during dehydration. **Biochimica et Biophysica Acta (BBA) - Biomembranes**, v. 1808, n. 6, p. 1520–1528, jun. 2011. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0005273610003858>. Acesso em: 07 set. 2021.

DUPONT, S. *et al*. Survival kit of *Saccharomyces cerevisiae* for anhydrobiosis. **Applied Microbiology and Biotechnology**, v. 98, n. 21, p. 8821–8834, 30 nov. 2014. Disponível em: <http://link.springer.com/10.1007/s00253-014-6028-5>. Acesso em: 15 set. 2021.

DUSKOVA, M. *et al*. The role of glycerol transporters in yeast cells in various physiological and stress conditions. **FEMS Microbiology Letters**, v. 362, n. 3, p. 1–8, 1 fev. 2015. Disponível em: <https://academic.oup.com/femsle/article/362/3/1/1803156>. Acesso em: 10 ago. 2021.

ELIZONDO, H.; LABUZA, T. P. Death kinetics of yeast in spray drying. **Biotechnology and Bioengineering**, v. 16, n. 9, p. 1245–1259, set. 1974. Disponível em: <http://doi.wiley.com/10.1002/bit.260160908>. Acesso em: 17 jun. 2021.

EVANGELISTA, S. R. *et al*. Microbiological diversity associated with the spontaneous wet method of coffee fermentation. **International Journal of Food Microbiology**, v. 210, p. 102–112, out. 2015. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S016816051530026X>. Acesso em: 19 mar. 2021.

EVANGELISTA, S. R. *et al*. Improvement of coffee beverage quality by using selected yeasts strains during the fermentation in dry process. **Food Research International**, v. 61, p. 183–195, jul. 2014. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S096399691300642X>. Acesso em: 19 mar.

2021.

FILKOVÁ, I.; MUJUMDAR, A. S. Industrial spray drying systems. *In*: **Handbook of Industrial Drying**. [s.l.] CRC Press, 1995. p. 263–308.

FRANK, H. A.; LUM, N. A.; DELACRUZ, A. S. Bacteria responsible for mucilage-layer decomposition in kona coffee cherries. **Applied microbiology**, v. 13, p. 201–7, mar. 1965. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/14325879>. Acesso em: 05 jul. 2021.

FU, N.; CHEN, X. D. Towards a maximal cell survival in convective thermal drying processes. **Food Research International**, v. 44, n. 5, p. 1127–1149, jun. 2011. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0963996911002122>. Acesso em: 14 jul. 2021.

GARRE, E. *et al*. Oxidative stress responses and lipid peroxidation damage are induced during dehydration in the production of dry active wine yeasts. **International Journal of Food Microbiology**, v. 136, n. 3, p. 295–303, jan. 2010. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0168160509005558>. Acesso em: 05 jul. 2021.

GHARSALLAOUI, A. *et al*. Applications of spray-drying in microencapsulation of food ingredients: An overview. **Food Research International**, v. 40, n. 9, p. 1107–1121, nov. 2007. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0963996907001238>. Acesso em: 14 jul. 2021.

GIBBS, B. F. *et al*. Encapsulation in the food industry: a review. **International Journal of Food Sciences and Nutrition**, v. 50, n. 3, p. 213–224, 6 jan. 1999. Disponível em: <http://www.tandfonline.com/doi/full/10.1080/096374899101256>. Acesso em: 13 ago. 2021.

GÓMEZ-PASTOR, R. *et al*. Recent advances in yeast biomass production. *In*: **Biomass—Detection, Production and Usage**. Rijeka: InTech, 2011.

HOLZAPFEL, W. Use of starter cultures in fermentation on a household scale. **Food Control**, v. 8, n. 5–6, p. 241–258, out. 1997. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0956713597000170>. Acesso em: 12 ago. 2021.

HOLZAPFEL, W. H. Appropriate starter culture technologies for small-scale fermentation in developing countries. **International Journal of Food Microbiology**, v. 75, n. 3, p. 197–212, maio 2002. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0168160501007073>. Acesso em: 25 maio 2021.

HORACZEK, A.; VIERNSTEIN, H. *Beauveria brongniartii* subjected to spray-drying in a composite carrier matrix system. **Journal of Microencapsulation**, v. 21, n. 3, p. 317–330, 3 maio 2004. Disponível em: <http://www.tandfonline.com/doi/full/10.1080/02652040410001673892>. Acesso em: 27 ago. 2021.

JENKINS, D. M.; POWELL, C. D.; SMART, K. A. Dried Yeast: Impact of Dehydration and

Rehydration on Brewing Yeast DNA Integrity. **Journal of the American Society of Brewing Chemists**, v. 68, n. 3, p. 132–138, 5 jun. 2010. Disponível em: <https://www.tandfonline.com/doi/full/10.1094/ASBCJ-2010-0629-01>. Acesso em: 18 jun. 2021.

KANAKDANDE, D.; BHOSALE, R.; SINGHAL, R. S. Stability of cumin oleoresin microencapsulated in different combination of gum arabic, maltodextrin and modified starch. **Carbohydrate Polymers**, v. 67, n. 4, p. 536–541, fev. 2007. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0144861706003055>. Acesso em: 25 jun. 2021.

KEARNEY, N. *et al*. Development of a spray dried probiotic yoghurt containing *Lactobacillus paracasei* NFBC 338. **International Dairy Journal**, v. 19, n. 11, p. 684–689, nov. 2009. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0958694609000971>. Acesso em: 20 mar. 2021.

KETS, E. P. W.; BONT, J. A. M. Protective effect of betaine on survival of *Lactobacillus plantarum* subjected to drying. **FEMS Microbiology Letters**, v. 116, n. 3, p. 251–255, mar. 1994. Disponível em: <https://academic.oup.com/femsle/article-lookup/doi/10.1111/j.1574-6968.1994.tb06711.x>. Acesso em: 23 maio 2021.

KETS, E.; TEUNISSEN, P.; DE BONT, J. Effect of compatible solutes on survival of lactic Acid bacteria subjected to drying. **Applied and environmental microbiology**, v. 62, n. 1, p. 259–61, jan. 1996. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/16535214>. Acesso em: 23 maio 2021.

LEE, L. W. *et al*. Coffee fermentation and flavor – An intricate and delicate relationship. **Food Chemistry**, v. 185, p. 182–191, out. 2015. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0308814615004963>. Acesso em: 07 set. 2021.

LESLIE, S. B. *et al*. Trehalose lowers membrane phase transitions in dry yeast cells. **Biochimica et Biophysica Acta (BBA) - Biomembranes**, v. 1192, n. 1, p. 7–13, jun. 1994. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/0005273694901368>. Acesso em: 25 maio 2021.

LIEVENSE, L. C.; VAN'T RIET, K. Convective drynig of bacteria I: The drying process. *In*: **Advances in Biochemical Engineering Biotechnology**. Berlin, Heidelberg: Springer, 1993. p. 45–63.

LIEVENSE, L. C.; VAN'T RIET, K. Convective drying of bacteria II. Factors influencing survival. *In*: **Advances in Biochemical Engineering/Biotechnology**. Berlin, Heidelberg: Springer, 1994. p. 71–89.

LIN, T. *et al*. Comparative analysis of spray-drying microencapsulation of *Bifidobacterium adolescentis* and *Lactobacillus acidophilus* cultivated in different growth media. **Journal of Food Process Engineering**, v. 42, n. 7, 11 nov. 2019. Disponível em: <https://onlinelibrary.wiley.com/doi/abs/10.1111/jfpe.13258>. Acesso em: 18 jun. 2021.

LINDERS, L. J. M. *et al*. Carbohydrates and the dehydration inactivation of *Lactobacillus plantarum*: The role of moisture distribution and water activity. **Journal of Food**

Engineering, v. 31, n. 2, p. 237–250, fev. 1997. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0260877496000775>. Acesso em: 16 out. 2021.

MADIGAN, M. T. *et al*. **Brock biology of microorganisms**. 14. ed. [s.l.] Benjamin-Cummings, 2016.

MARQUES, L. G.; SILVEIRA, A. M.; FREIRE, J. T. Freeze-Drying Characteristics of Tropical Fruits. **Drying Technology**, v. 24, n. 4, p. 457–463, maio 2006. Disponível em: <http://www.tandfonline.com/doi/abs/10.1080/07373930600611919>. Acesso em: 07 set. 2021.

MARTÍN, M. J. *et al*. Microencapsulation of bacteria: A review of different technologies and their impact on the probiotic effects. **Innovative Food Science & Emerging Technologies**, v. 27, p. 15–25, fev. 2015. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S146685641400160X>. Acesso em: 15 fev. 2021.

MARTINEZ, S. J. *et al*. Effect of Bacterial and Yeast Starters on the Formation of Volatile and Organic Acid Compounds in Coffee Beans and Selection of Flavors Markers Precursors During Wet Fermentation. **Frontiers in Microbiology**, v. 10, 26 jun. 2019. Disponível em: <https://www.frontiersin.org/article/10.3389/fmicb.2019.01287/full>. Acesso em: 25 jul. 2021.

MARTINEZ, S. J. *et al*. Different inoculation methods for semi-dry processed coffee using yeasts as starter cultures. **Food Research International**, v. 102, p. 333–340, dez. 2017. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S096399691730683X>. Acesso em: 25 jul. 2021.

MARTINS, P. M. M. *et al*. Coffee growing altitude influences the microbiota, chemical compounds and the quality of fermented coffees. **Food Research International**, v. 129, p. 108872, mar. 2020b. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0963996919307586>. Acesso em: 17 set. 2021.

MARTINS, P. M. M. *et al*. Production of coffee (*Coffea arabica*) inoculated with yeasts: impact on quality. **Journal of the Science of Food and Agriculture**, v. 99, n. 13, p. 5638–5645, 2019. Disponível em: <http://doi.wiley.com/10.1002/jsfa.9820> Acesso em: 17 set. 2021.

MENG, X. C. *et al*. Anhydrobiotics: The challenges of drying probiotic cultures. **Food Chemistry**, v. 106, n. 4, p. 1406–1416, fev. 2008. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0308814607005936>. Acesso em: 28 out. 2021.

MORENO, I. *et al*. S*accharomyces cerevisiae* Rds2 transcription factor involvement in cell wall composition and architecture. **International microbiology : the official journal of the Spanish Society for Microbiology**, v. 11, n. 1, p. 57–63, mar. 2008. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/18683633>. Acesso em: 16 out. 2021.

MORGAN, C. A. *et al*. Preservation of micro-organisms by drying; A review. **Journal of Microbiological Methods**, v. 66, n. 2, p. 183–193, ago. 2006. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0167701206000583>. Acesso em: 25 jul. 2021.

MORTAZAVIAN, A. *et al*. Principles and methods of microencapsulation of probiotic microorganisms. **Iranian Journal of Biotechnology**, v. 5, n. 1, p. 1–18, 2007.

MULLER, J. A. *et al*. Reconstitution conditions for dried probiotic powders represent a critical step in determining cell viability. **Journal of Applied Microbiology**, v. 108, n. 4, p. 1369–1379, abr. 2010. Disponível em: <http://doi.wiley.com/10.1111/j.1365-2672.2009.04533.x>. Acesso em: 19 mar. 2021.

MYLONAS, C.; KOURETAS, D. Lipid peroxidation and tissue damage. *In*: **In vivo (Athens, Greece)**. 13. ed. [s.l: s.n.]p. 295–309.

NAZZARO, F. *et al*. Microencapsulation in food science and biotechnology. **Current Opinion in Biotechnology**, v. 23, n. 2, p. 182–186, abr. 2012. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0958166911006847>. Acesso em: 19 ago. 2021.

O'SULLIVAN, J. J. *et al*. Atomisation technologies used in spray drying in the dairy industry: A review. **Journal of Food Engineering**, v. 243, p. 57–69, fev. 2019. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0260877418303649>. Acesso em: 19 ago. 2021.

OBUCHI, K. *et al*. Calorimetric characterization of critical targets for killing and acquired thermotolerance in yeast. **Yeast**, v. 16, n. 2, p. 111–119, 30 jan. 2000. Disponível em: <http://doi.wiley.com/10.1002/%28SICI%291097-0061%2820000130%2916%3A2%3C111%3A%3AAID-YEA507%3E3.0.CO%3B2-V>. Acesso em: 05 jul. 2021.

OLIVEIRA, A. C. *et al*. Microencapsulation of *B. lactis* (BI 01) and *L. acidophilus* (LAC 4) by Complex Coacervation Followed by Spouted-Bed Drying. **Drying Technology**, v. 25, n. 10, p. 1687–1693, out. 2007. Disponível em: <http://www.tandfonline.com/doi/abs/10.1080/07373930701590939>. Acesso em: 18 jun. 2021.

PARVEZ, S. *et al*. Probiotics and their fermented food products are beneficial for health. **Journal of Applied Microbiology**, v. 100, n. 6, p. 1171–1185, jun. 2006. Disponível em: <http://doi.wiley.com/10.1111/j.1365-2672.2006.02963.x>. Acesso em: 25 jun. 2021.

PATEL, R. P.; PATEL, M. P.; SUTHAR, A. M. Spray drying technology: an overview. **Indian Journal of Science and Technology**, v. 2, n. 10, 2009.

PECH-CANUL, A. de la C. *et al*. A Brief Review of Edible Coating Materials for the Microencapsulation of Probiotics. **Coatings**, v. 10, n. 3, p. 197, 25 fev. 2020. Disponível em: <https://www.mdpi.com/2079-6412/10/3/197>. Acesso em: 16 out. 2021.

PEIGHAMBARDOUST, S. H.; GOLSHAN TAFTI, A.; HESARI, J. Application of spray drying for preservation of lactic acid starter cultures: a review. **Trends in Food Science & Technology**, v. 22, n. 5, p. 215–224, maio 2011. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0924224411000100>. Acesso em: 18 jun. 2021.

PEREIRA, P. V *et al*. Effect of altitude and terrain aspect on the chemical composition of *Coffea canephora* cherries and sensory characteristics of the beverage. **Journal of the Science of Food and Agriculture**, p. jsfa.10885, 27 out. 2020. Disponível em: <https://onlinelibrary.wiley.com/doi/10.1002/jsfa.10885>. Acesso em: 19 out. 2021.

POIRIER, I. *et al*. *Saccharomyces cerevisiae* viability is strongly dependant on rehydration kinetics and the temperature of dried cells. **Journal of Applied Microbiology**, v. 86, n. 1, p. 87–92, jan. 1999. Disponível em: <http://doi.wiley.com/10.1046/j.1365-2672.1999.00638.x>. Acesso em: 15 fev. 2021.

PUERTA, G. I. **Fundamentos del proceso de fermentación en el beneficio del café**. [s.l.] Centro Nacional de Investigaciones de Café (Cenicafé), 2013.
RAPOPORT, A. Anhydrobiosis and Dehydration of Yeasts. *In*: **Biotechnology of Yeasts and Filamentous Fungi**. Cham: Springer International Publishing, 2017. p. 87–116.

RAPOPORT, A. *et al*. Anhydrobiosis: Inside yeast cells. **Biotechnology Advances**, v. 37, n. 1, p. 51–67, jan. 2019. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0734975018301794>. Acesso em: 15 jan. 2021.

REDDY, K. B. P. K.; MADHU, A. N.; PRAPULLA, S. G. Comparative survival and evaluation of functional probiotic properties of spray-dried lactic acid bacteria. **International Journal of Dairy Technology**, v. 62, n. 2, p. 240–248, maio 2009. Disponível em: <http://doi.wiley.com/10.1111/j.1471-0307.2009.00480.x>. Acesso em: 20 jul. 2021.

RIBEIRO, L. S. *et al*. Microbiological and chemical-sensory characteristics of three coffee varieties processed by wet fermentation. **Annals of Microbiology**, v. 68, n. 10, p. 705–716, 2018. Acesso em: 16 set. 2021.

RIBEIRO, L. S. *et al*. Behavior of yeast inoculated during semi-dry coffee fermentation and the effect on chemical and sensorial properties of the final beverage. **Food Research International**, v. 92, p. 26–32, 2017a. Disponível em: <http://dx.doi.org/10.1016/j.foodres.2016.12.011>. Acesso em: 16 set. 2021.

RIBEIRO, L. S. *et al*. Controlled fermentation of semi-dry coffee (*Coffea arabica*) using starter cultures: A sensory perspective. **LWT - Food Science and Technology**, v. 82, p. 32–38, set. 2017b. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0023643817302281>. Acesso em: 16 set. 2021.

RODRÍGUEZ-PORRATA, B. *et al*. Enhancing yeast cell viability after dehydration by modification of the lipid profile. **World Journal of Microbiology and Biotechnology**, v. 27, n. 1, p. 75–83, 12 jan. 2011. Disponível em: <http://link.springer.com/10.1007/s11274-010-0428-1>. Acesso em: 19 ago. 2021.

ROSOLEN, M. D. *et al*. Symbiotic microencapsulation of *Lactococcus lactis* subsp. *lactis* R7 using whey and inulin by spray drying. **LWT - Food Science and Technology**, v. 115, p. 108411, nov. 2019. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0023643819307534>. Acesso em: 09 set. 2021.

SANTIVARANGKNA, C.; HIGL, B.; FOERST, P. Protection mechanisms of sugars during different stages of preparation process of dried lactic acid starter cultures. **Food**

Microbiology, v. 25, n. 3, p. 429–441, maio 2008. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0740002008000038>. Acesso em: 15 jan. 2021.

SANTIVARANGKNA, C.; KULOZIK, U.; FOERST, P. Effect of carbohydrates on the survival of *Lactobacillus helveticus* during vacuum drying. **Letters in Applied Microbiology**, v. 42, n. 3, p. 271–276, mar. 2006. Disponível em: <http://doi.wiley.com/10.1111/j.1472-765X.2005.01835.x>. Acesso em: 15 jan. 2021.

SANTIVARANGKNA, C.; KULOZIK, U.; FOERST, P. Alternative Drying Processes for the Industrial Preservation of Lactic Acid Starter Cultures. **Biotechnology Progress**, v. 23, n. 2, p. 302–315, 9 abr. 2007. Disponível em: <http://doi.wiley.com/10.1021/bp060268f>. Acesso em: 15 jan. 2021.

SANTIVARANGKNA, C.; KULOZIK, U.; FOERST, P. Inactivation mechanisms of lactic acid starter cultures preserved by drying processes. **Journal of Applied Microbiology**, v. 105, n. 1, p. 1–13, jul. 2008. Disponível em: <http://doi.wiley.com/10.1111/j.1365-2672.2008.03744.x>. Acesso em: 15 jan. 2021.

SCHWAN, R. F.; WHEALS, A. E. Mixed microbial fermentations of chocolate and coffee. *In*: **Yeasts in food**. [s.l.] Elsevier, 2003. p. 429–449.

SHIMA, J.; ANDO, A.; TAKAGI, H. Possible roles of vacuolar H+-ATPase and mitochondrial function in tolerance to air-drying stress revealed by genome-wide screening of *Saccharomyces cerevisiae* deletion strains. **Yeast**, v. 25, n. 3, p. 179–190, mar. 2008. Disponível em: <http://doi.wiley.com/10.1002/yea.1577>. Acesso em: 25 jun. 2021.

SIBIRNY, A. A. Yeast peroxisomes: structure, functions and biotechnological opportunities. **FEMS Yeast Research**, v. 16, n. 4, p. fow038, jun. 2016. Disponível em: <https://academic.oup.com/femsyr/article-lookup/doi/10.1093/femsyr/fow038>. Acesso em: 19 out. 2021.

SILVA, C. F. *et al*. Microbial diversity during maturation and natural processing of coffee cherries of *Coffea arabica* in Brazil. **International Journal of Food Microbiology**, v. 60, n. 2–3, p. 251–260, set. 2000. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0168160500003159>. Acesso em: 25 jan. 2021.

SILVA, C. F. *et al*. Evaluation of a potential starter culture for enhance quality of coffee fermentation. **World Journal of Microbiology and Biotechnology**, v. 29, n. 2, p. 235–247, 2013. Acesso em: 16 set. 2021.

SILVA, P. T. da *et al*. Microencapsulation: concepts, mechanisms, methods and some applications in food technology. **Ciência Rural**, v. 44, n. 7, p. 1304–1311, jul. 2014. Disponível em: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0103-84782014000701304&lng=en&tlng=en>. Acesso em: 09 set. 2021.

SILVA, J. *et al*. Effect of the pH of growth on the survival of *Lactobacillus delbrueckii* subsp. *bulgaricus* to stress conditions during spray-drying. **Journal of Applied Microbiology**, v. 98, n. 3, p. 775–782, mar. 2005. Disponível em: <http://doi.wiley.com/10.1111/j.1365-2672.2004.02516.x>. Acesso em: 09 set. 2021.

SOOTTITANTAWAT, A. *et al*. Influence of emulsion and powder size on the stability of encapsulated d-limonene by spray drying. **Innovative Food Science & Emerging Technologies**, v. 6, n. 1, p. 107–114, mar. 2005. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S1466856404000840>. Acesso em: 05 jul. 2021.

STEFANELLO, R. F. *et al*. Survival and stability of *Lactobacillus fermentum* and *Wickerhamomyces anomalus* strains upon lyophilisation with different cryoprotectant agents. **Food Research International**, v. 115, p. 90–94, jan. 2019. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0963996918305817>. Acesso em: 19 out. 2021.

STRASSER, S. *et al*. Influence of lyophilization, fluidized bed drying, addition of protectants, and storage on the viability of lactic acid bacteria. **Journal of Applied Microbiology**, v. 107, n. 1, p. 167–177, jul. 2009. Disponível em: <http://doi.wiley.com/10.1111/j.1365-2672.2009.04192.x>. Acesso em: 19 ago. 2021.

SURYABHAN, P.; LOHITH, K.; ANU-APPAIAH, K. A. Sucrose and sorbitol supplementation on maltodextrin encapsulation enhance the potential probiotic yeast survival by spray drying. **LWT - Food Science and Technology**, v. 107, p. 243–248, jun. 2019. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0023643819301835>. Acesso em: 15 fev. 2021.

TAN, D. T.; POH, P. E.; CHIN, S. K. Microorganism preservation by convective air-drying—A review. **Drying Technology**, v. 36, n. 7, p. 764–779, 19 maio 2018. Disponível em: <https://www.tandfonline.com/doi/full/10.1080/07373937.2017.1354876>. Acesso em: 15 fev. 2021.

TEERA-ARUNSIRI, A.; SUPHANTHARIKA, M.; KETUNUTI, U. Preparation of Spray-Dried Wettable Powder Formulations of *Bacillus thuringiensis*-Based Biopesticides. **Journal of Economic Entomology**, v. 96, n. 2, p. 292–299, 1 abr. 2003. Disponível em: <https://academic.oup.com/jee/article-lookup/doi/10.1093/jee/96.2.292>. Acesso em: 28 jun. 2021.

TSAOUSI, K.; DIMITRELLOU, D.; KOUTINAS, A. A. Low-temperature thermal drying of *Saccharomyces cerevisiae* starter culture for food production. **Food Chemistry**, v. 110, n. 3, p. 547–553, out. 2008. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0308814608001623>. Acesso em: 27 jun. 2021.

UZUNOVA-DONEVA, T.; DONEV, T. Influence of the freezing rate on the survival of strains *Saccharomyces cerevisiae* after cryogenic preservation. **Journal of Culture Collections**, v. 3, p. 78–83, 2002. Acesso em: 22 jul. 2021.

VAN DE GUCHTE, M. *et al*. Stress responses in lactic acid bacteria. **Antonie van Leeuwenhoek**, v. 82, n. 1–4, p. 187–216, ago. 2002. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/12369188>. Acesso em: 19 out. 2021.

VANDEN BRABER, N. L. *et al*. Effect of microencapsulation in whey protein and water-soluble chitosan derivative on the viability of the probiotic *Kluyveromyces marxianus* VM004 during storage and in simulated gastrointestinal conditions. **LWT - Food Science and Technology**, v. 118, p. 108844, jan. 2020. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0023643819311867>. Acesso em: 15 fev. 2021.

VILELA, D. M. *et al*. Molecular ecology and polyphasic characterization of the microbiota associated with semi-dry processed coffee (*Coffea arabica* L.). **Food Microbiology**, v. 27, n. 8, p. 1128–1135, dez. 2010. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0740002010002091>. Acesso em: 16 out. 2021.

WANG, Y.-C.; YU, R.-C.; CHOU, C.-C. Viability of lactic acid bacteria and bifidobacteria in fermented soymilk after drying, subsequent rehydration and storage. **International Journal of Food Microbiology**, v. 93, n. 2, p. 209–217, jun. 2004. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0168160503006068>. Acesso em: 09 set. 2021.

WELSH, D. T. Ecological significance of compatible solute accumulation by micro-organisms: from single cells to global climate. **FEMS Microbiology Reviews**, v. 24, n. 3, p. 263–290, jul. 2000. Disponível em: <https://academic.oup.com/femsre/article-lookup/doi/10.1111/j.1574-6976.2000.tb00542.x>. Acesso em: 13 ago. 2021.

YING, D. Y. *et al*. Microencapsulated *Lactobacillus rhamnosus* GG Powders: Relationship of Powder Physical Properties to Probiotic Survival during Storage. **Journal of Food Science**, v. 75, n. 9, p. E588–E595, nov. 2010. Disponível em: <http://doi.wiley.com/10.1111/j.1750-3841.2010.01838.x>. Acesso em: 16 set. 2021.

SEGUNDA PARTE – ARTIGOS

ARTIGO 1 - MICROENCAPSULATION BY SPRAY DRYING OF COFFEE EPIPHYTIC YEASTS *SACCHAROMYCES CEREVISIAE* CCMA 0543 AND *TORULASPORA DELBRUECKII* CCMA 0684

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Abstract

The objective of this work was to evaluate the microencapsulation feasibility of *Saccharomyces cerevisiae* CCMA 0543 and *Torulaspora delbrueckii* CCMA 0684 in three different compositions of wall material by spray-dryer. The yeasts (10^9 cells/mL) were microencapsulated separately using maltodextrin (15%), maltodextrin (15%) with sucrose (2%), or maltose (2%) as wall material. The viability was evaluated for six months at two different temperatures (7 and 25 °C). The yield, cell viability after spray drying, and characterization of the microcapsules were performed. Results indicate that cell viability ranged between 94.06 and 97.97%. After six months, both yeasts stored at 7°C and 25°C presented 10^7 and 10^2 cells/mL, respectively. Regarding Fourier-transform infrared spectroscopy analysis, all microencapsulated yeasts presented typical spectra footprints of maltodextrin. After six months of storage, *S. cerevisiae* CCMA 0543 obtained a 10.8% increase in cell viability using maltodextrin with maltose as wall material in comparison with maltodextrin and maltodextrin with sucrose. However, *T. delbrueckii* CCMA 0684 obtained a 13.5% increase in cell viability using only maltodextrin. The study showed that maltodextrin as a wall material was efficient in the microencapsulation of yeasts. It is possible to assume that maltose incorporation increased the cell viability of *S. cerevisiae* CCMA 0543 during storage.

Keywords: Starter culture; Maltodextrin; Maltose; Sucrose.

1. Introduction

The storage of microorganisms in liquid form is not economically advantageous. Long-term storage reduces cell viability and strains' genetic stability, which may be associated with loss of cell robustness in industrial fermentation processes [1]. There are also transport and contamination issues that increase its difficulty of use.

Different drying technologies are mentioned as efficient processes to preserve microorganisms, such as freeze-drying [2], spray drying [3], and fluidized bed drying [4]. The purpose of these processes is to keep cell viability of the yeasts, to ease of handling, lower maintenance, transport, and storage costs. In addition, it allows storage for more extended periods, preserving cell viability and negligible risk of contamination. Thus, drying has been a viable alternative for culture storage [5-7].

Several studies have shown the potential of yeast for the coffee's production with exotic flavor profiles and high sensory scores looked for specialty coffee markets [8–12]. The specialty coffee market is expected to grow by $64.82 billion during 2021-2025, stimulating several companies to invest in this sector [13–15]. *Saccharomyces cerevisiae* CCMA 0543 and *Torulaspora delbrueckii* CCMA 0684 were isolated from coffee fruit and used as starter cultures during coffee fermentation. However, in liquid form, the use of pure culture is also unfeasible for application by the coffee producer. In addition, there is difficulty in transport and storage.

The dehydration of yeast cells causes them to enter a state of anhydrobiosis in which their metabolism is temporarily and reversibly suspended. This unique state among organisms is currently used to produce active dry yeast (ADY). The most used application is in bakery. However, they are also commercialized for beverages, such as beer, wine, whiskey, cachaça**,** and others. Strains of *S. cerevisiae* are produced in larger quantities by the yeast industry. However, in recent decades, the use of non-conventional yeasts, such as *T. delbrueckii*, has

been increasing in some areas of biotechnology [10-15]. Thus, the possibility of obtaining these yeast strains in a viable dry state becomes increasingly likely.

Different dehydration methods are used to obtain active dry yeast preparations and dry starter cultures for various biotechnological processes. All these approaches have their advantages and disadvantages [22]. However, during the last few years, some studies have been dedicated to improving and optimizing these drying methods to use them efficiently [23,24].

Freeze-drying is a drying method that allows the commercialization of yeast due to its excellent quality based on long-term cell viability. However, this method's energy consumption and time increase its final cost [25]. The fluidized bed technology is already used in industry, and among the different products that have been dehydrated are yeasts. The main problem of industrial fluidized bed dryers is the scaling up of processes. However, [26] reported that the multi-stage fluidized bed drying process is an effective commercial method for drying baker's yeast. Spray drying is another process that allows the production of active dry microbial preparations on an industrial scale, such as yeast. This technique allows high production rates with low operating costs [24]. Some of its advantages include good quality of the final product, low cost, greater control of variables, such as inlet and outlet temperature and feed flow, and continuously operation [27].

There are some studies on drying probiotic bacteria from spray drying [28,29], but there are few studies related to yeast drying. Most studies have evaluated the microencapsulation behavior of *Saccharomyces cerevisiae* strains [7,24,30]. However, a study with *Torulaspora delbrueckii* strains has not yet been reported.

One of the problems with this method is finding the ideal conditions that allow greater cell viability. The survival of these microorganisms depends on several factors, such as temperature, membrane characteristics, and cell morphology. During drying, removing the

water present inside the cells without damaging the cell membrane is crucial, so wall materials are essential to maintain their integrity [31].

Maltodextrin is considered the principal wall material due to its effectiveness, low cost, good solubility, and moisture control [7,30,32]. It is known that disaccharides, such as sucrose and maltose, may act as an alternative to water molecules, supporting the original conformation of the lipid bilayer of the cell membrane [33,34]. Also, they can delay the denaturation of proteins through hydrogen bonds with proteins, preserving cell structure [35]. Few studies have used sucrose [36] and maltose [24] to dry yeasts, and both wall materials have achieved good results. [7] increased the viability of probiotic yeast *S. cerevisiae* (strain KTP) by 35% with the sucrose addition during microencapsulation by spray drying.

Spray drying technology is widely used in the food industry to dry liquid foods and it have been shown the potential to dry microorganisms. The low cost and efficiency for drying become the product accessible for consumers. However, the process condition as wall materials must be optimized for each strain. Therefore, the objective of this work was to evaluate the microencapsulation feasibility of *Saccharomyces cerevisiae* CCMA 0543 and *Torulaspora delbrueckii* CCMA 0684 in three different compositions of wall material by spray-dryer.

2. Material and methods

2.1 Microorganisms

Saccharomyces cerevisiae CCMA 0543 (access number KF728798.1) and *Torulaspora delbrueckii* CCMA 0684 (access number KM402082.1) previously isolated from coffee fruits were used [37,38]. These strains belong to the Culture Collection of Agricultural Microbiology (CCMA, Federal University of Lavras, Lavras, Minas Gerais, Brazil) (http://www.ccma.dbi.ufla.br/). The isolates (1 mL) stored at -80°C in YEPD containing 20%

glycerol (w/w) were reactivated, without agitation for 48 h at 28°C, in tubes containing 9 mL of the liquid medium of molasses at 10°Brix [in g L^{-1}: liquid cane molasses 137.5 (ECL Eireli – supplier *Mellaço de Cana*), calcium chloride 1.1 (Dinâmica), urea 2 (Ambion), Polysorbate 80 0.5 (Dinâmica) and pH 3.5]. The molasses medium was used to growth of the yeasts being incubate at 28°C for 48 h and 120 rpm. Then, the cultures (10 mL) were transferred to 250 mL flasks containing 90 mL of medium. Subsequently, the cultures (100 mL) were transferred to 2 L flasks containing 900 mL of the medium. Finally, the cultures (1000 mL) were transferred to 20 L flasks containing 9 L of the same medium. At the end of growth, the yeasts reach the stationary phase. At the end of each step, the °Brix was measured, and the yeasts were enumerated by micro drop plating on YEPG agar [in g L^{-1}: yeast extract 10 (Himedia), glucose 20 (Dynamics), peptone 20 (Himedia), and agar 20 (Himedia)]. The plates were incubated at 28°C for 48h. The cells were recovered by centrifugation (3200 x g; 10 min) and resuspended in sterile water to a concentration of 10^9 CFU mL^{-1}.

2.2 Microencapsulation by spray drying

2.2.1 Preparation of the wall material

According to the methodology described in the literature [7], the wall material solutions were prepared with modifications. Three different compositions of wall material were used for each yeast: maltodextrin DE10 (15%) (represented as MD), maltodextrin DE10 (15%) with sucrose (2%) (represented as MD + SU), and maltodextrin DE10 (15%) with maltose (2%) (represented as MD + MA). The wall materials were dissolved in water, then autoclaving at 121°C for 15 minutes. After cooling, the cells were resuspended in each of the three investigated solutions. Yeasts at 10^9 cells/mL were added separately. The ratio of yeast to wall material was 1:1 (yeast cell dry mass: wall material dry mass). The feed suspension was mixed for 5 min with a magnetic stirrer and then sprayed drying.

151

152 **2.2.2 Spray drying conditions**

153 *Saccharomyces cerevisiae* CCMA 0543 and *Torulaspora delbrueckii* CCMA 0684 were
154 microencapsulated using a laboratory scale spray dryer (model MSD 1.0; Labmaq Brasil,
155 Ribeirão Preto, Brazil). The equipment was operated at a constant air inlet temperature of
156 80°C. The feed solutions (900 mL) were kept under constant magnetic stirring at room
157 temperature (approximately 25°C). These were fed into the main chamber through a
158 peristaltic pump, with a feed rate of 0.6 L h^{-1} dryer, the drying airflow rate of 2.4 x 10^3 L h^{-1},
159 and the atomization air flow rate of 5.94 x 10^6 L h^{-1} were used [39]. The resulting powder
160 (microcapsules) was collected at the cyclone base and stored in a 250g package made of
161 polyester + metallization + polyethylene. Thus, the incubation was in the absence of light.
162 Then the packages were vacuum-sealed. The samples were stored at refrigerated temperature
163 (7°C) and room temperature (25°C) for further analysis [32]. The spray drying was performed
164 in triplicate.

165

166 **2.3 Viability of microencapsulated cells during drying and storage**

167 The viability of the starter cultures, *Saccharomyces cerevisiae* CCMA 0543 and
168 *Torulaspora delbrueckii* CCMA 0684, were performed after the drying process and monitored
169 during 6 months of storage microcapsules at 7 and 25°C. Before viability analysis, samples
170 were homogenized by vortex, and sample powder (1 g) was mixed with 9 mL of peptone
171 water [in g L^{-1}: peptone 1 (Himedia)] [30]. The quantification of the microorganisms was
172 performed with serial dilution, followed by micro-drop plating on YEPG agar [40]. After
173 inoculation, the plates were incubated at 28°C for 48 hours. The experiment was carried out in
174 triplicate.

175

176 **2.4 Yield and cell viability after spray drying**

177 The powder yield (%, w/w) was calculated according to Eq. (1):

178

179 $$Powder\ yield\ (\%) = [Wp\ (1 - Xw, powder)/Ts]\ x\ 1($$ (1)

180

181 where *Wp* is the weight of the powder collected after drying, *Xw,powder* is th fraction of
182 water present in the powder and *Ts* is the total dry solids of the feed [30].

183 The cell viability after spray drying (%) was calculated according to Eq. (2):

184

185 $$Cell\ viability\ after\ spray\ drying\ (\%) = \{[(\mathrm{Log}\ N\ x\ Wp)\ /\ V\ ]/\log No\}\ x\ 1($$ (2)

186

187 where *N* is the number of viable cells (CFU mL^{-1} of wet material) in the powder, *Wp* is the
188 weight of the powder collected after drying, *V* is the volume of feed suspension, and *No* is the
189 number of viable cells (CFU mL^{-1}) in the initial suspensions [3].

190

191 **2.5 Characterization of microcapsules**

192 **2.5.1 Water activity, moisture content, hygroscopicity, and water solubility**

193 The water activities (a_w) of the wall materials and powder were measured using a water
194 activity meter (Aqualab 4TE, Decagon Devices, Pullman, Washington, USA). The moisture
195 content of the solutions, wall material, and powder was determined by the gravimetric method
196 [41]. Approximately 3 g of the sample was weighed in porcelain capsules of known mass. The
197 capsules were carried to the oven at 105 °C until constant weight. Moisture content was
198 expressed as a percentage of the initial weight. The hygroscopicity of the wall materials and
199 microcapsules was performed according to [39]. Samples (0.3 g) were placed in Petri dishes
200 and stored in a desiccator with no silica at 25 °C, containing a saturated sodium chloride

201 solution. After one week, the hygroscopicity was weighed and expressed as g of water
202 absorbed by the sample per 100 g dry solids. The water solubility of the wall materials and
203 microcapsules were characterized according to [42]. Samples (0.5 g) were placed in flasks
204 containing 50 mL of distilled water and homogenized in an orbital shaker at 100 rpm for 30
205 minutes at room temperature. Then, the solution was centrifuged at 3500 rpm for 5 minutes.
206 The supernatant was transferred to a porcelain capsule of a known weight. Then, it was placed
207 in an oven at 105 °C until constant weight.
208

209 **2.5.2 Morphology and size of microcapsules**

210 The morphology of microcapsules was evaluated using a scanning electron microscope
211 (SEM) (model Jeol JSM 6360 LV) with an acceleration voltage of 20 kV. A secondary
212 electron detector was used. The analysis was carried out at a high vacuum and no tilt was
213 applied. The microcapsules were mounted on aluminum bases adhered by double-sided
214 carbon adhesive tape. Then they were coated with gold with a vacuum spray applicator [29].
215 The size of the microcapsules was evaluated with the ImageJ 2014 software (Rasban,
216 National Institute of Health, USA) [3], with a diameter of 300 particles of each treatment was
217 measured.
218

219 **2.6 Fourier-transform infrared spectroscopy**

220 The wall materials and the powders resulting from the dried yeasts were evaluated by
221 analyzing vibrational spectroscopy in the infrared. An FT-IR Varian 600-IR spectrometer
222 with Fourier transform (FTIR) was used, with GladiATR accessory from Pike Technologies
223 coupled for measurements by attenuated total reflection (ATR) 45° with zinc selenide crystal.
224 For each sample, the FTIR spectra were represented an average of 32 scans in a range of 400
225 to 4000 cm^{-1} at a resolution of 4 cm^{-1} [43].

2.7 Statistical analysis

Cell viability, yield, water activity, moisture, hygroscopicity, solubility, and size data of the microencapsulated cells were evaluated by analyzing variance (ANOVA). Scott-Knott test was used to compare the means, adopting a significance level of 5 % probability. The tests were performed with Sisvar 5.6 software [44]. FTIR was analyzed by principal component analysis (PCA) using Chemoface software [45].

3. Results and discussion

3.1 Evaluation of the drying process

Spray drying microencapsulation of coffee epiphytic yeasts, *S. cerevisiae* CCMA 0543 and *T. delbrueckii* CCMA 0684, was performed. As previously mentioned, maltodextrin is considered the principal wall material due to its benefits. Therefore, the 15% concentration of maltodextrin was present in all treatments. Sucrose and maltose, both disaccharides, were added to verify the possible protection of cells during microencapsulation. These disaccharides may act against thermic and osmotic stresses, showing a protective effect on yeast cells and acting as stabilizers of membranes and proteins [34].

Spray drying conditions were fixed so that the influence of wall materials on yeast cell viability was evaluated. After drying, the population count was analyzed (Fig. 1). All treatments maintained the population count at 10^9 CFU mL^{-1}. *S. cerevisiae* CCMA 0543 did not obtain a significant difference between the treatment count after drying (Fig. 1a and 1b). However, there was a significant difference between the wall materials with *T. delbrueckii* CCMA 0684. Cell viability after drying was on overage 2.2% higher for *T. delbrueckii* CCMA 0684 microencapsulated only with 15% maltodextrin (Fig. 1c and 1d).

Powder yield and cell viability after spray drying were calculated (Table 1). There was no significant difference in the powder yield, ranging from 32.11 to 33.97%. However, there was a significant difference in cell viability between yeasts and the wall materials used. For example, *S. cerevisiae* CCMA 0543 obtained a 3.1% higher cell viability using MD + SU (97.10%). *T. delbrueckii* CCMA 0684 achieved 1.9% greater cell viability using only MD (97.97%).

The cell viability after spray drying varied between 94.06 to 97.97%, which is considered satisfactory (Table 1). The high cell viability after drying is correlated with the low temperature of the outlet air of the equipment. In all treatments, this varied between 55 and 59 °C. The outlet air temperature indicates the high temperature that the cells may have been exposed inside the dryer. Some studies have shown that the variation in the outlet temperature between 40 to 60 °C produced a satisfactory survival rate after spray drying [46,47]. Yeast cell components, such as DNA, RNA, proteins, and enzymes, can be negatively affected by high temperatures (45-75°C). Furthermore, high temperatures may degrade essential cellular structures such as the ribosome resulting in cell death [48,49].

Therefore, there was a significant difference between the yeasts in MD and MD + MA wall materials. In both wall materials, the cell viability was higher with *T. delbrueckii* CCMA 0684 (Table 1). Thus, cell viability was 3.9% higher for MD and 1.8% for MD + MA, which might have occurred due to the difference between yeast species and cell resistance during the drying process.

The stress tolerance of microorganisms may vary from species to species [50]. For example, [23] studied the physiological behavior of three non-*Saccharomyces* yeast strains during drying and compared them with the *S. cerevisiae* strain. Regarding cell viability, *T. delbrueckii* was also more resistant than *S. cerevisiae*. After the most drastic dehydration kinetics, 73 and 25% of viable cells were observed for *T. delbrueckii* and *S. cerevisiae*,

respectively. The most significant accumulation of trehalose found in *T. delbrueckii* and *S. cerevisiae* cells were 195 and 173 μg, respectively. The authors concluded that cell viability may be associated with a greater amount of trehalose synthesized by cells.

3.1 Characterization of wall materials and microcapsules

The water activity, moisture, and hygroscopic of the wall materials were measured (Table 2). There was a significant difference in the result of all analyzes. Maltodextrin had the lowest a_w (0.26), while maltose had the highest (0.38). The moisture content was higher for maltodextrin (1.17%) and lower for sucrose (0.08%). There was no significant difference in the hygroscopicity of maltose and sucrose wall materials. However, maltodextrin was significantly more hygroscopic, with an increase of 96%.

The characterization of wall material is important so that it is possible to understand the effectiveness of the thermal drying process and the storage period. The powders obtained after yeast microencapsulation were also characterized (Table 1).

Water activity is essential in estimating stability during storage [51]. A_w ranged between 0.25 and 0.28 (Table 1). The value found was satisfactory since it is generally accepted that the ideal stability is obtained between 0.2 and 0.3 [52]. There was a significant difference in water activity between yeasts and wall materials. The microencapsulation of *S. cerevisiae* CCMA 0543 showed lower a_w using MD and MD + SU, both with 0.25. *T delbrueckii* CCMA 0684 showed lower a_w with MD (0.25). The water activity with the MD + SU and MD + MA wall materials was significantly higher with *T. delbrueckii* CCMA 0684 (0.28). The addition of 2% sucrose and 2% maltose may have contributed to the increase in water activity, since maltose showed the highest water activity, followed by sucrose and maltodextrin (Table 2), corroborating with the results obtained.

There was no significant difference in the moisture of the powders. These ranged from 0.59 to 0.65% (Table 1). The recommended value for powdered foods is a maximum of 4%, corroborating with the data found in this study [53]. The characteristics of the drying air strongly influence the water content of a powder. Besides, there is a correlation between moisture content and a_w, thus significantly influencing powder storage. This affects stability, influencing microbiological and physicochemical parameters such as non-enzymatic browning, enzymatic activity, and cell viability [51].

There was a significant difference in hygroscopicity and water solubility between yeasts and wall materials (Table 1). *S. cerevisiae* CCMA 0543 obtained less hygroscopicity with MD (10.87 g 100 g^{-1}) and MD + SU (10.74 g 100 g^{-1}) and *T. delbrueckii* CCMA 0684 with MD (4.8 g 100 g^{-1}). There was a significant difference between yeasts for all treatments. Treatments with *S. cerevisiae* CCMA 0543 had hygroscopicity between 10.74 and 12.06 g 100 g^{-1}, while *T. delbrueckii* CCMA 0684 varied between 4.8 and 6.15 g 100 g^{-1}.

Thus, according to these results, it may be that the yeast species used has influenced the hygroscopicity of the powders obtained. For example, a powder is considered non-hygroscopic if the percentage of hygroscopicity is less than 10%, as was observed for *T. delbrueckii* CCMA 0684, and slightly hygroscopic between 10.1 to 15%, as observed for *S. cerevisiae* CCMA 0543. Non-hygroscopic powders are desirable because they allow microcapsule stability and facilitate handling. Besides that, the absorption of water in a powder is an important parameter for choosing appropriate packaging that will depend on the composition of the powder and the particle size [51].

The solubility of the powders varied between 74.01 and 85.41% (Table 1). *S. cerevisiae* CCMA 0543 obtained greater solubility with MD + MA (78.22%) and *T. delbrueckii* CCMA 0684 with MD + SU (84.54%) and MD + MA (85.41%). There was a significant difference in results using MD + SU and MD + MA between yeasts. In both, the solubility was higher for

T. delbrueckii CCMA 0684, increasing 12.5% and 8.4%, respectively. Solubility is an essential parameter in controlling the quality of powders that must be incorporated in the aqueous phase [51], as is the case of these yeasts for later inoculation in coffee fruit fermentation.

The size of the microcapsules ranged from 3.43 to 3.96 µm (Table 1). There was no significant difference between treatments with *S. cerevisiae* CCMA 0543. The same data was found by [7]. [32] also obtained a score of 3.47 µm for the microencapsulation of *S. cerevisiae* var. *boulardii* with maltodextrin. However, there was a difference with *T. delbrueckii* CCMA 0684, 7.5% lower with MD (3.43 µm). Adding 2% sucrose and 2% maltose might increase the microcapsules' size due to the increase in the solids number. There was also a significant difference between yeasts, with MD being 9.3% higher for *S. cerevisiae* CCMA 0543 (3.78 µm) (Table 1). The size of yeast cells may influence the size of microcapsules. According to [54], *Torulaspora* species have spherical to ellipsoidal cells that are slightly smaller than *S. cerevisiae*, with dimensions of approximately 2-4 x 3-5 µm.

The size of the microparticles is an important characteristic because it affects physical and functional properties such as solubility and hygroscopicity as these depend on the contact surface. The size of the droplets mainly influences this during spraying and the viscosity of the solution. Thus, the high spray pressure and low viscosity reduce the size of the particles [51]. Also, as found in this study, smaller-sized particles are preferred in food formulations to ensure product homogeneity [55].

The micrographs of the microencapsulated yeasts with the different wall materials can be seen in Fig. 2. Regardless of yeast or wall material, all treatments presented irregular matrix shapes and undefined shapes. Some microparticles are smooth, and others are deformed and notched with concavities and varying sizes. These morphologies are characteristic of spray-dried powders [3,30,39]. Surface imperfections may be related to a slow film formation

process during the drying of the atomized droplets [56]. The microcapsules showed no fissure or rupture, which confirms good structural integrity. Besides, it can be observed that the addition of 2% sucrose (Fig. 2b and 2e) and 2% maltose (Fig. 2c and 2f) were not enough to influence the morphology of the microcapsules.

3.2 Viability of microencapsulated cells during storage

The viability of the microencapsulated powders of *S. cerevisiae* CCMA 0543 (Fig. 1a and 1b) and *T. delbrueckii* CCMA 0684 (Fig. 1c and 1d) were evaluated over six months. The powders were stored at two different temperatures: refrigeration (7°C) and room (25°C). There was variation in viability during storage at different temperatures for both yeasts.

The viability of *S. cerevisiae* CCMA 0543 stored at 25°C obtained a significant difference between treatments after the second month of storage (Fig. 1a). The most significant viability was with MD + MA as the wall material. This difference remained at 8.59 x 10^2 CFU mL^{-1} until the sixth month. Viability with the other treatments was less than 10^2 CFU mL^{-1}. Thus, on a logarithmic basis, at the end of six months and room temperature, the cell viability of *S. cerevisiae* CCMA 0543 was 31.8% higher with MD + MA wall material.

The viability of *S. cerevisiae* CCMA 0543 stored at 7°C showed a significant difference from the first month (Fig. 1b). This difference varied between treatments during the other months of storage. At the end of the sixth month, the most significant viability was found with MD + MA (2.51x10^7 CFU mL^{-1}) as the wall material, corroborating the results obtained at 25°C. The MD and MD + SU treatments obtained 4.31x10^6 CFU mL^{-1}.

Therefore, on a logarithmic basis, a 10.8% increase in cell viability of *S. cerevisiae* CCMA 0543 was obtained after 6 months of storage in the presence of MD + MA. Thus, maltose proved to be an important disaccharide in protecting against stress from *S. cerevisiae* CCMA 0543 during the spray drying process, increasing cell viability. In addition,

disaccharides can retard the denaturation of proteins through hydrogen bonds, preserving cell structure [35].

The cell count of *T. delbrueckii* CCMA 0684 stored at 25°C showed a significant difference only in the fifth month for MD + MA ($7.97x10^3$ CFU mL^{-1}) (Fig 1c). However, there was no difference between the three treatments in the sixth month. Viability ranged from $3.57x10^2$ to $4.78x10^2$ CFU mL^{-1}.

The viability of *T. delbrueckii* CCMA 0684 stored at 7°C showed a significant difference with MD + SU ($2.23x10^8$ CFU mL^{-1}) (Fig. 1d). However, the most significant viability was found in the sixth month using MD ($2.64x10^7$ CFU mL^{-1}) as the wall material. Thereby, on a logarithmic basis, at the end of 6 months, *T. delbrueckii* CCMA 0684 microencapsulated with MD obtained 13.5% greater cell viability.

At the end of six months, it was possible to observe a variation of approximately 10^5 CFU mL^{-1} between the two storage temperatures for both yeasts. The viability was around 10^2 CFU mL^{-1} at room temperature, while it was around 10^7 CFU mL^{-1} at refrigeration temperature. So, the rate of decrease in cell viability is related to the storage temperature. Lower temperatures can prolong storage time without significant loss of cell viability [57]. Over time, the decrease in cell viability occurs due to the natural degradation of macromolecules essential to life. Lipids and proteins undergo oxidation and denaturation over a prolonged storage period [50].

Thus, the decrease in cell viability is related to microbial metabolism. For this reason, wall materials are added to protect the cell from adverse conditions, such as temperature, oxygen, and humidity, increasing the stability of the microcapsule during storage. That is why the choice of wall material is so important [50,58].

Finally, it was observed that the best wall material for both yeasts at the end of six months was different. As previously mentioned, *S. cerevisiae* CCMA 0543 obtained greater cell viability using MD + MA as a wall material at both storage temperatures (7 and 25°C).

399 However, *T. delbrueckii* CCMA 0684 obtained a significant difference only in refrigeration
400 temperature (7°C), in which it obtained greater cell viability using only MD. Thus, the
401 addition of 2% maltose contributed to *S. cerevisiae's* viability throughout the microcapsules'
402 storage. Probably this disaccharide was able to replace the water molecules during the drying
403 process, delaying protein denaturation over time. Furthermore, in this drying process,
404 maltodextrin is a viable and economical alternative.

405 *S. cerevisiae* CCMA 0543 and *T. delbrueckii* CCMA 0684 belong to genera that are not
406 genetically close to each other [59,60]. Thus, it was expected to obtain different responses to
407 stress between the two strains evaluated. However, the phenomenon of yeast cells' adaptation
408 to stress is very complex. Thus, several responses occur during cell growth and dehydration.
409 For example, yeasts may accumulate compatible solutes, such as trehalose, by providing
410 stressful environments throughout biomass formation. This mechanism aims to survive a
411 possible period of stress, such as dehydration [23]. In addition, trehalose is associated with
412 several cell protection mechanisms such as stabilizing membranes through interaction with
413 hydrogen bonds and phospholipids, reducing lipid peroxidation, and antioxidant action [33].

414 Cell survival is the most significant parameter because the drying technique will be
415 helpful only if the microorganisms maintain their function and reproducibility. Consequently,
416 it is necessary to maintain essential cellular structures intact after drying and entirely
417 functional after rehydration. However, stress tolerance and cell viability during dehydration
418 are related to the physiological characteristics of each yeast strain [7,50,61].

419

420 **3.3 FTIR analysis**

421 FTIR was carried out to understand the characteristics of intermolecular interactions
422 between wall materials and encapsulated material. FTIR spectra ranged from -4000 to -400
423 cm^{-1} related to wall materials (see Figure 4a) and microencapsulated yeasts (see Figure 4b).

Once sucrose and maltose are disaccharides, both present a similar spectral region. The first characteristic peak occurs in the spectral region -3750 to -3000 cm^{-1} correlated to hydrogen bonds (O - H). The region between -1474 and -1199 cm^{-1} may represent the bending modes of O − C − H, C − C – H, C − O – H. Bands between -1153 to -904 cm^{-1} regions are assigned to C – O and C – C stretching modes. The spectral region between -900 and 750 cm^{-1} corresponds to the anomeric region characteristic of the saccharides configuration [62,63]. Finally, it is known that absorption peaks at -994 cm^{-1} are characteristic of glycosidic links of sucrose, while maltose has a pattern peak at 1032 cm^{-1} [64,65].

Regarding maltodextrin (Fig. 3a), the first characteristic peak occurs at the frequency -3309 cm^{-1} due to hydrogen bonds (O – H), also observed in sucrose and maltose. In addition, the spectra showed bands at -2911 cm^{-1} (elongation C – H); -1637 cm^{-1} (elongation C = O); -1147, -1078, and -993 cm^{-1} (C - O elongation). The other spectra observed at -923, -844, -757, -703, and -570 cm^{-1} are correlated to vibrations of the pyranose ring skeleton of the maltodextrin glucose monomers [66,67].

Concerning the treatments, it is possible to observe a pattern in FTIR spectra for all studied treatments (Fig. 3b). All peaks present a similar footprint observed in maltodextrin spectra, indicating that maltodextrin rules the FTIR spectra. It probably happened due to the higher concentration of maltodextrin (15%) in comparison to maltose and sucrose (2%).

Therefore, PCA was applied to evaluate the variations in spectra between samples (Fig. 4). Scatter plots were used to interpret the differences and similarities between the samples, so each significant component was characterized by the scale coefficient. Similar samples are usually grouped about the components used.

The main components responded to 90.84 % of the total spectral variation in microencapsulated powders, explained by PC1 and PC2 at 62.20 % and 28.64 %, respectively (Fig. 4a). There were high correlations of spectra between samples: *S. cerevisiae* CCMA

449 0543+MD+SU and *T. delbrueckii* CCMA 0684+MD and between *S. cerevisiae* CCMA
450 0543+MD and *T. delbrueckii* CCMA 0684+MD+SU, probably because they are in the same
451 quadrant and there are acute angles between them.

452 However, the microencapsulated powders of both yeasts with MD + MA were the most
453 discrepant and, consequently, it was not possible to correlate with the other treatments (Fig.
454 4a). The discrepancy found might occurred due to differences in the intensity of some spectra
455 regions (Fig. 3a). This result was confirmed by the PCA loading graph (Fig. 4b and 4c),
456 which showed the relationship between the main component and the original variables.

457 According to the graph, it is possible to identify which variables are responsible for the
458 differences observed in the samples. The variable that has great amplitude, whether positive
459 or negative, is significant. PC1 (Fig. 4b) and PC2 (Fig. 4c) showed that the spectra regions
460 between -3580 and -2990 cm^{-1} and between -1170 and 925 cm^{-1} are responsible for the
461 differences between samples. Probable chemical groups present in these regions are hydrogen
462 bonds (O - H) and C - O elongation, respectively. The graph can confirm this result. In both
463 regions, the spectra of *S. cerevisiae* CCMA 0543 MD + MA and *T. delbrueckii* CCMA 0684
464 MD + MA showed greater and lesser intensity, respectively (Fig. 3a). Therefore, FTIR spectra
465 allowed to observe differences between samples.

466

467 **4. Conclusion**

468 Maltodextrin, maltose, and sucrose were evaluated as wall material for *S. cerevisiae*
469 CCMA 0543 and *T. delbrueckii* CCMA 0684. The yeasts responded differently to the
470 microencapsulation process since stress tolerance and cell viability during the drying process
471 are related to the physiological characteristics of each yeast strain. The best wall material for
472 microencapsulation of *S. cerevisiae* CCMA 0543 was 15% maltodextrin with 2% maltose,
473 and for *T. delbrueckii* CCMA 0684, it was 15% maltodextrin. Maltose proved to be an

474 essential wall material for cell protection of *S. cerevisiae* CCMA 0543 during the spray drying
475 process. In both processes, the best storage temperature for microencapsulated powders was
476 7°C. Therefore, the drying methodology used for *S. cerevisiae* CCMA 0543 and *T.*
477 *delbrueckii* CCMA 0684 may help formulate a product for use in coffee fermentation and the
478 production of specialty coffees. Processing parameters should be optimized to improve the
479 cell viability of epiphytic coffee yeasts at room temperature.
480

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Data Availability

Data will be made available on reasonable request.

Declarations

Conflict of Interest

The authors declare no competing interests.

References

1. Boulton C, Quain D (2001) Yeast management. In: Boulton C, Quain D (ed) Brewing Yeast and Fermentation, 1st edn. Blackwell Science Ltd, Oxford, pp 468-509
2. Stefanello RF, Nabeshima EH, Iamanaka BT, et al (2019) Survival and stability of *Lactobacillus fermentum* and *Wickerhamomyces anomalus* strains upon lyophilisation with different cryoprotectant agents. Food Res Int 115:90-94. https://doi.org/10.1016/j.foodres.2018.07.044
3. Vanden Braber NL, Díaz Vergara LI, Rossi YE, et al (2020) Effect of microencapsulation in whey protein and water-soluble chitosan derivative on the viability of the probiotic *Kluyveromyces marxianus* VM004 during storage and in simulated gastrointestinal conditions. LWT - Food Sci Technol 118:108844. https://doi.org/10.1016/j.lwt.2019.108844
4. Strasser S, Neureiter M, Geppl M, Braun R, Danner H (2009) Influence of lyophilization, fluidized bed drying, addition of protectants, and storage on the viability of lactic acid bacteria. J Appl Microbiol 107(1):167-177.

https://doi.org/10.1111/j.1365-2672.2009.04192.x

5. Elizondo H, Labuza TP (1974) Death kinetics of yeast in spray drying. Biotechnol Bioeng 16(9):1245-1259. https://doi.org/10.1002/bit.260160908

6. Morgan CA, Herman N, White PA, Vesey G (2006) Preservation of micro-organisms by drying; A review. J Microbiol Methods 66(2):183-193. https://doi.org/10.1016/j.mimet.2006.02.017

7. Suryabhan P, Lohith K, Anu-Appaiah KA (2019) Sucrose and sorbitol supplementation on maltodextrin encapsulation enhance the potential probiotic yeast survival by spray drying. LWT - Food Sci Technol 107:243-248. https://doi.org/10.1016/j.lwt.2019.03.002

8. Bressani APP, Martinez SJ, Sarmento ABI, Borém FM, Schwan RF. Influence of yeast inoculation on the quality of fermented coffee (*Coffea arabica* var. Mundo Novo) processed by natural and pulped natural processes. Int J Food Microbiol 343:109107. https://doi.org/10.1016/j.ijfoodmicro.2021.109107

9. Da Mota MCB, Batista NN, Rabelo MHS, Ribeiro DE, Borém FM, Schwan RF (2020) Influence of fermentation conditions on the sensorial quality of coffee inoculated with yeast. Food Res Int 136:109482. https://doi.org/10.1016/j.foodres.2020.109482

10. Elhalis H, Cox J, Frank D, Zhao J (2021) Microbiological and Chemical Characteristics of Wet Coffee Fermentation Inoculated With *Hansinaspora uvarum* and *Pichia kudriavzevii* and Their Impact on Coffee Sensory Quality. Front Microbiol 12. https://doi.org/10.3389/fmicb.2021.713969

11. da Silva BL, Pereira PV, Bertoli LD, et al (2021) Fermentation of *Coffea canephora* inoculated with yeasts: Microbiological, chemical, and sensory characteristics. Food Microbiol 98:103786. https://doi.org/10.1016/j.fm.2021.103786

12. Prakash I, R SS, P SH, et al (2022) Metabolomics and volatile fingerprint of yeast

fermented robusta coffee: A value added coffee. LWT - Food Sci Technol 154:112717. https://doi.org/10.1016/j.lwt.2021.112717

13. TechNavio (2021) Global Specialty Coffee Shops Market 2021-2025. https://www.researchandmarkets.com/reports/4990317/global-specialty-coffee-shops-market-2021-2025

14. Adroit (2021) Specialty Coffee Market by Grade (80-84.99, 80-84.99 and 90-100), Application (Home and Commercial) and by Region (North America, Europe, Asia-Pacific, Middle East & Africa, and South America), Global Forecast - 2019 to 2028. https://www.adroitmarketresearch.com/industry-reports/specialty-coffee-market

15. Saerens S, Swiegers JH. Enhancement of coffee quality and flavor by using *Pichia Kluyveri* yeast starter culture for coffee fermentation. Published online 2014. https://patents.justia.com/patent/20160058028

16. Alcazar-Valle M, Gschaedler A, Gutierrez-Pulido H, Arana-Sanchez A, Arellano-Plaza M (2019) Fermentative capabilities of native yeast strains grown on juices from different Agave species used for tequila and mezcal production. Brazilian J Microbiol 50(2):379-388. https://doi.org/10.1007/s42770-019-00049-7

17. Vaquero C, Loira I, Heras JM, Carrau F, González C, Morata A (2021) Biocompatibility in Ternary Fermentations With *Lachancea thermotolerans*, Other Non-*Saccharomyces and Saccharomyces cerevisiae* to Control pH and Improve the Sensory Profile of Wines From Warm Areas. Front Microbiol 12. https://doi.org/10.3389/fmicb.2021.656262

18. Bressani APP, Martinez SJ, Batista NN, Simão JBP, Dias DR, Schwan RF (2021) Co-inoculation of yeasts starters: A strategy to improve quality of low altitude Arabica coffee. Food Chem 361:130133. https://doi.org/10.1016/j.foodchem.2021.130133

19. Li Z, Song K, Li H, Ma R, Cui M (2019). Effect of mixed *Saccharomyces cerevisiae* Y10 and *Torulaspora delbrueckii* Y22 on dough fermentation for steamed bread

making. Int J Food Microbiol 303:58-64. https://doi.org/10.1016/j.ijfoodmicro.2019.05.00

20. Visintin S, Ramos L, Batista N, Dolci P, Schwan F, Cocolin L (2017). Impact of *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* starter cultures on cocoa beans fermentation. Int J Food Microbiol 257:31-40. https://doi.org/10.1016/j.ijfoodmicro.2017.06.004

21. Yang W, Liu S, Marsol-Vall A, et al (2021) Chemical composition, sensory profile and antioxidant capacity of low-alcohol strawberry beverages fermented with *Saccharomyces cerevisiae* and *Torulaspora delbrueckii*. LWT - Food Sci Technol 149:111910. https://doi.org/10.1016/j.lwt.2021.111910

22. Rapoport A (2017). Anhydrobiosis and Dehydration of Yeasts. In: Sibirny AA (ed) Biotechnology of Yeasts and Filamentous Fungi, 1st edn. Springer International Publishing, Switzerland, pp 87-116

23. Câmara A de A, Maréchal P-A, Tourdot-Maréchal R, Husson F (2019) Dehydration stress responses of yeasts *Torulaspora delbrueckii*, *Metschnikowia pulcherrima* and *Lachancea thermotolerans*: Effects of glutathione and trehalose biosynthesis. Food Microbiol 79:137-146. https://doi.org/10.1016/j.fm.2018.12.008

24. Aponte M, Troianiello GD, Di Capua M, Romano R, Blaiotta G (2016) Impact of different spray-drying conditions on the viability of wine *Saccharomyces cerevisiae* strains. World J Microbiol Biotechnol 32(1):13. https://doi.org/10.1007/s11274-015-1956-5

25. Marques LG, Silveira AM, Freire JT (2006) Freeze-Drying Characteristics of Tropical Fruits. Dry Technol 24(4):457-463. https://doi.org/10.1080/07373930600611919

26. Akbari H, Karimi K, Lundin M, Taherzadeh MJ (2012) Optimization of baker's yeast drying in industrial continuous fluidized bed dryer. Food Bioprod Process 90(1):52-57.

https://doi.org/10.1016/j.fbp.2010.12.005

27. Martín MJ, Lara-Villoslada F, Ruiz MA, Morales ME (2015) Microencapsulation of bacteria: A review of different technologies and their impact on the probiotic effects. Innov Food Sci Emerg Technol 27:15-25. https://doi.org/10.1016/j.ifset.2014.09.010

28. Alfaro-Galarza O, López-Villegas EO, Rivero-Perez N, et al (2020) Protective effects of the use of taro and rice starch as wall material on the viability of encapsulated *Lactobacillus paracasei* subsp. *paracasei*. LWT - Food Sci Technol 117:108686. https://doi.org/10.1016/j.lwt.2019.108686

29. Andrade DP, Ramos CL, Botrel DA, Borges SV, Schwan RF, Ribeiro Dias D (2019) Stability of microencapsulated lactic acid bacteria under acidic and bile juice conditions. Int J Food Sci Technol 54(7):2355-2362. https://doi.org/10.1111/ijfs.14114

30. Chandralekha A, Tavanandi AH, Amrutha N, Hebbar HU, Raghavarao KSMS, Gadre R (2016) Encapsulation of yeast (*Saccharomyces cerevisiae*) by spray drying for extension of shelf life. Dry Technol 34(11):1307-1318. https://doi.org/10.1080/07373937.2015.1112808

31. Behboudi-Jobbehdar S, Soukoulis C, Yonekura L, Fisk I (2013) Optimization of Spray-Drying Process Conditions for the Production of Maximally Viable Microencapsulated *L. acidophilus* NCIMB 701748. Dry Technol 31(11):1274-1283. https://doi.org/10.1080/07373937.2013.788509

32. Arslan S, Erbas M, Tontul I, Topuz A (2015) Microencapsulation of probiotic *Saccharomyces cerevisiae* var. *boulardii* with different wall materials by spray drying. LWT - Food Sci Technol 63(1):685-690. https://doi.org/10.1016/j.lwt.2015.03.034

33. Crowe LM (2002) Lessons from nature: the role of sugars in anhydrobiosis. Comp Biochem Physiol Part A Mol Integr Physiol 131(3):505-513. https://doi.org/10.1016/S1095-6433(01)00503-7

626 34. Crowe JH, Hoekstra FA, Crowe LM (1992) Anhydrobiosis. Annu Rev Physiol
627 54(1):579-599. https://doi.org/10.1146/annurev.ph.54.030192.003051
628 35. Peighambardoust SH, Golshan Tafti A, Hesari J (2011) Application of spray drying for
629 preservation of lactic acid starter cultures: a review. Trends Food Sci Technol
630 22(5):215-224. https://doi.org/10.1016/j.tifs.2011.01.009
631 36. N'Guessan FK, Coulibaly HW, Alloue-Boraud MWA, Cot M, Djè KM (2016)
632 Production of freeze-dried yeast culture for the brewing of traditional sorghum beer,
633 tchapalo. Food Sci Nutr 4(1):34-41. https://doi.org/10.1002/fsn3.256
634 37. Silva CF, Schwan RF, Sousa Dias Ë, Wheals AE (2000). Microbial diversity during
635 maturation and natural processing of coffee cherries of *Coffea arabica* in Brazil. Int J
636 Food Microbiol 60(2-3):251-260. https://doi.org/10.1016/S0168-1605(00)00315-9
637 38. Vilela DM, Pereira GV de M, Silva CF, Batista LR, Schwan RF (2010). Molecular
638 ecology and polyphasic characterization of the microbiota associated with semi-dry
639 processed coffee (*Coffea arabica* L.). Food Microbiol 27(8):1128-1135.
640 https://doi.org/10.1016/j.fm.2010.07.024
641 39. Braga ABAC, Costa CJM, Pomella AWV, Ribeiro EJ, Santos LD, Zotarelli MF (2019)
642 Evaluation of lethality temperature and use of different wall materials in the
643 microencapsulation process of *Trichoderma asperellum* conidias by spray drying.
644 Powder Technol 347:199-206. https://doi.org/10.1016/j.powtec.2019.02.037
645 40. Herigstad B, Hamilton M, Heersink J (2001) How to optimize the drop plate method
646 for enumerating bacteria. J Microbiol Methods 44(2):121-129.
647 https://doi.org/doi:10.1016/S0167-7012(00)00241-4
648 41. Horwitz W (2005) Official Methods of Analysis. Association of Official Analytical
649 Chemists, Washington
650 42. Cano-Chauca M, Stringheta PC, Ramos AM, Cal-Vidal J (2005) Effect of the carriers

651 on the microstructure of mango powder obtained by spray drying and its functional
652 characterization. Innov Food Sci Emerg Technol 6(4):420-428.
653 https://doi.org/10.1016/j.ifset.2005.05.003
654 43. de la Cruz-Gavia A, Pérez-Alonso C, Barrera-Díaz CE, Alvarez-Ramírez J, Carrillo-
655 Navas H, Guadarrama-Lezama AY (2018). Survival of *Saccharomyces cerevisiae*
656 microencapsulated with complex coacervate after freezing process. Food Hydrocoll
657 82:45-52. https://doi.org/10.1016/j.foodhyd.2018.03.045
658 44. Ferreira DF. Sisvar: a Guide for its Bootstrap procedures in multiple comparisons
659 (2014). Ciência e Agrotecnologia 38(2):109-112. https://doi.org/10.1590/S1413-
660 70542014000200001
661 45. Nunes CA, Freitas MP, Pinheiro ACM, Bastos SC (2012). Chemoface: a novel free
662 user-friendly interface for chemometrics. J Braz Chem Soc 23(11):2003-2010.
663 https://doi.org/10.1590/S0103-50532012005000073
664 46. Horaczek A, Viernstein H (2004) *Beauveria brongniartii* subjected to spray-drying in a
665 composite carrier matrix system. J Microencapsul 21(3):317-330.
666 https://doi.org/10.1080/02652040410001673892
667 47. REDDY KBPK, MADHU AN, PRAPULLA SG (2009) Comparative survival and
668 evaluation of functional probiotic properties of spray-dried lactic acid bacteria. Int J
669 Dairy Technol 62(2):240-248. https://doi.org/10.1111/j.1471-0307.2009.00480.x
670 48. Obuchi K, Iwahashi H, Lepock JR, Komatsu Y (2000) Calorimetric characterization of
671 critical targets for killing and acquired thermotolerance in yeast. Yeast 16(2):111-119.
672 https://doi.org/10.1002/(SICI)1097-0061(20000130)16:2<111::AID-
673 YEA507>3.0.CO;2-V
674 49. Rapoport A, Golovina EA, Gervais P, Dupont S, Beney L (2019) Anhydrobiosis:
675 Inside yeast cells. Biotechnol Adv 37(1):51-67.

https://doi.org/10.1016/j.biotechadv.2018.11.003

50. Fu N, Chen XD (2011) Towards a maximal cell survival in convective thermal drying processes Food Res Int 44(5):1127-1149. https://doi.org/10.1016/j.foodres.2011.03.053

51. Schuck P, Dolivet A, Jeantet R (2012) Analytical Methods for Food and Dairy Powders. Wiley-Blackwell, France.

52. Esftathiou T, Feuardent C, Méjean S, Schuck P (2002) The use of carbonyl analysis to follow the main reactions involved in the process of deterioration of dehydrated dairy products: prediction of most favourable degree of dehydration. Lait 82(4):423-439. https://doi.org/10.1051/lait:2002021

53. Ananta E, Volkert M, Knorr D (2005) Cellular injuries and storage stability of spray-dried *Lactobacillus rhamnosus* GG. Int Dairy J 15(4):399-409. https://doi.org/10.1016/j.idairyj.2004.08.004

54. Benito S (2018) The impact of *Torulaspora delbrueckii* yeast in winemaking. Appl Microbiol Biotechnol 102(7):3081-3094. https://doi.org/10.1007/s00253-018-8849-0

55. Burgain J, Gaiani C, Linder M, Scher J (2011) Encapsulation of probiotic living cells: From laboratory scale to industrial applications. J Food Eng 104(4):467-483. https://doi.org/10.1016/j.jfoodeng.2010.12.031

56. Ré MI (1998) Microencapsulation by spray drying. Dry Technol 16(6):1195-1236. https://doi.org/10.1080/07373939808917460

57. Dimitrellou D, Tsaousi K, Kourkoutas Y, Panas P, Kanellaki M, Koutinas AA (2008) Fermentation efficiency of thermally dried immobilized kefir on casein as starter culture. Process Biochem 43(12):1323-1329. https://doi.org/10.1016/j.procbio.2008.07.017

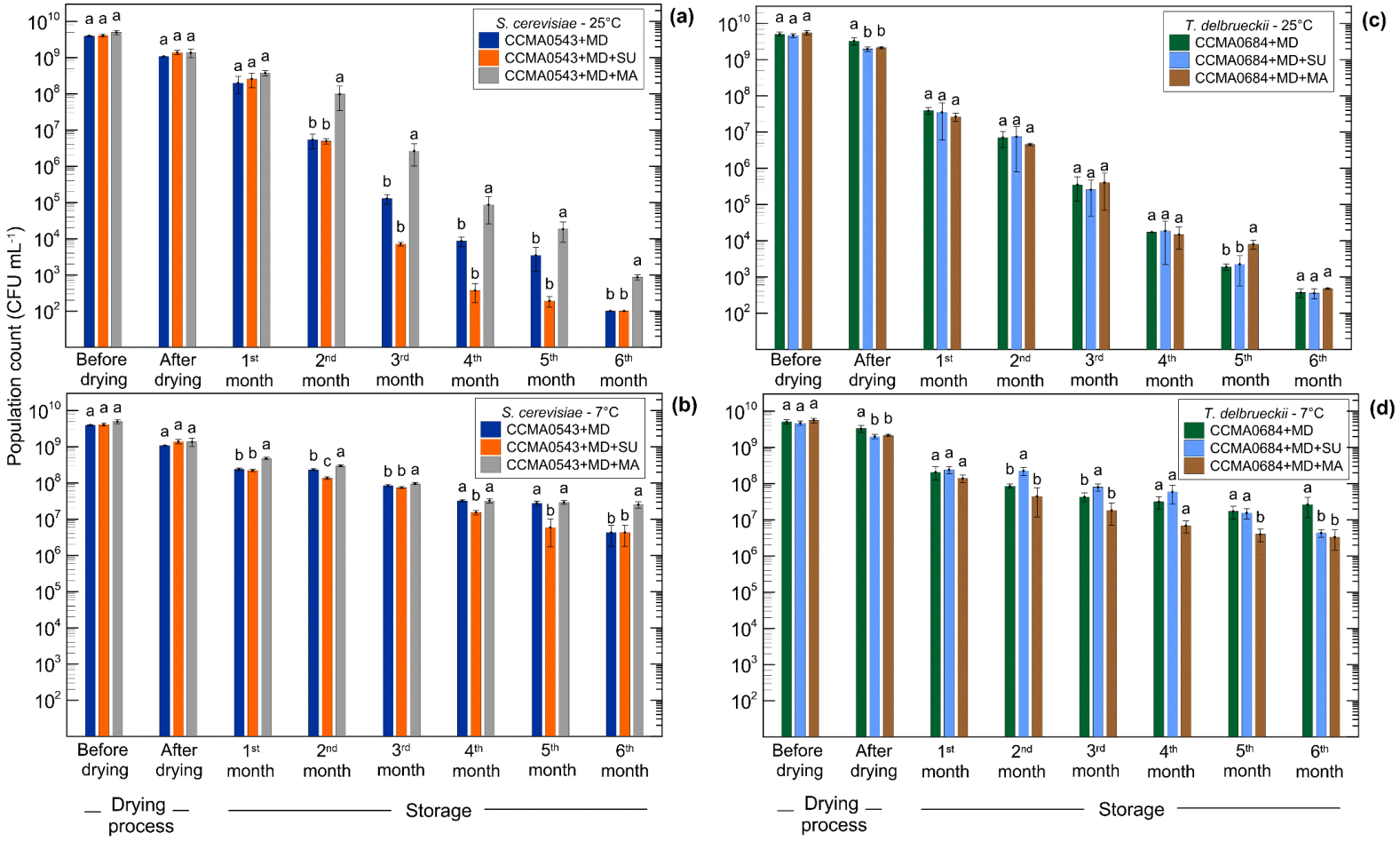
58. Gharsallaoui A, Roudaut G, Chambin O, Voilley A, Saurel R (2007) Applications of spray-drying in microencapsulation of food ingredients: An overview. Food Res Int

701 40(9):1107-1121. https://doi.org/10.1016/j.foodres.2007.07.004
702 59. Rozpędowska E, Piškur J, Wolfe KH (2011) Genome Sequences of Saccharomycotina:
703 Resources and Applications in Phylogenomics. In: Kurtzman CP, Fell JW, Boekhout T
704 (ed) The Yeasts: A Taxonomy Study. 5th edn. Elsevier, London, pp 145-157
705 60. Oda Y, Fukunaga M (1999) Isolation and characterization of MELt gene from
706 *Torulaspora delbrueckii* IFO 1255. Yeast 15:1797-1801.
707 https://doi.org/10.1002/(SICI)1097-0061(199912)15:16<1797::AID-
708 YEA498>3.0.CO;2-G
709 61. Alonso S (2016) Novel preservation techniques for microbial cultures. In: Pilar M (ed)
710 Novel Food Fermentation Technologies, 1st edn. Springer, Cham, pp 7-33
711 62. Tewari J, Irudayaraj J (2004) Quantification of saccharides in multiple floral honeys
712 using fourier transform infrared microattenuated total reflectance spectroscopy. J Agric
713 Food Chem 52(11):3237-3243. https://doi.org/10.1021/jf035176+
714 63. Tul'chinsky VM, Zurabyan SE, Asankozhoev KA, Kogan GA, Khorlin AY (1976)
715 Study of the infrared spectra of oligosaccharides in the region 1,000-40 cm−1.
716 Carbohydr Res 51(1):1-8. https://doi.org/10.1016/S0008-6215(00)84031-8
717 64. Vasko PD, Blackwell J, Koenig JL (1971) Infrared and raman spectroscopy of
718 carbohydrates. Carbohydr Res 19(3):297-310. https://doi.org/10.1016/S0008-
719 6215(00)86160-1
720 65. Wang J, Kliks MM, Jun S, Jackson M, Li QX (2010) Rapid Analysis of Glucose,
721 Fructose, Sucrose, and Maltose in Honeys from Different Geographic Regions using
722 Fourier Transform Infrared Spectroscopy and Multivariate Analysis. J Food Sci
723 75(2):C208-C214. https://doi.org/10.1111/j.1750-3841.2009.01504.x
724 66. Kang Y-R, Lee Y-K, Kim YJ, Chang YH (2019) Characterization and storage stability
725 of chlorophylls microencapsulated in different combination of gum Arabic and

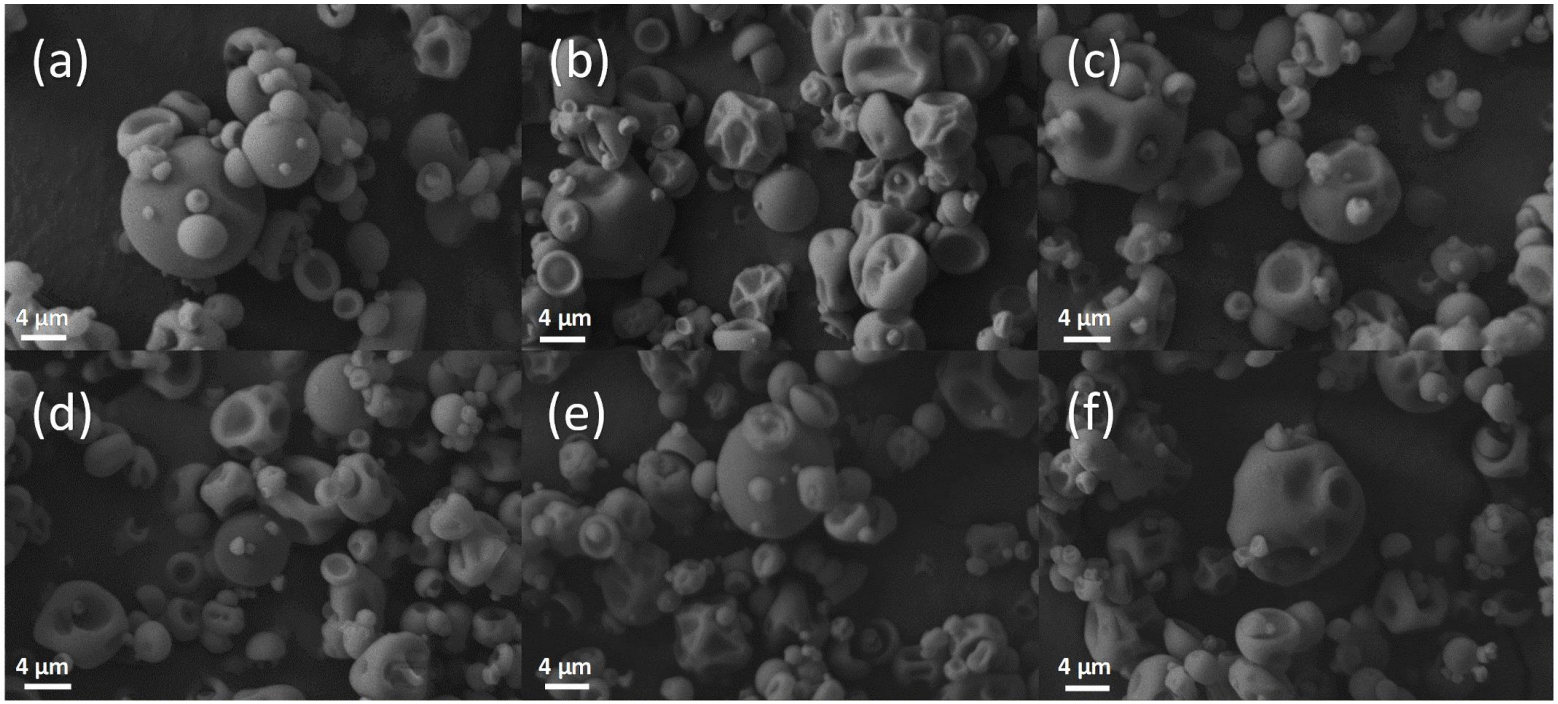
726 maltodextrin. Food Chem 272:337-346.
727 https://doi.org/10.1016/j.foodchem.2018.08.063
728 67. Tabani H, Mahyari M, Sahragard A, Fakhari AR, Shaabani A (2015) Evaluation of
729 sulfated maltodextrin as a novel anionic chiral selector for the enantioseparation of
730 basic chiral drugs by capillary electrophoresis. Electrophoresis 36(2):305-311.
731 https://doi.org/10.1002/elps.201400370
732

Figure Captions

Fig. 1 Viability of microencapsulated *S. cerevisiae* CCMA 0543 (a,b) and *T. delbrueckii* CCMA 0684 (c,d) during drying and storage at 25°C (a,c) and 7°C (b,d)

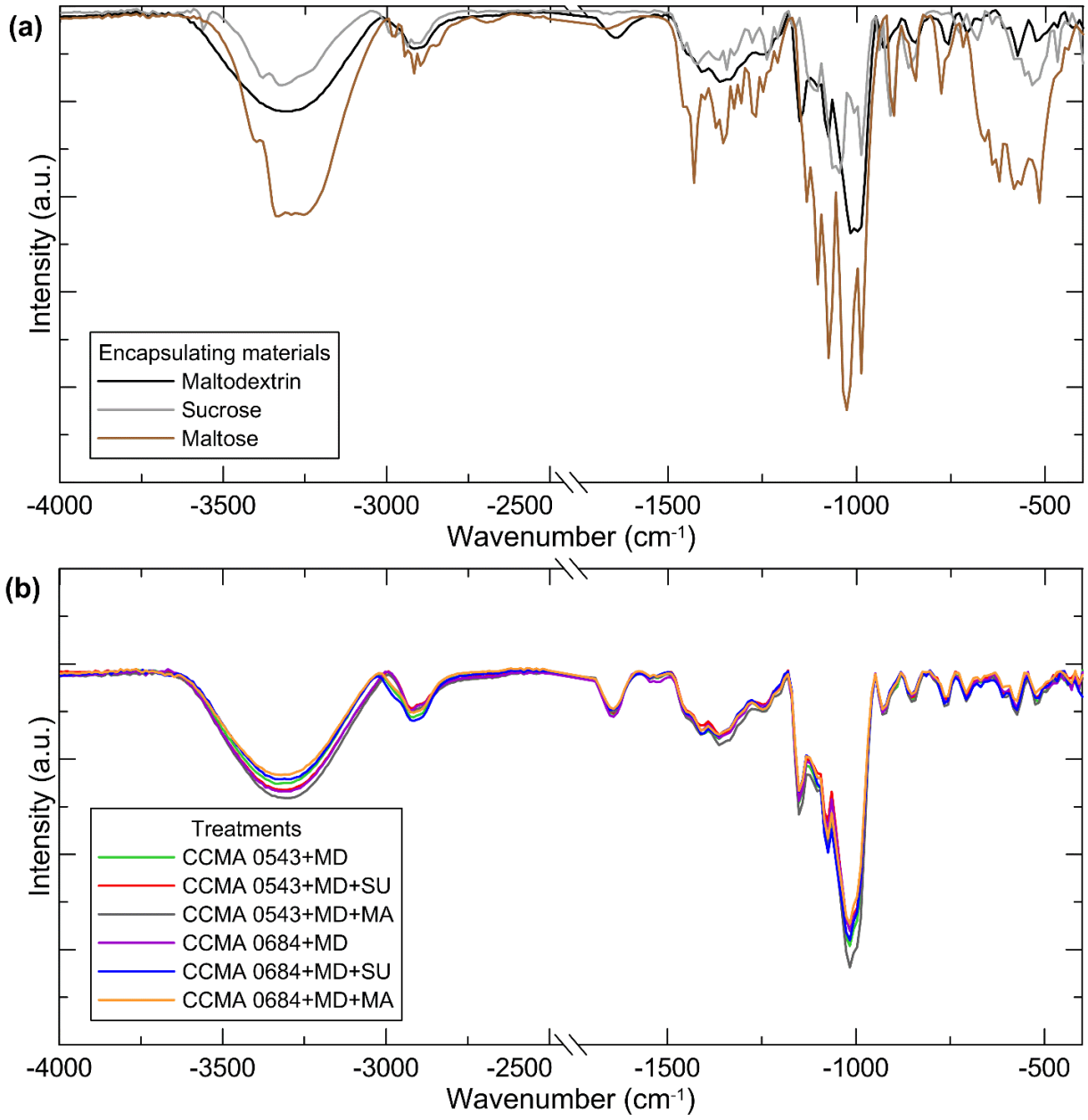


737 **Fig. 2** Scanning electron microscope of: (a) *S. cerevisiae* CCMA 0543+MD, (b) *S. cerevisiae* CCMA 0543+MD+SU, (c)
738 *S. cerevisiae* CCMA 0543+MD+MA, (d) *T. delbrueckii* CCMA 0684+MD, (e) *T. delbrueckii* CCMA 0684+MD+SU and
739 (f) *T. delbrueckii* CCMA 0684+MD+MA. MD: maltodextrin; MA: maltose, and SU: sucrose



740

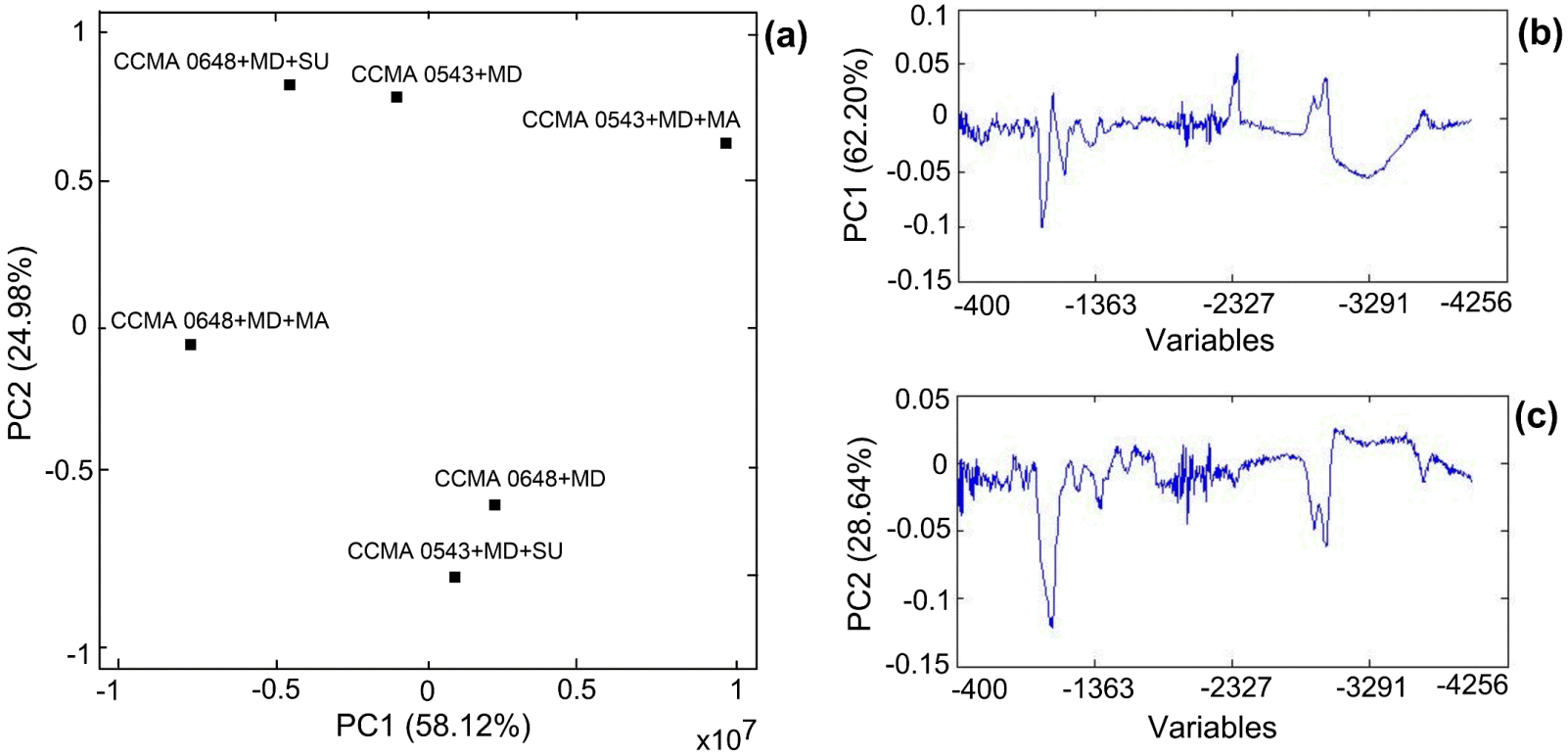
741 **Fig. 3** FTIR spectra of encapsulation materials (a) and treatments (b)



742

743 **Fig. 4** Score plot for the first two PCs of microencapsulation cells (a) and FTIR variables loading of a PC1 (b) and PC2

744 (c)



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Tables

Table 1. Characterization of the powders obtained after drying the yeasts by spray drying with different encapsulating materials.

Characterization after spray drying	Yeasts	Wall materials		
		15% MD[a]	15% MD + 2% SU[b]	15% MD + 2% MA[c]
Powder yield (%)	*S. cerevisiae* CCMA 0543	33.51aA	33.97aA	33.59aA
	T. delbrueckii CCMA 0684	33.86aA	33.68aA	32.11aA
Cell viability after spray drying (%)	*S. cerevisiae* CCMA 0543	94.10aB	97.10aA	94.06bB
	T. delbrueckii CCMA 0684	97.97bA	96.11aB	95.76aB
Water activity	*S. cerevisiae* CCMA 0543	0.25aA	0.25aA	0.27aB
	T. delbrueckii CCMA 0684	0.25aA	0.28bB	0.27aB
Moisture content (%)	*S. cerevisiae* CCMA 0543	0.61aA	0.59aA	0.62aA
	T. delbrueckii CCMA 0684	0.65aA	0.63aA	0.61aA
Hygroscopicity (g 100 g^{-1})	*S. cerevisiae* CCMA 0543	10.87bA	10.74bA	12.06bB
	T. delbrueckii CCMA 0684	4.8aA	7.11aB	6.15aB
Water solubility (%)	*S. cerevisiae* CCMA 0543	74.09aB	74.01bB	78.22bA
	T. delbrueckii CCMA 0684	75.94aB	84.54aA	85.41aA
Particle size D_{50} (µm)	*S. cerevisiae* CCMA 0543	3.78bA	3.94aA	3.96aA
	T. delbrueckii CCMA 0684	3.43aA	3.87aB	3.71aB

Data are presented as mean. a–b for each column, mean values with different lowercase letters are significant at $p < 0.05$ by Scott–Knott test. A–B For each row, mean values with different capital letters are significant at $p < 0.05$ by Scott–Knott test.

[a]Maltodextrin

[b]Sucrose

[c]Maltose

762 **Table 2.** Characterization of wall materials.

Wall materials	Water activity	Moisture content (%)	Hygroscopicity (g 100 g^{-1})
Maltodextrin	0.26a	1.17c	8.82b
Sucrose	0.30b	0.08a	0.32a
Maltose	0.38c	1.04b	0.19a

763 Data are presented as mean. a–c for each column, mean values with different letters are
764 significant at $p < 0.05$ by Scott–Knott test.

ARTIGO 2 - MICROENCAPSULATION OF EPIPHYTIC COFFEE YEASTS BY SPRAY DRYING USING DIFFERENT WALL MATERIALS: IMPLEMENTATION IN COFFEE MEDIUM

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ABSTRACT

The storage of microorganisms in liquid form is presented as the main drawback for the commercialization of epiphytic coffee yeasts. The objective of this work was to evaluate the fermentative performance of microencapsulated yeasts by spray drying in a coffee peel and pulp media (CPM). The yeasts, *Saccharomyces cerevisiae* CCMA 0543, *Torulaspora delbrueckii* CCMA 0684, and *Meyerozyma caribbica* CCMA 1738, were microencapsulated using maltodextrin DE10 (MD), high maltose (MA), and whey powder (WP) as wall materials. A Central Composite Rotational Design (CCRD) was used to investigate the effect of operating parameters on cell viability, drying yield, and water activity of the microcapsules. Yeasts reached cell viability and drying yields above 90 and 50%, respectively. WP maintained the cell viability of the three yeasts over 90 days of storage at room temperature (25 °C) and was selected as a wall material for the three yeasts. *M. caribbica* showed to be more sensitive to the spray drying process and less resistant to storage. Some differences were found in the fermentation of the CPM medium, but the microencapsulated yeasts maintained their biotechnological characteristics. Therefore, the microencapsulation of epiphytic coffee yeasts by spray drying was promising to be used in the coffee fermentation process.

Keywords: Starter culture; Coffee fermentation; CCRD; Whey powder; Active dry yeast

1. Introduction

Saccharomyces cerevisiae, Torulaspora delbrueckii, Pichia kluyveri, Pichia kudriavzevii, Candida parapsilosis, Meyerozyma caribbica, and *Hanseniaspora uvarum* are used as starter cultures in coffee fermentation and have shown promising results (Bressani et al., 2021; da Silva et al., 2021; Elhalis et al., 2021; Shankar et al., 2022). The yeasts inoculated in the coffee fermentation are grown in a specific culture medium until reaching the appropriate cell concentration (Evangelista et al., 2014). However, the microorganism's storage in liquid form is not indicated in the long term, as the cells lose viability. The main problems associated with the commercialization of fresh yeasts are shelf life, risk of contamination, transportation, and refrigerated storage costs (Márquez-Montes et al., 2007; Tan et al., 2018).

Thus, the drying process may be used for the long-term preservation of epiphytic coffee yeasts, contributing to transport, storage, reducing odor, and the possibilities of contamination (Tan et al., 2018). Due to these advantages, active dry yeasts can be applied in several areas of biotechnology, and some yeasts are commercially available, such as *Saccharomyces cerevisiae, Torulaspora delbrueckii, Metschnikowia pulcherrima, Lachancea thermotolerans, Metschnikowia fructicola,* and *Pichia kluyveri* (Hansen, 2022; Laffort, 2022; Lallemand, 2022).

There are different methods for microorganism drying, such as freeze-drying, spray drying, and fluidized bed, but some may be more suitable depending on the purpose (Frakolaki et al., 2021; Tan et al., 2018). Spray drying is a unit operation by which a liquid product is atomized in a hot gas current to obtain a powder instantaneously. This technique can be used continuously and quickly, allowing high production rates with low operating costs. Furthermore, it allows modifying drying parameters such as inlet and outlet temperature, feed rate, atomization air pressure, and residence time within the dryer chamber (Gharsallaoui et al., 2007; Martín et al., 2015).

However, the disadvantage is the high temperature used, which may affect the cell viability of the yeast. For example, DNA, RNA, proteins, and enzymes are some heat-sensitive cellular components. Based on that, an alternative to reduce cell impact is microencapsulation, in which drying conditions and wall material can be optimized to achieve maximum cell survival (Fu and Chen, 2011; Liu et al., 2019; Martín et al., 2015). Another advantage is that microencapsulated cells may be protected from factors such as moisture and heat, increasing stability and maintaining viability throughout storage. Regarding this, the choice of wall material has a significant influence on the properties of the encapsulated material and the efficiency of the process (Gharsallaoui et al., 2007).

Thus, our group isolated and identified three potential yeasts starter to coffee fermentation: *Saccharomyces cerevisiae* CCMA 0543, *Torulaspora delbrueckii* CCMA 0684, and *Meyerozyma caribbica* CCMA 1738 (Bressani et al., 2021; Da Mota et al., 2020; da Silva et al., 2021). However, microorganisms' storage in liquid form has presented as the main drawback for the commercialization of these starter cultures. That said, spray drying is widely used in the food industry to dry liquid foods and has shown the potential to dry microorganisms. The drying efficiency and the low cost of the process make the product accessible for commercialization. However, the process condition and wall material must be optimized for each strain. Based on that, the objective of this work was to evaluate the effect of operating parameters in the drying process using different wall materials to obtain microencapsulated yeasts.

2. Material and methods

2.1 Microorganisms

Yeasts previously isolated from coffee fruits were used in this study. *Saccharomyces cerevisiae* CCMA 0543 and *Torulaspora delbrueckii* CCMA 0684 were isolated from *Coffea*

arabica (Silva et al., 2000; Vilela et al., 2010), while *Meyerozyma caribbica* CCMA 1738 was isolated from *Coffea canephora* (Pereira et al., 2021). These strains belong to the Culture Collection of Agricultural Microbiology (CCMA, Federal University of Lavras, Lavras, Minas Gerais, Brazil). The isolates, stored at -80 °C, were reactivated and grown in molasses liquid medium [in g/L: molasses 137.5 (ECL Eireli – supplier *Mellaço de Cana*), calcium chloride 1.1 (Dinâmica), urea 2 (Ambion), Polysorbate 80 0.5 (Dinâmica) and pH 3.5] until reached a 10^8 CFU/mL. The yeasts were incubated at 28 °C for 48 h in an orbital shaker set at 120 rpm at each growth stage. At the end of growth, the yeasts reach the stationary phase. The cells were recovered by centrifugation (7000 ×*g*; 10 min) and resuspended in sterile water to a concentration of 10^9 CFU/mL.

2.2 Microencapsulation by spray drying

2.2.1 Preparation of the solution

Three different wall materials were studied and analyzed separately for each yeast: maltodextrin DE10 (MD) (Ingredion, Brazil), high maltose (MA) (Ingredion, Brazil), and whey powder (WP) (*Jaciara Indústria e Comércio*, Brazil). First, the wall materials were diluted in water, and the concentrations used were detailed in the topic (2.2.2). The MD and MA solutions were autoclaved at 121 °C for 15 min. The solutions with WP were pasteurized at 75 °C for 30 min, followed by rapid cooling (Andrade et al., 2019). Then, the yeasts were added separately at a 10^8 CFU/mL concentration. Finally, the feed solutions (300 mL) were mixed for 5 min with a magnetic stirrer for homogenization.

2.2.2 Experimental design

Saccharomyces cerevisiae CCMA 0543, *Torulaspora delbrueckii* CCMA 0684, and *Meyerozyma caribbica* CCMA 1738 were microencapsulated using a more extensive scale spray dryer (model SD 5.0; Labmaq do Brasil, Ribeirão Preto, Brazil). In all experiments, a

feed rate of 1.5 L/h, drying airflow of $1.5x10^5$ L/h, and atomization air flow rate of 60 L/h were used. These parameters were found in preliminary tests (data not shown). The most critical variables investigated in the microencapsulation process were the wall material concentration and drying air inlet temperature using the Central Composite Rotational Design (CCRD), with three replicas at the central point, totaling 11 treatments for each yeast and wall material. The response variables were cell viability, drying yield, and water activity of the microcapsules. The ranges of variation between the lower and upper limits for the independent variables were determined according to preliminary tests and other studies described in the literature (Braga et al., 2019; Huang et al., 2017; Vanden Braber et al., 2020). Supplementary material: Table S1 shows the complete CCRD in which the levels used for wall material concentration ranged from 17.93 to 32.07% and the air inlet temperature from 61.70 to 118.28 °C.

2.3 Cell viability, drying yield, and water activity

After drying, the viability of microencapsulated cells was performed. Sample powder (1 g) was hydrated in 9 mL of peptone water [in g/L: peptone 1 (Himedia)] for 30 min and vortexed to obtain a homogeneous suspension (Chandralekha et al., 2016). The quantification of microorganisms was performed with serial dilution, followed by micro-drop plating on YEPG culture medium pH 3.5 [in g/L: yeast extract 10 (Himedia), glucose 20 (Dinamica), peptone 20 (Himedia), and agar 20 (Himedia)], incubated at 28 °C for 48 h. The experiment was carried out in triplicate. Then, the cell viability of microencapsulated yeasts (%) was calculated from the number of viable cells (CFU/mL of wet material) in the powder by the number of viable cells (CFU/mL) in the initial suspensions (Vanden Braber et al., 2020). The drying process yield (%, w/w) was calculated according to Eq. (1):

$$Drying\ yield\ (\%) = [Wp\ (1 - Xw, powder)/Ts]\ x\ 100 \quad (1)$$

where *Wp* is the weight of the powder collected after drying, *Xw,powder* is th fraction of water present in the powder and *Ts* is the total dry solids of the feed (Chandralekha et al., 2016). The water activities (a_w) were measured using a water activity meter (Aqualab 4TE, Decagon Devices, Pullman, Washington, USA).

2.4 Process optimization and experimental validation

The region with the highest cell viability and drying yield for each yeast was obtained by the response surface methodology described by Box et al. (1978) based on CCRD data. Then, the model equations were obtained, which are presented as contour curves in Figure 1. The criteria to be achieved by optimizing the processes were: high microorganism viability, the low water activity of microencapsulated powders, and high drying yield. Thus, system responses were optimized simultaneously within the values studied, and cell viability responses were analyzed, considering the maximum water activity of 0.22 (Esftathiou et al., 2002).

For model validation, the drying of each yeast with the best parameters found for wall material concentration and drying air inlet temperature was performed.

2.5 Characterization of microcapsules

The moisture content of the feed solutions, wall material, and powder was determined by the gravimetric method (AOAC, 2005). Approximately 3 g of the sample was weighed in porcelain capsules of known mass. The capsules were carried to the oven at 105 °C until constant weight. Moisture content was expressed as a percentage of the initial weight. The hygroscopicity of the wall materials and microcapsules was performed according to Braga et al. (2019). Samples (0.3 g) were placed in Petri dishes and stored in a desiccator with no silica at 25 °C, containing a saturated sodium chloride solution. After one week, the hygroscopicity

was weighed and expressed as g of water absorbed by the sample per 100 g dry solids. The water solubility of the wall materials and microcapsules were characterized according to Cano-Chauca et al. (2005). Samples (0.5 g) were placed in flasks containing 50 mL of distilled water and homogenized in an orbital shaker at 100 rpm for 30 minutes at room temperature. Then, the solution was centrifuged at 3500 rpm for 5 minutes. The supernatant was transferred to a porcelain capsule of a known weight. Then, it was placed in an oven at 105 °C until constant weight. The morphology of microcapsules was evaluated using a scanning electron microscope (SEM) (model Jeol JSM 6360 LV) with an acceleration voltage of 20 kV. A secondary electron detector was used. The analysis was carried out at a high vacuum and without tilt application. The microcapsules were mounted on aluminum bases adhered by double-sided carbon adhesive tape. Then they were coated with gold (3nm) with a vacuum spray applicator (Andrade et al., 2019). The size of the microcapsules was evaluated with the ImageJ 2014 software (Rasban, National Institute of Health, USA) (Vanden Braber et al., 2020). The diameter of 300 particles of each treatment was measured.

2.6 Viability of microencapsulated cells during storage

The viability of the starter cultures, *S. cerevisiae* CCMA 0543, *T. delbrueckii* CCMA 0684, and *M. caribbica* CCMA 1738, was monitored every 15 days during three months of storage at 7 and 25 °C. The powders were stored in 250 g packages made of polyester, metallization, and polyethylene. Then they were vacuum-packed. The resulting powder was divided so that each package was opened only once. The cell viability was performed according to topic 2.3.

2.7 Application of microencapsulated yeasts in the fermentative process

The yeast fermentation was carried out in a culture medium containing coffee peel and pulp (CPM), according to Ribeiro et al. (2020), with modifications. For *S. cerevisiae* CCMA 0543 and *T. delbrueckii*, CCMA 0684 was used, *Coffea arabica*, and for *M. caribbica*, CCMA 1738 was used *Coffea canephora*. For the preparation of CPM medium, 200 g of coffee peel and pulp were added to 1 L of distilled water. Then, they were homogenized in a blender for 5 minutes, filtered, and 15 g of glucose and 15 g of fructose were added. The initial pH was adjusted to 5.5 with HCl (0.1 M). The medium was sterilized at 121 °C for 15 min. The treatments performed were *S. cerevisiae* CCMA 0543, *T. delbrueckii* CCMA 0684 and *M. caribbica* CCMA 1738 fresh, and *S. cerevisiae* CCMA 0543, *T. delbrueckii* CCMA 0684, and *M. caribbica* CCMA 1738 microencapsulated with WP. All yeasts were inoculated separately. Fresh yeasts were grown to 10^8 cells/mL in YEPG. Subsequently, the cells were centrifuged and resuspended in a CPM medium. Microencapsulated yeasts (10^8 cells/mL) were reactivated in a CPM medium at 40 °C for 30 min. The inoculum (10^7 cells/mL) was transferred separately to flasks containing CPM medium (200 mL) and kept at 28 °C for 48 h. For chemical and microbiological analysis, samples were collected at 0, 24, and 48 h of fermentation. The fermentations were carried out in triplicate.

2.8 Analysis of sugars, alcohol, and organic acids using high-performance liquid chromatography (HPLC)

Sugars, alcohol, and organic acids were analyzed. The samples were evaluated at the initial (0 h) and the end of the fermentation process (48 h). Samples of fermented coffee medium were centrifuged (12,745 ×*g* at 4 °C for 10 min). For acid analysis, the perchloric acid solution was used to adjust the supernatant pH to 2.11 and then re-centrifuged under the same conditions. The supernatant was filtered through a 0.22 µm cellulose acetate membrane. The filtered extract was stored at -18 °C until analysis (Ribeiro et al., 2020). The extracts

were analyzed using an HPLC system (Shimadzu) according to Da Mota et al. (2020). A Shim-pack SCR-101H (7.9 mm x 30 cm) column was used for acids with a 100 mM perchloric acid solution as the mobile phase and a flow rate of 0.6 mL/min. A Shim-pack SCR-101C (7.9 mm x 30 cm) column was used for sugars and alcohols with ultra-pure water as the mobile phase and a flow rate of 0.5 mL/min. The oven temperature was maintained at 50°C for analysis of the acids, detected with a UV detector at 210 nm, and at 80°C for analysis of the sugars and alcohols, detected with a refractive index detector. The compounds quantification was performed using calibration curves constructed with different concentrations of standard compounds [malic and citric acids were purchased from Merck (Darmstadt, Germany), sucrose, glucose, fructose, alcohol, and lactic, acetic, succinic, and tartaric acids were purchased from Sigma-Aldrich (Saint Louis, MO, United States), butyric acid were purchased from Riedel-deHaen (Seelze, Germany)]. All analyses were performed in triplicate (Evangelista et al., 2014).

2.9 Specific growth rate and generation time

Fresh and microencapsulated yeasts' specific growth rate and generation time were evaluated in a CPM medium. First, fresh yeasts were grown in YEPG, centrifuged, and resuspended in a CPM medium. Microencapsulated yeasts were reactivated in a CPM medium at 40 °C for 30 min. Then, the yeasts (10^4 CFU/mL) were added separately in a CPM medium (200 mL) and incubated at 28 °C. Samples were taken every 1.5 hours during nine hours for cell counting by micro-drop plating, as already mentioned. The experiment was carried out in triplicate. The specific growth rate was obtained according to the regression equation between population count (CFU/mL) and time (h). The generation time was calculated according to Eq. (2):

$$G = \ln 2/\mu \quad (2)$$

where G is the generation time of each yeast and μ is the specific growth rate (Zhu et al., 2012).

2.10 Statistical analysis

The viability of the optimized powders during storage, the wall materials and microcapsules characterization, growth rate, generation time, and the concentrations of carbohydrates and organic acids were evaluated by variance analysis (ANOVA). Scott-Knott test was used to compare means with a 5% significance level.

3. Results and discussion

3.1 Optimization of drying process conditions

The simultaneous effect of drying air inlet temperature and wall material concentration on the cell viability of *S. cerevisiae* CCMA 0543, *T. delbrueckii* CCMA 0684, and *M. caribbica* CCMA 1738, water activity, and drying yield were evaluated using the CCRD (Supplementary material: Table S1). The cell viability of the encapsulated yeasts ranged from 28.18% to 96.15%, while the a_w ranged from 0.05 to 0.37 and the drying yield ranged from 30.84 to 63.52%.

The highest cell viability achieved by microencapsulated *S. cerevisiae* CCMA 0543 with MD, MA, and WP wall materials was 87.81% (treatment 3), 93.71% (treatment 1), and 95.68% (treatment 7), respectively. Conversely, the lowest a_w with MD, MA, and WP was 0.11 (treatment 2), 0.13 (treatment 4), and 0.14 (treatments 4 and 8), and the highest drying yield was 60.46% (treatment 3), 63.52% (treatment 3), and 57.67 (treatment 6), respectively (Supplementary material: Table S1).

Regarding microencapsulated *T. delbrueckii* CCMA 0684, the highest cell viability achieved with MD, MA, and WP was 85.76% (treatment 7), 93.63% (treatment 7), and

96.15% (treatment 3), respectively. On the contrary, the lowest a_w with MD, MA, and WP was 0.10 (treatments 10 and 11), 0.05 (treatment 8), and 0.18 (treatment 4), and the highest drying yield was 61.89 (treatment 6), 57.17 (treatment 6), 58.49 (treatment 3), respectively (Supplementary material: Table S1).

On the other hand, the highest cell viability achieved by microencapsulated *M. caribbica* CCMA 1738 with MD, MA, and WP was 59.05% (treatment 7), 94.60% (treatment 7), and 86.58% (treatment 7), respectively. Conversely, the lowest a_w with MD, MA, and WP was 0.07 (treatment 8), 0.11 (treatment 4), and 0.14 (treatment 8), and the highest drying yield was 60.72 (treatment 6), 59.79 (treatment 3), 61.27 (treatment 3), respectively (Supplementary material: Table S1).

The contour curves were constructed with the results of cell viability, a_w, and drying yield obtained in the CCRD experiments and are essential to selecting the operating regions with the best results (Fig. 1). Cell viability and a_w of microencapsulated yeasts decreased with increasing inlet temperature of drying air. As the temperature increases, cell death will increase due to thermal inactivation and rapid cell dehydration (Aguirre-Güitrón et al., 2018). Temperature-sensitive components include DNA, RNA, proteins, membranes, and ribosomes. In addition, dehydration stress causes osmotic and oxidative stresses, mainly affecting the cytoplasmic membrane (Garre et al., 2010). On the other hand, the drying yield increased with the increasing concentration of wall materials, probably due to the increase in solids concentration in the solution (Fig. 1).

The region marked in red in Figure 1 is higher cell viability and drying yield with water activity at a maximum of 0.22. This region was obtained for each yeast and wall material in the conditions studied. A_w influences microbiological and physicochemical parameters such as cell viability, enzymatic activity, and non-enzymatic browning. Consequently, a_w is an important parameter as it significantly influences the stability of the powder and cells during

storage (Schuck et al., 2012). It is generally accepted that optimal stability is achieved with a_w between 0.2 and 0.3. Based on this, a_w was set to 0.22 (Esftathiou et al., 2002).

The region for *S. cerevisiae* +MD was obtained with 97.80% of the maximum point of cell viability and drying yield (Fig. 1A1). The region includes drying air inlet temperature and wall material concentration from 71.22 to 77.50 °C and 31.62 to 32.07%, respectively. Results validation was performed at 71 °C and 32.05%. The *S. cerevisiae* + MA region was obtained with 93.40% and ranged from 84.58 to 85.20 °C and 29.34 from 29.68% (Fig. 1A2). The validation was performed at 85 °C and 29.51%. The *S. cerevisiae* + WP region was obtained with 96.40% and ranged from 86.67 to 87.01 °C and 27.78 to 28.06% (Fig. 1A3). The validation was performed at 87 °C and 27.92%.

Concerning *T. delbrueckii* + MD, the region was obtained with 85.20% of the maximum point of cell viability and drying yield (Fig. 1B1). The region includes drying air inlet temperature and wall material concentration from 82.65 to 84.69 °C and 30.26 to 30.31%, respectively. Results validation was performed at 83 °C and 30.30%. The region for *T. delbrueckii* + MA was obtained at 97.00% and ranged from 74.84 to 75.69 °C and 27.93 to 28.65% (Fig. 1B2). The validation was performed at 75 °C and 28.30%. The *T. delbrueckii* + WP region was obtained with 93.80% and ranged from 88.54 to 89.45 °C and 31.83 to 32.06% (Fig. 1B3). The validation was performed at 88 °C and 32.06%.

Finally, the region for *M. caribbica* + MD was obtained with 94.60% of the maximum point of cell viability and drying yield (Fig. 1C1). The region includes drying air inlet temperature and wall material concentration from 61.7 to 61.81 °C and 29.01 to 29.38%, respectively. The validation was performed at 62 °C and 29.2%. The region for *M. caribbica* + MA was obtained with 88.20% and ranged from 84.64 to 85.09 °C and 21.54 to 23.41% (Fig. 1C2). Results validation was performed at 85 °C and 22.47%. The region for *M.*

caribbica + WP was obtained with 92.80% and ranged from 82.88 to 83.45 °C and 30.40 to 30.54% (Fig. 1C3). The validation was performed at 83 °C and 30.47%.

The experimental results were similar to the model, indicating that the models represent microencapsulation of the three epiphytic coffee yeasts by spray drying using MD, MA, or WP as wall materials (Table 1). After drying the optimal points, cell viability above 90% was achieved, showing promising results. Total solids contents between 20 and 30% were reported in the literature as ideal to ensure high cell viability (Huang et al., 2017), corroborating the results obtained in this study. The drying air outlet temperature ranged between 45 and 68 °C, and this parameter also has an impact on cell viability. The drying yields obtained were above 50% (Table 1). This parameter is important because it shows the productivity of the drying process, being an essential factor for the industry due to the cost-effectiveness (Huang et al., 2017).

The cell viability of *S. cerevisiae* CCMA 0543, *T. delbrueckii* CCMA 0684 and *M. caribbica* CCMA 1738 achieved 94% (with WP), 96.58% (with MA), and 76.56% (with WP), respectively. *M. caribbica* showed to be more sensitive to the spray drying process. These differences occur because microorganisms' tolerance and stress response vary between species and strains (Fu and Chen, 2011). Torrellas et al. (2020) evaluated the cell viability of eight non-*Saccharomyces* wine species after the drying process. *M. pulcherrima, M. fructicola,* and *S. bacillaris* showed cell viability above 80% and high catalase activity and glutathione levels. On the other hand, *K. wickerhamii (23.18%)* and *H. vineae (1.83%)* showed low viability and low concentration of trehalose, which may explain the worse performance. Enzymatic activities (catalase, superoxide dismutase, and glutathione reductase) and protective molecules (trehalose and glutathione) are the primary defense mechanisms that help to maintain redox balance or eliminate reactive oxygen species (Herrero et al., 2008).

During yeast dehydration and rehydration, essential structural and functional changes occur in virtually all organelles and cellular components. Therefore, mechanical, structural, and oxidative restrictions can cause cell death (Rapoport, 2019). A strategy used to improve cell protection is the addition of specific components that have protective properties (Huang et al., 2017), such as maltose, maltodextrin, and whey powder used in this work.

High maltose, maltodextrin, and whey powder proved to be efficient for drying the epiphytic coffee yeasts, probably due to the stabilization of parts of the cell membrane, resulting in greater viability (Aguirre-Güitrón et al., 2018). However, MD proved to be the wall material with the lowest cell protection since the cell viability of the three yeasts was lower than with MA and WP (Table 1). This comparison is necessary because cell survival is the most crucial parameter since the drying technique will only be helpful if the microorganisms maintain their function and reproducibility (Tan et al., 2018).

3.2 Characterization of microcapsules

The moisture, solubility, hygroscopicity, and a_w of the wall materials were evaluated (Table 2). There was a statistical difference among MA, MD, and WP in all parameters evaluated, except for the moisture content, which varied between 5.02 and 6.74%. WP powder was the wall material with the lowest a_w (0.33) and hygroscopicity (5.73 g/100g). On the other hand, MD showed higher a_w (0.49) and lower solubility (91.51%). Finally, MA showed the highest solubility (96.53%) and hygroscopicity (20.76 g/100g) (Table 2), which is classified as a very hygroscopic powder (Schuck et al., 2012). That said, the characterization of the wall material is essential to understanding the drying process's effectiveness and influence during the storage of the microcapsules (Gharsallaoui et al., 2007).

The microencapsulated yeasts obtained from the optimal condition determined in this study of the drying process were characterized in terms of moisture content, hygroscopicity,

water solubility, and particle size (Table 3). The moisture of the microcapsules varied between 4.04 and 8.53%. *M. caribbica* CCMA 1738 + MD showed a statistical difference and obtained the highest moisture content (8.53%), probably due to the low inlet temperature of the drying air used (62 °C). The water content of a powder is strongly influenced by the thermodynamic characteristics of the drying air and droplet size. As with a_w, moisture content significantly influences powder storage capacity (Schuck et al., 2012).

Yeasts did not significantly influence the hygroscopicity of the powders, in contrast to the wall material. The yeasts microencapsulated with MA obtained higher hygroscopicity, varying between 24.26 and 26.92 g/100g (Table 3). A powder is considered extremely hygroscopic if the percentage is greater than 25% (Schuck et al., 2012), as occurred with the yeasts microencapsulated with MA. This result was expected because MA proved to be a very hygroscopic powder (20.76 g/100g) (Table 2). On the other hand, there was no significant difference between microencapsulated yeasts with MD and WP, ranging from 8.73 to 12.98 g/100g (Table 3). These values are characterized as slightly hygroscopic powders. In this way, fewer hygroscopic powders are desired as they facilitate handling and help stabilize the microcapsules (Schuck et al., 2012).

There was no statistical difference between yeasts and wall materials (Table 3). The water solubility ranged from 79.89 to 92.07%. This parameter is essential in powders that will be incorporated into the aqueous phase (Schuck et al., 2012). The yeasts will be reactivated in water for later use in the coffee fermentation. Thus, a high solubility is desired to contribute to cell reactivation and homogeneous coffee distribution.

The size of the microcapsules ranged from 3.57 to 4.97 μm (Table 3). However, there was a statistical difference between yeasts and wall materials. This variation was expected because the concentration of MA, MD, and WP was different for each yeast. Therefore, the size of

microcapsules is crucial because it affects physical and functional properties, such as solubility and hygroscopicity, as these depend on the contact surface (Schuck et al., 2012).

The micrographs of the microencapsulated yeasts with MD, MA, and WP can be seen in Supplementary material: Fig. S1. The wall material influenced the shape of the microcapsules. The three yeasts microencapsulated with MD showed undefined and irregular matrix shapes. Microcapsules are deformed, with concavities, invaginations, and sizes. These imperfections observed on the surfaces may be related to a slow film formation process during drying (Ré, 1998). On the other hand, yeasts microencapsulated with MA and WP showed spherical and smooth microcapsules of different sizes. Regardless of the wall material used, the microcapsules did not show ruptures or cracks, confirming good structural integrity.

3.3 Viability of microencapsulation yeasts during storage

The cell viability data were represented as relative viability taken to the number of cells evaluated before drying as 100% (Fig. 2). *S. cerevisiae* CCMA 0543, *T. delbrueckii* CCMA 0684, and *M. caribbica* CCMA 1738 were evaluated during 90 days of storage, and storage temperatures were evaluated at 25 °C and 7 °C. There was a statistical difference in cell viability of all microencapsulated yeasts during storage concerning the wall material used.

During the entire storage at 25 °C, *S. cerevisiae* CCMA 0543, *T. delbrueckii* CCMA 0684, and *M. caribbica* CCMA 1738 had higher cell viability with WP. After 90 days of storage, *S. cerevisiae* + WP had a 13.02% decrease in cell viability. In contrast, *S. cerevisiae* + MA and *S. cerevisiae* + MD decreased by 30.51 and 45.57%, respectively (Fig. 2.A1). *T. delbrueckii* + WP decreased 7.38%. In comparison, *T. delbrueckii* + MA and *T. delbrueckii* + MD decreased 43.18 and 46.29%, respectively (Fig. 2.B1). Finally, *M. caribbica* + WP had a decrease of 57.82%. At the same time, *M. caribbica* + MA and *M. caribbica* + MD had viability lower than 20% (Fig. 2.C1).

Thus, after 90 days of storage at 25 °C, there was a reduction in the cell viability of *S. cerevisiae* + WP, *T. delbrueckii* + WP and *M. caribbica* + WP of 13.02, 7.38 and 57.82% (Fig. 2). Based on these results, it could be observed that *M. caribbica* CCMA 1738 was also less resistant to storage than *S. cerevisiae* CCMA 0543 and *T. delbrueckii* CCMA 0684.

On the other hand, after 90 days of storage at 7 °C, there was a statistical difference in the cell viability of yeasts stored with different wall materials. *S. cerevisiae* microencapsulated with WP (92.39%) obtained higher cell viability, followed by MA (88.69%) and MD (79.52) (Fig. 2.A2). Regarding *T. delbrueckii*, there was no statistical difference between WP (92.46%) and MA (94.53%), followed by MD (76.70%) (Fig. 2.B2). *M. caribbica* also obtained greater viability with WP (66.64%) and MA (66.24%), followed by MD (49.97%) (Fig. 2.C2). Thus, it could be observed that the shelf life of microencapsulated yeasts depends on the strain used since the decrease in cell viability is related to microbial metabolism and the wall material used in the drying process (Chandralekha et al., 2016; Rapoport et al., 2019).

During storage, microorganisms' cell viability decreases due to the natural degradation of macromolecules essential to life, such as lipids and proteins. Although room temperature storage presents a significant challenge, the industry does not indicate refrigeration temperature storage. As a result, storage and refrigerated transport costs are high (D'Alessandro et al., 2021; Fu and Chen, 2011; Huang et al., 2017). Thus, storing microencapsulated epiphytic coffee yeasts at room temperature is economically viable. Although maltodextrin has been used as a carrier material in many studies, the polysaccharide shows a decreased tendency to bind to the cell membrane and can reduce cell viability (Chandralekha et al., 2016; Semyonov et al., 2010; Sohail et al., 2013), with was observed in our results presented here (Fig. 2). Based on this, WP maintained the cell viability of the three yeasts over 90 days of storage at room temperature. Therefore, WP was selected as a wall material for the three yeasts.

The whey powder used in this work is predominantly composed of carbohydrates (5%) and proteins (3%). Proteins exhibit good film-forming ability. Thus, a protective coating on the cell wall can be formed during spray drying. This coating prevents cell injury by stabilizing membrane constituents (Liu et al., 2019). Furthermore, lactose appears to support this protective effect synergistically. López-Rubio et al. (2012) observed that bacteria encapsulated only with carbohydrates were less protected than those encapsulated with whey protein.

3.4 Generation time and microencapsulated yeast behavior in the coffee medium

The specific growth rate and generation time of the fresh and microencapsulated yeasts with WP was evaluated in the CPM medium (Table 4), and it was found a statistical difference between the behavior of the yeasts in the fresh microencapsulated state. *S. cerevisiae* CCMA 0543 and *T. delbrueckii* CCMA 0684 had higher specific growth rates and shorter generation times in the fresh state. However, *M. caribbica* CCMA 1738 showed the opposite behavior (Table 4). The specific growth rate of microencapsulated yeasts may be lower than that of fresh yeasts depending on the type and level of damage caused during drying (Márquez-Montes et al., 2007; Rapoport, 2017). In a rich medium, intact and injured cells can reproduce, but injured cells will probably have a longer latency due to the repair process (Fu and Chen, 2011). On the other hand, the increase in biomass yield with dry yeasts compared with fresh yeasts has also been reported in the literature (Rapoport, 2017).

Then, the behavior of the yeasts in the two states (fresh and microencapsulated) was compared during fermentation in the CPM medium. The fermentation of the three yeasts lasted 48 hours. In general, pH started at 5.5, decreasing to 4.0, and Brix started at 5.0, decreasing to 1.0 °Brix (Fig. 3).

After 48h of fermentation, there was no statistical difference in the population count between fresh and microencapsulated yeasts (Fig. 3). Thus, despite having shown different behavior concerning generation time, the microencapsulation of yeasts with WP did not change the population growth during fermentation in the CPM medium. This result is significant because the spray drying process will be only helpful if the epiphytic coffee yeasts maintain their function and reproducibility in coffee (Tan et al., 2018).

Carbohydrate consumption and production of alcohols and organic acids during growth in the CPM medium were also evaluated (Fig. 4). However, there were some statistical differences in compound production between fresh and microencapsulated yeasts. Sucrose, glucose, and fructose were analyzed as total reducing sugar (TRS). Ethanol and citric, malic, and succinic acids were detected in all fermentations.

S. cerevisiae showed no statistical difference in ethanol and succinic acid production. Microencapsulated *S. cerevisiae* had higher consumption of TRS (0.07 g/L) and higher production of malic (0.25 g/L). However, *T. delbrueckii* did not obtain statistical differences only for ethanol production. Microencapsulated *T. delbrueckii* showed higher consumption of TRS (0.023 g/L) and higher production of malic (0.87 g/L). Finally, *M. caribbica* showed no significant difference in citric and malic acids. Microencapsulated *M. caribbica* showed higher production of ethanol (0.61 g/L) and succinic (0.67 g/L) acids.

In microencapsulated yeast preparations for industrial purposes, the cells must maintain a high cell viability rate during storage. In addition, it is essential that the yeasts not lose their main biotechnological characteristics, as these will influence the characteristics of the product obtained (Rapoport, 2019).

Kim et al. (2019) observed that dehydration of non-*Saccharomyces* yeasts did not change the fermentation characteristics of persimmon wine and apple cider. On the other hand, Torrellas et al. (2020) observed a loss of fermentative capacity of eight non-*Saccharomyces*

wine yeasts after drying. In addition, laboratory-scale wines produced with active dry yeast showed an improvement in fermentation behavior (Roca Domènech et al., 2018), corroborating the data found in this study.

Therefore, some differences were found in the fermentation of the CPM medium with *S. cerevisiae* CCMA 0543, *T. delbrueckii* CCMA 0684, and *M. caribbica* CCMA 1738. Nevertheless, the microencapsulated yeasts maintained their biotechnological characteristics. Based on this, in the next step, it is necessary to evaluate them during coffee fermentation in bioreactors to assess whether the differences obtained will impact the formation of volatile compounds and the sensory quality of the beverage.

4 Conclusion

The region with the highest cell viability and drying yield with water activity lower than 0.22 were obtained for each yeast with wall-specific material studied. The experimental results were similar to the model, indicating that the models represent microencapsulation of *S. cerevisiae* CCMA 0543, *T. delbrueckii* CCMA 0684, and *M. caribbica* CCMA 1738 by spray drying. Cell viability above 90% was achieved, showing promising results. However, *M. caribbica* showed to be more sensitive to the spray drying process and less resistant to storage. WP maintained the cell viability of the three yeasts over 90 days of storage at room temperature and was selected as a wall material for the three yeasts. Finally, some differences were found in the fermentation of the CPM medium. However, the microencapsulated yeasts maintained their biotechnological characteristics. Therefore, the microencapsulation of epiphytic coffee yeasts by spray drying was promising to be used in coffee fermentation. Other studies need to be carried out to evaluate the differences in the formation of volatile compounds and the sensory quality of the beverage.

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References

Aguirre-Güitrón, L., Calderón-Santoyo, M., Ortiz-Basurto, R.I., Bautista-Rosales, P.U., Ragazzo-Sánchez, J.A., 2018. Optimisation of the spray drying process of formulating the post-harvest biocontrol agent *Meyerozyma caribbica*. Biocontrol Sci. Technol. 28, 574–590. https://doi.org/10.1080/09583157.2018.1468997

Andrade, D.P., Ramos, C.L., Botrel, D.A., Borges, S.V., Schwan, R.F., Ribeiro Dias, D., 2019. Stability of microencapsulated lactic acid bacteria under acidic and bile juice conditions. Int. J. Food Sci. Technol. 54, 2355–2362. https://doi.org/10.1111/ijfs.14114

AOAC, 2005. Official Methods of Analysis, 18th ed, Analytical Chemistry. American Chemical Society (ACS). https://doi.org/10.1021/ac50052a726

Box, G.E.P., Hunter, W.G., Hunter, S.J., 1978. Statistics for experimenters. New York: John Wiley Co.

Braga, A.B.A.C., Costa, C.J.M., Pomella, A.W.V., Ribeiro, E.J., Santos, L.D., Zotarelli, M.F., 2019. Evaluation of lethality temperature and use of different wall materials in the microencapsulation process of *Trichoderma asperellum* conidias by spray drying.

Powder Technol. 347, 199–206. https://doi.org/10.1016/j.powtec.2019.02.037

Bressani, A.P.P., Martinez, S.J., Sarmento, A.B.I., Borém, F.M., Schwan, R.F., 2021. Influence of yeast inoculation on the quality of fermented coffee (*Coffea arabica* var. Mundo Novo) processed by natural and pulped natural processes. Int. J. Food Microbiol. 343, 109107. https://doi.org/10.1016/j.ijfoodmicro.2021.109107

Cano-Chauca, M., Stringheta, P.C., Ramos, A.M., Cal-Vidal, J., 2005. Effect of the carriers on the microstructure of mango powder obtained by spray drying and its functional characterization. Innov. Food Sci. Emerg. Technol. 6, 420–428. https://doi.org/10.1016/j.ifset.2005.05.003

Chandralekha, A., Tavanandi, A.H., Amrutha, N., Hebbar, H.U., Raghavarao, K.S.M.S., Gadre, R., 2016. Encapsulation of yeast (*Saccharomyces cerevisiae*) by spray drying for extension of shelf life. Dry. Technol. 34, 1307–1318. https://doi.org/10.1080/07373937.2015.1112808

D'Alessandro, M., Pisanu, F., Baldo, D., Parolin, C., Filippini, G., Vitali, B., Lanciotti, R., Patrignani, F., 2021. Unravelling the functional and technological potential of soy milk based microencapsulated *Lactobacillus crispatus* and *Lactobacillus gasseri*. J. Funct. Foods 87, 104745. https://doi.org/10.1016/j.jff.2021.104745

Da Mota, M.C.B., Batista, N.N., Rabelo, M.H.S., Ribeiro, D.E., Borém, F.M., Schwan, R.F., 2020. Influence of fermentation conditions on the sensorial quality of coffee inoculated with yeast. Food Res. Int. 136, 109482. https://doi.org/10.1016/j.foodres.2020.109482

da Silva, B.L., Pereira, P.V., Bertoli, L.D., Silveira, D.L., Batista, N.N., Pinheiro, P.F., de Souza Carneiro, J., Schwan, R.F., de Assis Silva, S., Coelho, J.M., Bernardes, P.C., 2021. Fermentation of *Coffea canephora* inoculated with yeasts: Microbiological, chemical, and sensory characteristics. Food Microbiol. 98, 103786. https://doi.org/10.1016/j.fm.2021.103786

575 Elhalis, H., Cox, J., Frank, D., Zhao, J., 2021. Microbiological and Chemical Characteristics
576 of Wet Coffee Fermentation Inoculated With *Hanseniaspora uvarum* and *Pichia*
577 *kudriavzevii* and Their Impact on Coffee Sensory Quality. Front. Microbiol. 12.
578 https://doi.org/10.3389/fmicb.2021.713969
579 Esftathiou, T., Feuardent, C., Méjean, S., Schuck, P., 2002. The use of carbonyl analysis to
580 follow the main reactions involved in the process of deterioration of dehydrated dairy
581 products: prediction of most favourable degree of dehydration. Lait 82, 423–439.
582 https://doi.org/10.1051/lait:2002021
583 Evangelista, S.R., Silva, C.F., Miguel, M.G.P. da C., Cordeiro, C. de S., Pinheiro, A.C.M.,
584 Duarte, W.F., Schwan, R.F., 2014. Improvement of coffee beverage quality by using
585 selected yeasts strains during the fermentation in dry process. Food Res. Int. 61, 183–
586 195. https://doi.org/10.1016/j.foodres.2013.11.033
587 Frakolaki, G., Giannou, V., Kekos, D., Tzia, C., 2021. A review of the microencapsulation
588 techniques for the incorporation of probiotic bacteria in functional foods. Crit. Rev. Food
589 Sci. Nutr. 61, 1515–1536. https://doi.org/10.1080/10408398.2020.1761773
590 Fu, N., Chen, X.D., 2011. Towards a maximal cell survival in convective thermal drying
591 processes. Food Res. Int. 44, 1127–1149. https://doi.org/10.1016/j.foodres.2011.03.053
592 Garre, E., Raginel, F., Palacios, A., Julien, A., Matallana, E., 2010. Oxidative stress responses
593 and lipid peroxidation damage are induced during dehydration in the production of dry
594 active wine yeasts. Int. J. Food Microbiol. 136, 295–303.
595 https://doi.org/10.1016/j.ijfoodmicro.2009.10.018
596 Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., Saurel, R., 2007. Applications of
597 spray-drying in microencapsulation of food ingredients: An overview. Food Res. Int. 40,
598 1107–1121. https://doi.org/10.1016/j.foodres.2007.07.004
599 Hansen, C., 2022. Yeasts. URL https://www.chr-hansen.com/pt/food-cultures-and-

enzymes/fermented-beverages/cards/collection-cards/speciality-yeast

Herrero, E., Ros, J., Bellí, G., Cabiscol, E., 2008. Redox control and oxidative stress in yeast cells. Biochim. Biophys. Acta - Gen. Subj. 1780, 1217–1235. https://doi.org/10.1016/j.bbagen.2007.12.004

Huang, S., Vignolles, M.-L., Chen, X.D., Le Loir, Y., Jan, G., Schuck, P., Jeantet, R., 2017. Spray drying of probiotics and other food-grade bacteria: A review. Trends Food Sci. Technol. 63, 1–17. https://doi.org/10.1016/j.tifs.2017.02.007

Kim, D.-H., Lee, S.-B., Jeon, J.-Y., Park, H.-D., 2019. Development of air-blast dried non-*Saccharomyces* yeast starter for improving quality of Korean persimmon wine and apple cider. Int. J. Food Microbiol. 290, 193–204. https://doi.org/10.1016/j.ijfoodmicro.2018.10.002

Laffort, 2022. Catalogue Laffort. URL https://laffort.com/wp-content/uploads/Catalogues/PT_Catalogue_Laffort_2017_2018.pdf

Lallemand, 2022. Yeasts. URL https://www.lallemandwine.com/pt-pt/brazil/produtos/catalogue/leveduras/

Liu, H., Cui, S.W., Chen, M., Li, Y., Liang, R., Xu, F., Zhong, F., 2019. Protective approaches and mechanisms of microencapsulation to the survival of probiotic bacteria during processing, storage and gastrointestinal digestion: A review. Crit. Rev. Food Sci. Nutr. 59, 2863–2878. https://doi.org/10.1080/10408398.2017.1377684

López-Rubio, A., Sanchez, E., Wilkanowicz, S., Sanz, Y., Lagaron, J.M., 2012. Electrospinning as a useful technique for the encapsulation of living bifidobacteria in food hydrocolloids. Food Hydrocoll. 28, 159–167. https://doi.org/10.1016/j.foodhyd.2011.12.008

Márquez-Montes, R., Robles-Olvera, V., Luna-Solano, G., Salgado-Cervantes, M.A., 2007. Metabolic Activity of the Dehydrated Yeast by Spray Drying. Dry. Technol. 25, 1281–

1285. https://doi.org/10.1080/07373930701438824

Martín, M.J., Lara-Villoslada, F., Ruiz, M.A., Morales, M.E., 2015. Microencapsulation of bacteria: A review of different technologies and their impact on the probiotic effects. Innov. Food Sci. Emerg. Technol. 27, 15–25. https://doi.org/10.1016/j.ifset.2014.09.010

Pereira, P.V., Bravim, D.G., Grillo, R.P., Bertoli, L.D., Osório, V.M., da Silva Oliveira, D., da Cruz Pedrozo Miguel, M.G., Schwan, R.F., de Assis Silva, S., Coelho, J.M., Bernardes, P.C., 2021. Microbial diversity and chemical characteristics of *Coffea canephora* grown in different environments and processed by dry method. World J. Microbiol. Biotechnol. 37, 51. https://doi.org/10.1007/s11274-021-03017-2

Rapoport, A., 2019. Anhydrobiosis in Non-conventional Yeasts, in: Non-Conventional Yeasts: From Basic Research to Application. Springer International Publishing, Cham, pp. 341–359. https://doi.org/10.1007/978-3-030-21110-3_10

Rapoport, A., 2017. Anhydrobiosis and Dehydration of Yeasts, in: Biotechnology of Yeasts and Filamentous Fungi. Springer International Publishing, Cham, pp. 87–116. https://doi.org/10.1007/978-3-319-58829-2_4

Rapoport, A., Golovina, E.A., Gervais, P., Dupont, S., Beney, L., 2019. Anhydrobiosis: Inside yeast cells. Biotechnol. Adv. 37, 51–67. https://doi.org/10.1016/j.biotechadv.2018.11.003

Ré, Mi, 1998. Microencapsulation By Spray Drying. Dry. Technol. 16, 1195–1236. https://doi.org/10.1080/07373939808917460

Ribeiro, L.S., da Cruz Pedrozo Miguel, M.G., Martinez, S.J., Bressani, A.P.P., Evangelista, S.R., Silva e Batista, C.F., Schwan, R.F., 2020. The use of mesophilic and lactic acid bacteria strains as starter cultures for improvement of coffee beans wet fermentation. World J. Microbiol. Biotechnol. 36, 186. https://doi.org/10.1007/s11274-020-02963-7

Roca Domènech, G., López Martínez, G., Barrera, E., Poblet, M., Rozès, N., Cordero-Otero,

650 R., 2018. Enhancing the tolerance of the *Starmerella bacillaris* wine strain to
651 dehydration stress. Ann. Microbiol. 68, 667–676. https://doi.org/10.1007/s13213-018-
652 1373-8
653 Shankar, R.S., Sneha H.P., Prakash, I., Khan, M., H N, P.K., Om, H., Basavaraj, K., Murthy,
654 P.S., 2022. Microbial ecology and functional coffee fermentation dynamics with *Pichia*
655 *kudriavzevii*. Food Microbiol. 105, 104012. https://doi.org/10.1016/j.fm.2022.104012
656 https://doi.org/10.1016/j.fm.2022.104012
657 Schuck, P., Dolivet, A., Jeantet, R., 2012. Analytical Methods for Food and Dairy Powders.
658 John Wiley & Sons.
659 Semyonov, D., Ramon, O., Kaplun, Z., Levin-Brener, L., Gurevich, N., Shimoni, E., 2010.
660 Microencapsulation of *Lactobacillus paracasei* by spray freeze drying. Food Res. Int. 43,
661 193–202. https://doi.org/10.1016/j.foodres.2009.09.028
662 Silva, C.F., Schwan, R.F., Sousa Dias, Ë., Wheals, A.E., 2000. Microbial diversity during
663 maturation and natural processing of coffee cherries of *Coffea arabica* in Brazil. Int. J.
664 Food Microbiol. 60, 251–260. https://doi.org/10.1016/S0168-1605(00)00315-9
665 Sohail, A., Turner, M.S., Coombes, A., Bhandari, B., 2013. The Viability of *Lactobacillus*
666 *rhamnosus* GG and *Lactobacillus acidophilus* NCFM Following Double Encapsulation
667 in Alginate and Maltodextrin. Food Bioprocess Technol. 6, 2763–2769.
668 https://doi.org/10.1007/s11947-012-0938-y
669 Tan, D.T., Poh, P.E., Chin, S.K., 2018. Microorganism preservation by convective air-
670 drying—A review. Dry. Technol. 36, 764–779.
671 https://doi.org/10.1080/07373937.2017.1354876
672 Torrellas, M., Rozès, N., Aranda, A., Matallana, E., 2020. Basal catalase activity and high
673 glutathione levels influence the performance of non-*Saccharomyces* active dry wine
674 yeasts. Food Microbiol. 92, 103589. https://doi.org/10.1016/j.fm.2020.103589

675 Vanden Braber, N.L., Díaz Vergara, L.I., Rossi, Y.E., Aminahuel, C.A., Mauri, A.N.,
676 Cavaglieri, L.R., Montenegro, M.A., 2020. Effect of microencapsulation in whey protein
677 and water-soluble chitosan derivative on the viability of the probiotic *Kluyveromyces*
678 *marxianus* VM004 during storage and in simulated gastrointestinal conditions. LWT
679 118, 108844. https://doi.org/10.1016/j.lwt.2019.108844
680 Vilela, D.M., Pereira, G.V. de M., Silva, C.F., Batista, L.R., Schwan, R.F., 2010. Molecular
681 ecology and polyphasic characterization of the microbiota associated with semi-dry
682 processed coffee (*Coffea arabica* L.). Food Microbiol. 27, 1128–1135.
683 https://doi.org/10.1016/j.fm.2010.07.024
684 Zhu, X., Kong, H., Gao, Y., Wu, M., Kong, F., 2012. Low concentrations of polycyclic
685 aromatic hydrocarbons promote the growth of *Microcystis aeruginosa*. J. Hazard. Mater.
686 237–238, 371–375. https://doi.org/10.1016/j.jhazmat.2012.08.029
687
688

689 **Figures caption**

690 **Figure 1**. Contour curves for wall material concentration (maltodextrin (1), high maltose (2), and whey powder (3)) and
691 inlet temperature related to cell viability, drying yield, and water activity: (A) *S. cerevisiae* CCMA 0543 (B) *T.*
692 *delbrueckii* CCMA 0684 and (C) *M. caribbica* CCMA 1738. The red region corresponds to the zone of higher cell
693 viability and drying yield with water activity at a maximum of 0.22.

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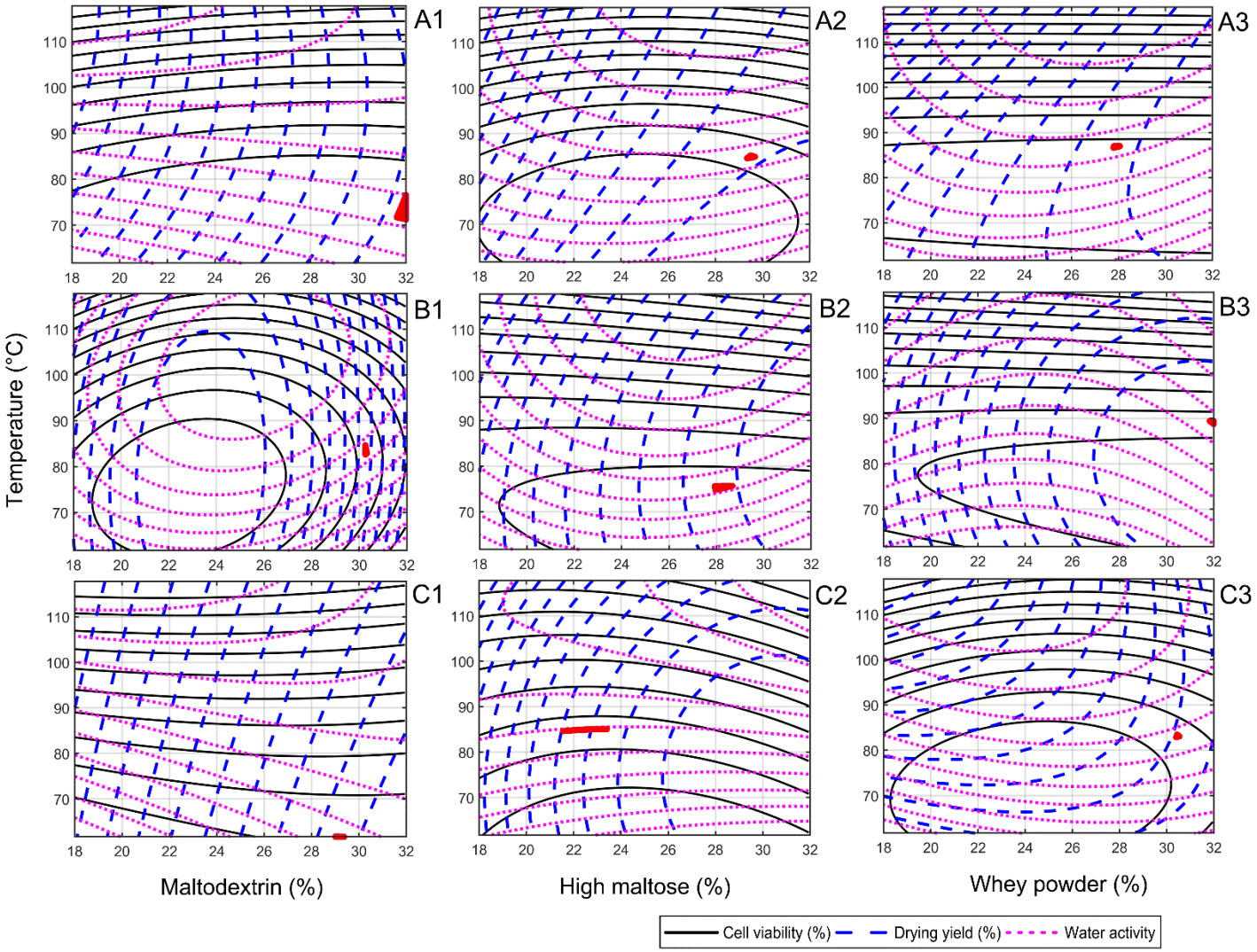

A1
A2
A3
B1
B2
B3
C1
C2
C3
Temperature (°C)
Maltodextrin (%)
High maltose (%)
Whey powder (%)
Cell viability (%)
Drying yield (%)
Water activity

695

Figure 2. Cell viability of microencapsulated *S. cerevisiae* CCMA 0543 (A), *T. delbrueckii* CCMA 0684 (B) and *M. caribbica* CCMA 1738 (C) during storage at 25 °C (1) and 7 °C (2). MA = maltose; MD = maltodextrin; WP = whey powder.

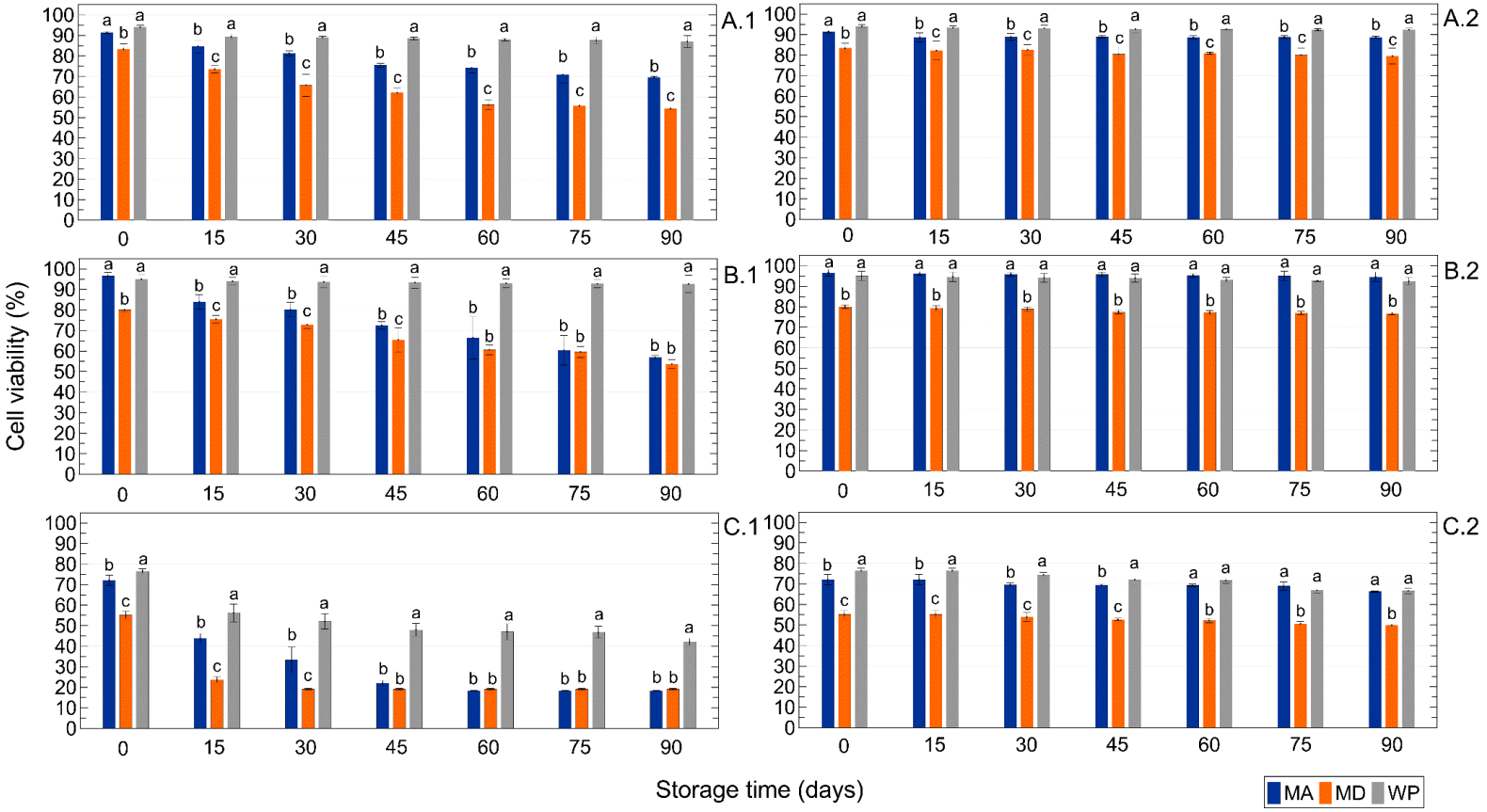


Figure 3. Population count, °Brix and pH during fermentation. Inoculation with (A) *S. cerevisiae* CCMA 0543, (B) *T. delbrueckii* CCMA 0684 and (C) *M. caribbica* CCMA 1738 in a fresh (1) and microencapsulated with whey powder (2).

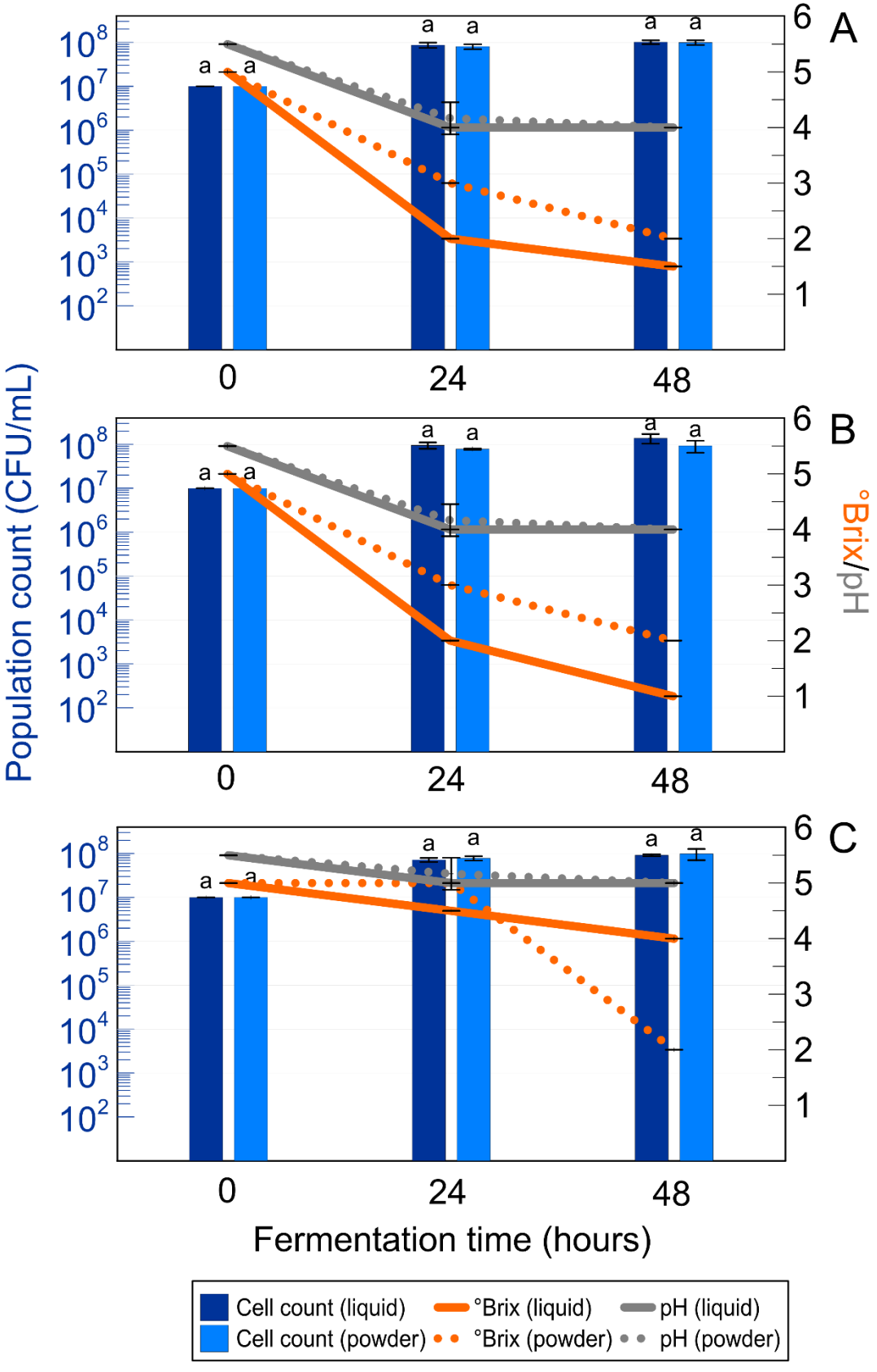
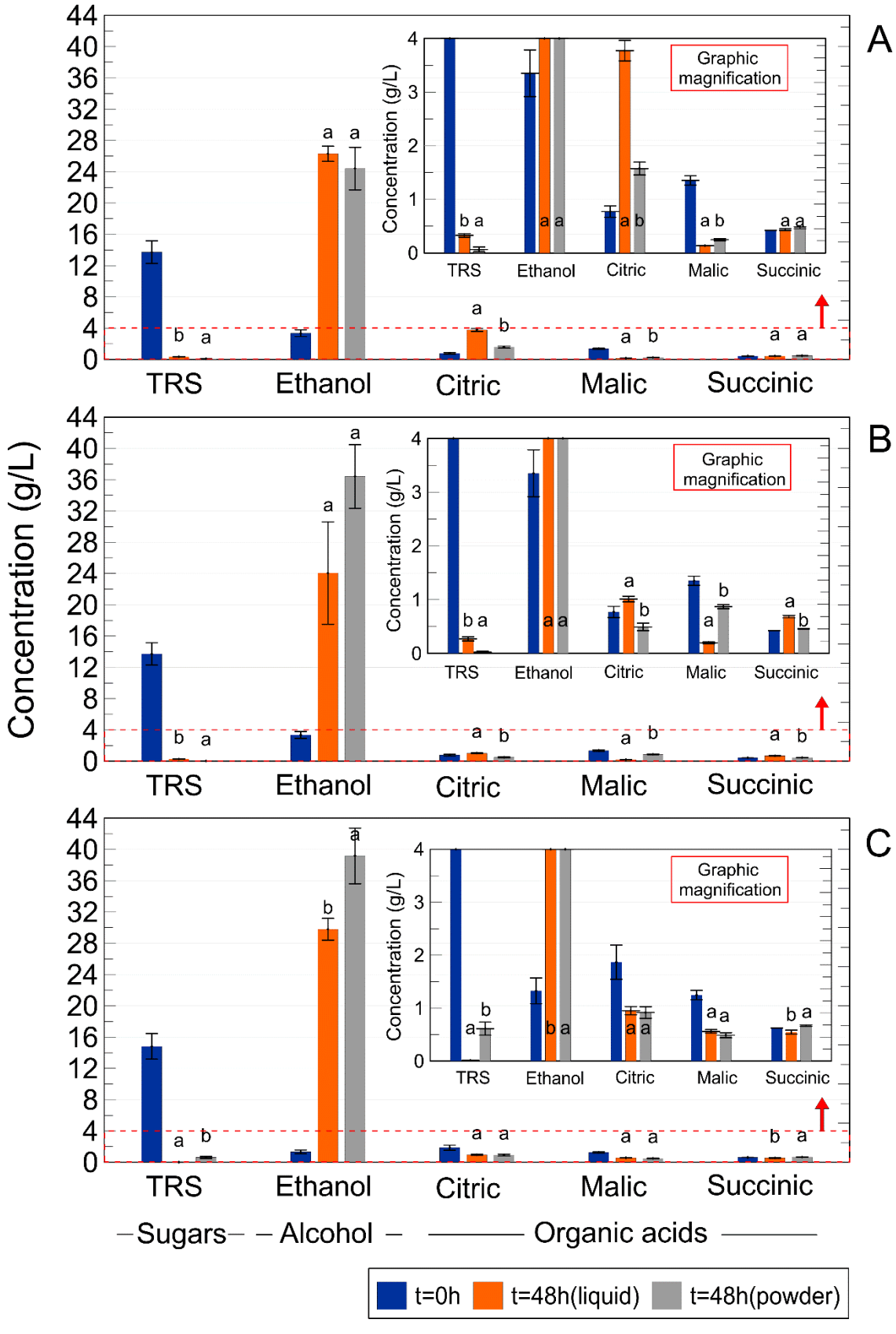


Figure 4. Concentrations of total reducing sugars (TRS), ethanol and acids (citric, malic, succinic, lactic and acetic) during fermentation. Inoculation with (A) *S. cerevisiae* CCMA 0543, (B) *T. delbrueckii* CCMA 0684 and (C) *M. caribbica* CCMA 1738.



708 **Tables**

709 **Table 1.** Comparison between predicted and experimental values from the model validation
710 test.

Treatments		Cell viability (%)	Water activity	Drying yield (%)
S. cerevisiae CCMA 0543 + maltose	Theoretical value	88.56[b]	0.22[a]	56.89[a]
	Experimental value	91.44 ± 0.58[a]	0.23 ± 0.01[a]	56.30 ± 0.07[b]
S. cerevisiae CCMA 0543 + maltodextrin	Theoretical value	85.83[a]	0.22[a]	60.26[a]
	Experimental value	83.39 ± 2.45[a]	0.23 ± 0.01[a]	57.57 ± 0.58[b]
S. cerevisiae CCMA 0543 + whey powder	Theoretical value	94.74[a]	0.22[a]	55.69[a]
	Experimental value	94.00 ± 0.93[a]	0.21 ± 0.01[a]	56.63 ± 0.65[a]
T. delbrueckii CCMA 0684 + maltose	Theoretical value	90.94[b]	0.22[a]	54.51[a]
	Experimental value	96.58 ± 1.60[a]	0.23 ± 0.01[a]	55.43 ± 1.93[a]
T. delbrueckii CCMA 0684 + maltodextrin	Theoretical value	72.72[b]	0.22[a]	57.30[a]
	Experimental value	80.04 ± 0.89[a]	0.22 ± 0.02[a]	58.33 ± 0.82[a]
T. delbrueckii CCMA 0684 + whey powder	Theoretical value	94.40[a]	0.22[a]	58.59[a]
	Experimental value	95.01 ± 2.30[a]	0.22 ± 0.01[a]	59.06 ± 0.65[a]
M. caribbica CCMA 1738 + maltose	Theoretical value	82.96[a]	0.22[a]	52.98[a]
	Experimental value	72.10 ± 2.55[b]	0.22 ± 0.02[a]	50.54 ± 1.55[a]
M. caribbica CCMA 1738 + maltodextrin	Theoretical value	57.46[a]	0.20[a]	59.39[a]
	Experimental value	55.16 ± 1.89[a]	0.40 ± 0.02[b]	52.18 ± 0.12[b]
M. caribbica CCMA 1738 + whey powder	Theoretical value	81.47[a]	0.22[a]	52.88[a]
	Experimental value	76.56 ± 1.07[b]	0.22 ± 0.01[a]	52.09 ± 2.43[a]

711 Data are presented as mean. a–b for each column, mean values with different letters are
712 significant at $p \leq 0.05$ by Scott–Knott test.
713

714 **Table 2.** Characterization of wall materials.

Wall materials	Moisture content (%)	Water solubility (%)	Hygroscopicity (g/100g)	Water activity
Maltose	5.33 ± 0.22[a]	96.53 ± 0.79[a]	20.76 ± 1.17[b]	0.36 ± 0.00[b]
Maltodextrin	6.74 ± 0.41[a]	91.51 ± 0.44[c]	7.11 ± 0.63[a]	0.49 ± 0.01[c]
Whey powder	5.02 ± 3.39[a]	95.05 ± 0.54 [b]	5.73 ± 2.25[a]	0.33 ± 0.01[a]

715 Data are presented as mean. a–c for each column, mean values with different letters are
716 significant at $p \leq 0.05$ by Scott–Knott test.

Table 3. Microcapsules characterization obtained after drying of optimum condition.

Characterization after spray drying	Yeasts	Wall materials		
		MA[a]	MD[b]	WP[c]
Moisture content (%)	*S. cerevisiae* CCMA 0543	4.04 ± 0.23^{aA}	6.33 ± 0.75^{aA}	5.24 ± 0.25^{aA}
	T. delbrueckii CCMA 0684	4.37 ± 0.45^{aA}	5.29 ± 0.04^{aA}	5.42 ± 0.22^{aA}
	M. caribbica CCMA 1738	4.67 ± 0.21^{aA}	8.53 ± 0.13^{bB}	5.98 ± 0.07^{aA}
Hygroscopicity (g/100g)	*S. cerevisiae* CCMA 0543	26.26 ± 1.51^{aB}	12.36 ± 1.09^{aA}	8.73 ± 1.01^{aA}
	T. delbrueckii CCMA 0684	26.92 ± 0.06^{aB}	12.98 ± 0.26^{aA}	10.54 ± 1.47^{aA}
	M. caribbica CCMA 1738	24.26 ± 0.24^{aB}	12.37 ± 3.73^{aA}	10.85 ± 1.43^{aA}
Water solubility (%)	*S. cerevisiae* CCMA 0543	90.23 ± 2.77^{aA}	79.89 ± 6.32^{aA}	86.58 ± 0.77^{aA}
	T. delbrueckii CCMA 0684	92.07 ± 1.63^{aA}	83.61 ± 1.46^{aA}	88.18 ± 2.40^{aA}
	M. caribbica CCMA 1738	89.43 ± 4.50^{aA}	82.31 ± 3.46^{aA}	87.27 ± 3.47^{aA}
Particle size D_{50} (µm)	*S. cerevisiae* CCMA 0543	4.53 ± 1.71^{bA}	4.97 ± 1.92^{cB}	4.48 ± 1.87^{bA}
	T. delbrueckii CCMA 0684	4.36 ± 1.75^{bA}	4.04 ± 2.52^{bA}	4.16 ± 2.03^{aA}
	M. caribbica CCMA 1738	3.57 ± 1.55^{aA}	3.59 ± 2.18^{aA}	4.67 ± 1.86^{bB}

Data are presented as mean. a–c for each column, mean values with different lowercase letters are significant at $p \leq 0.05$ by Scott–Knott test. A–C for each row, mean values with different capital letters are significant at $p \leq 0.05$ by Scott–Knott test.

[a]Maltose

[b]Maltodextrin

[c]Whey powder

723 **Table 4**. Growth rate and generation time of fresh and microencapsulated yeasts with WP.

Yeast	**μ (1/h)[a]**	**G (h)[b]**
S. cerevisiae CCMA 0543 fresh	0.423 ± 0.023[a]	1.641 ± 0.091[a]
S. cerevisiae CCMA 0543 powder	0.187 ± 0.002[b]	3.700 ± 0.036[b]
Yeast	**μ (1/h)[a]**	**tg (h)[b]**
T. delbrueckii CCMA 0684 fresh	0.410 ± 0.001[a]	1.685 ± 0.001[a]
T. delbrueckii CCMA 0684 powder	0.141 ± 0.005[b]	4.911 ± 0.164[b]
Yeast	**μ (1/h)[a]**	**tg (h)[b]**
M. caribbica CCMA 1738 fresh	0.236 ± 0.012[b]	2.944 ± 0.153[b]
M. caribbica CCMA 1738 powder	0.279 ± 0.015[a]	2.493 ± 0.135[a]

724 Data are presented as mean. a–b for each column, mean values with different lowercase letters
725 are significant at $p \leq 0.05$ by Scott–Knott test.
726 [a]Specific growth rate
727 [b]Generation time

728 **Supporting Information - Supplementary material**

729 **Table S1.** Central Composite Rotational Design used in microencapsulation assays by spray drying of *S. cerevisiae* CCMA 0543, *T. delbrueckii*
730 CCMA 0684 and *M. caribbica* CCMA 1738.

***S. cerevisiae* - CCMA 0543**

Experiments	Wall material (%)	Temperature (°C)	MD[a]			MA[b]			WP[c]		
			Viability (%)	A_w	Yield (%)	Viability (%)	A_w	Yield (%)	Viability (%)	A_w	Yield (%)
1	20.00 (-1)	70.00 (-1)	86.99	0.29	49.34	93.71	0.27	48.50	91.49	0.30	50.69
2	20.00 (-1)	110.00 (+1)	66.30	0.11	46.32	70.33	0.18	30.84	65.70	0.18	45.27
3	30.00 (+1)	70.00 (-1)	87.81	0.22	60.46	93.14	0.27	63.52	94.52	0.32	56.17
4	30.00 (+1)	110.00 (+1)	70.30	0.12	59.65	70.80	0.13	53.00	67.18	0.14	55.09
5	17.93 (-α)	90.00 (0)	72.59	0.17	45.23	78.38	0.23	32.55	95.25	0.24	49.75
6	32.07 (+α)	90.00 (0)	75.97	0.18	57.10	78.29	0.23	55.31	93.51	0.25	57.67
7	25.00 (0)	61.70 (-α)	85.95	0.31	54.62	93.16	0.34	52.92	95.68	0.34	55.73
8	25.00 (0)	118.28 (+α)	42.02	0.10	51.04	42.95	0.13	47.15	50.58	0.14	50.65
9	25.00 (0)	90.00 (0)	78.96	0.17	52.17	87.44	0.18	50.54	91.51	0.18	54.71
10	25.00 (0)	90.00 (0)	79.86	0.16	52.91	87.86	0.18	50.03	93.36	0.2	53.43
11	25.00 (0)	90.00 (0)	81.85	0.18	52.80	86.68	0.22	51.43	93.11	0.22	53.13

***T. delbrueckii* - CCMA 0684**

Experiments	Wall material (%)	Temperature (°C)	MD			MA			WP		
			Viability (%)	A_w	Yield (%)	Viability (%)	A_w	Yield (%)	Viability (%)	A_w	Yield (%)
1	20.00 (-1)	70.00 (-1)	81.17	0.31	51.01	89.37	0.33	48.80	95.98	0.33	50.19
2	20.00 (-1)	110.00 (+1)	63.15	0.21	52.50	71.85	0.16	46.77	78.78	0.20	50.06
3	30.00 (+1)	70.00 (-1)	64.73	0.35	60.56	89.87	0.29	53.88	96.15	0.29	58.49
4	30.00 (+1)	110.00 (+1)	55.98	0.15	59.82	63.88	0.08	52.99	71.95	0.18	57.89
5	17.93 (-α)	90.00 (0)	73.06	0.18	49.09	80.72	0.16	46.51	89.53	0.22	46.04
6	32.07 (+α)	90.00 (0)	71.56	0.21	61.89	81.64	0.18	57.17	95.27	0.23	57.65

7	25.00 (0)	61.70 (-α)	85.76	0.26	41.29	93.63	0.26	53.26	95.20	0.37	55.17
8	25.00 (0)	118.28 (+α)	55.33	0.21	45.13	46.41	0.05	50.80	59.50	0.19	50.62
9	25.00 (0)	90.00 (0)	85.49	0.15	41.93	84.94	0.12	51.14	91.12	0.27	54.80
10	25.00 (0)	90.00 (0)	77.06	0.10	46.64	81.23	0.14	53.22	94.42	0.26	58.31
11	25.00 (0)	90.00 (0)	81.03	0.10	45.45	84.78	0.14	53.01	93.89	0.25	54.57

***M. caribbica* - CCMA 1738**

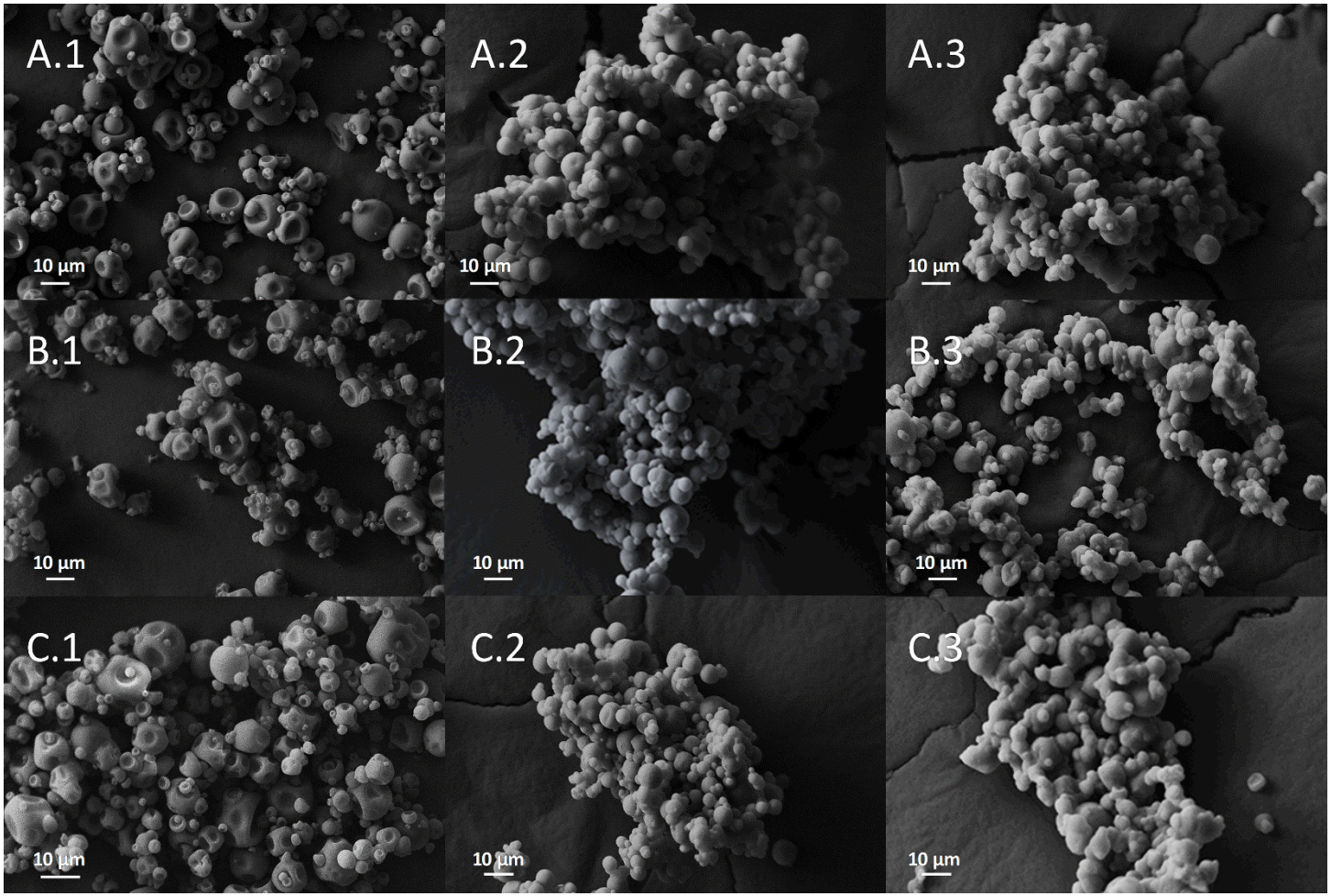
Experiments	Wall material (%)	Temperature (°C)	MD			MA			WP		
			Viability (%)	A_w	Yield (%)	Viability (%)	A_w	Yield (%)	Viability (%)	A_w	Yield (%)
1	20.00 (-1)	70.00 (-1)	57.07	0.25	46.62	87.39	0.31	47.06	85.35	0.31	50.04
2	20.00 (-1)	110.00 (+1)	40.24	0.10	44.04	66.51	0.18	41.39	57.76	0.16	32.36
3	30.00 (+1)	70.00 (-1)	55.53	0.17	58.84	89.21	0.31	59.79	82.19	0.26	61.27
4	30.00 (+1)	110.00 (+1)	41.30	0.10	55.60	62.94	0.11	55.17	58.30	0.15	55.12
5	17.93 (-α)	90.00 (0)	48.50	0.14	42.22	78.48	0.17	49.51	76.54	0.18	48.89
6	32.07 (+α)	90.00 (0)	47.56	0.14	60.72	69.70	0.19	56.00	78.63	0.23	47.30
7	25.00 (0)	61.70 (-α)	59.05	0.21	54.85	94.60	0.34	55.94	86.58	0.30	50.55
8	25.00 (0)	118.28 (+α)	28.18	0.07	50.16	53.09	0.14	53.29	53.93	0.14	44.01
9	25.00 (0)	90.00 (0)	48.05	0.12	51.17	76.86	0.21	54.58	84.04	0.18	48.56
10	25.00 (0)	90.00 (0)	48.68	0.15	52.97	78.71	0.21	55.36	81.90	0.20	47.54
11	25.00 (0)	90.00 (0)	48.85	0.14	52.42	83.28	0.18	56.11	78.87	0.17	43.71

731 [a]Maltodextrin

732 [b]High maltose

733 [c]Whey powder

734 **Fig. S1.** Scanning electron microscope of *S. cerevisiae* CCMA 0543 (A), *T. delbrueckii* CCMA 0684 (B) and *M.*
735 *caribbica* CCMA 1738 (C) microencapsulated with maltodextrin (1), maltose (2) and whey powder (3).



736

ARTIGO 3 - USE OF MICROENCAPSULATED STARTER CULTURES BY SPRAY DRYING IN COFFEE UNDER SELF-INDUCED ANAEROBIOSIS FERMENTATION (SIAF)

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Abstract

Using starter culture in liquid form is not economically viable in the coffee fermentation process. This work aimed to compare the fermentative performances of fresh and microencapsulated yeasts in coffee under self-induced anaerobic fermentation (SIAF). The inoculum permanence was monitored, and sugars, alcohols, acids, and volatile compounds were analyzed by chromatography. In addition, sensory analysis was performed on roasted beans. After 180 h of fermentation in the natural process, microencapsulated *Torulaspora delbrueckii* (MT) ($7.97x10^7$ cell/g) showed a higher population than fresh *Torulaspora delbrueckii* (FT) ($1.76x10^7$ cell/g). The same acids and volatile compounds were detected in coffees with fresh and microencapsulated yeast. However, the yeast state influenced the concentration of the compounds. In pulped coffee, the coffee inoculated with microencapsulated *Saccharomyces cerevisiae* (MS) obtained the highest concentration of alcohols (67%), ketones (60%), pyrazines (54%), pyrroles (58%), phenols (54%), esters (57%), and others (63%) compared with fresh *Saccharomyces cerevisiae* (FS). Furthermore, the coffee inoculated with MT obtained the highest concentration in 11 of the 12 chemical classes in both processes compared with FT. These differences ranged from 0.9 to 39.7%. Regarding sensory analysis, coffees inoculated with MS showed dominant notes of fruity, caramel, and nuts in the natural process. Otherwise, in pulped process, coffees inoculated with MT showed caramel, honey, and nuts. Therefore, the microencapsulated yeasts were metabolically active and may be considered with commercial potential. Considering the parameters analyzed, the most suitable yeast for natural and pulped processing would be MS and MT, respectively.

Keywords: Fresh yeast; Dried yeast; Active dry yeast, Starter culture; Whey powder; Coffee fermentation

1. Introduction

Self-induced anaerobic fermentation (SIAF) is a new technology used to ferment coffees in which anaerobiosis is formed gradually through microbial metabolism (da Mota et al., 2022; Cassimiro et al., 2022; Pereira et al., 2022). This technology improves the fermentative performance of yeasts and lactic acid bacteria during natural, pulped, and wet processing. Consequently, the production of volatile and non-volatile compounds is intensified, contributing to flavors and aromas in coffee beverages (Bressani et al., 2021; Elhalis et al., 2020; Martinez et al., 2021).

In addition to this technology, selected fresh yeasts have been used as starter cultures in the coffee fermentation process (da Silva et al., 2021; Elhalis et al., 2021; Martinez et al., 2021). The use of yeasts is an economically viable alternative to obtain coffee with different flavors and standardize the fermentation process (Bressani et al., 2021; Da Mota et al., 2020; da Silva et al., 2022). Also, it can bring benefits such as a reduction in fermentation time, inhibition of the growth of undesirable microorganisms, and production of promising compounds for the final quality of the beverage (Bressani et al., 2021).

However, the use of microorganisms in liquid form is not economically viable. During long-term storage, cell viability and strain stability decrease (Tan et al., 2018). In addition, the commercialization of fresh yeast results in shorter shelf life, greater risk of contamination, and higher cost with transportation and refrigerated storage (Márquez-Montes et al., 2007; Tan et al., 2018). These factors mentioned making it difficult to use and commercialize these starter cultures.

Based on this, drying may be an alternative for preserving coffee epiphytic yeasts. During this process, cells enter a state of anhydrobiosis, in which their metabolism is temporarily and reversibly suspended or slowed. This state is used for the industrial production of active dry yeasts (ADY) (Rapoport et al., 2019). As a result, ADY has more excellent stability and less

risk of contamination. In addition, it contributes to transport and storage, facilitates handling, and reduces odor (Márquez-Montes et al., 2007; Tan et al., 2018).

Studies showed that microencapsulation by spray drying is a preservation method that can be used for drying yeasts (López-Cruz et al., 2020; Suryabhan et al., 2019; Vanden Braber et al., 2020). Furthermore, this method can be used quickly and continuously, allowing ADY production on an industrial scale with low operating costs (Aponte et al., 2016). In addition, microencapsulation reduces the impact of high temperatures on cells and protects the microorganism from adverse environmental conditions such as temperature, moisture content, oxygen, and light. Consequently, it contributes to increased stability and maintenance of cell viability during storage (Desai & Park, 2005; Liu et al., 2019; Rokka & Rantamäki, 2010).

Our research group studied and optimized the microencapsulation by spray drying of two potential yeasts starter for coffee fermentation (Martins et al., 2022a, 2022b). In addition, the microencapsulated yeast fermentative performance was evaluated in coffee peel and pulp media. Some differences were found, but the microencapsulated yeasts maintained their biotechnological characteristics. Thus, microencapsulated yeasts have shown promise to be used in coffee fermentation (Martins et al., 2022b). However, a better understanding of the viability and vitality of epiphytic coffee yeasts after the drying process will help develop suitable ADY for coffee fermentation. Therefore, this work aimed to compare the fermentative performances of fresh and microencapsulated yeasts in coffee during self-induced anaerobiosis fermentation (SIAF).

2. Material and methods

2.1 Microorganisms

Saccharomyces cerevisiae CCMA 0543 (access number KF728798.1) and *Torulaspora delbrueckii* CCMA 0684 (access number KM402082.1) previously isolated from *Coffea*

arabica (Silva et al., 2000; Vilela et al., 2010) were used. These strains belong to the Culture Collection of Agricultural Microbiology (CCMA, Federal University of Lavras, Lavras, Minas Gerais, Brazil). The isolates (1 mL) stored at -80 °C in YEPD containing 20% glycerol (w/w) were reactivated and grown in molasses liquid medium [in g/L: molasses 137.5 (ECL Eireli – supplier *Mellaço de Cana*), calcium chloride 1.1 (Dinâmica), urea 2 (Ambion), Polysorbate 80 0.5 (Dinâmica) and pH 3.5] until reached a 10^8 CFU/mL. The yeasts were incubated at 28 °C for 48 h in an orbital shaker set at 120 rpm at each growth stage. At the end of growth, the yeasts reach the stationary phase. The cells were recovered by centrifugation (7000 ×*g*; 10 min) and resuspended in sterile water to a concentration of 10^9 CFU/mL (Martins et al., 2022a).

2.2 Microencapsulation by spray drying

2.2.1 Solution preparation

Whey powder (WP) (Jaciara Indústria e Comércio, Brazil) was used as wall material, and the concentrations were previously optimized for each yeast (Martins et al., 2022b). The concentrations used for *S. cerevisiae* CCMA 0543 and *T. delbrueckii* CCMA 0684 were 27.92 and 32%, respectively. First, the wall materials were diluted in water and pasteurized at 75 °C for 30 min, followed by rapid cooling (Andrade et al., 2019). Then, the yeasts were added separately at an 8 x 10^8 cells/mL concentration. Finally, the feed solutions (2 L) were mixed for 5 min with a magnetic stirrer for homogenization.

2.2.2 Spray drying conditions

Saccharomyces cerevisiae CCMA 0543 and *Torulaspora delbrueckii* CCMA 0684 were microencapsulated using a more extensive scale spray dryer (model SD 5.0; Labmaq do Brasil, Ribeirão Preto, Brazil). The drying air inlet temperature was previously optimized for

each strain (Martins et al., 2022b). Thus, the temperature for *S. cerevisiae* and *T. delbrueckii* was 87 and 88 °C, respectively. The feed solutions (2 L) were kept under constant magnetic stirring at room temperature. In drying both yeasts, a feed rate of 1.5 L/h, drying airflow of 1.5 x 10^5 L/h, and atomization air flow rate of 60 L/h were used (Martins et al., 2022b). The resulting powder was collected at the cyclone base and stored in a 500 g package made of polyester, metallization, and polyethylene. Then the packages were vacuum-sealed. The samples were stored at room temperature (25 °C).

2.3 Coffee processing and yeast inoculation

Coffees (*Coffea arabica* L.) of the Topazio Amarelo variety were mechanically collected at the Cafés Monte Alegre farm (S 1°59'56", W 54°4'58") located in Alfenas (Minas Gerais, Brazil) at 850 m above sea level. Two processing were carried out: natural and pulped processes. In the natural process, the coffee fruit was fermented with a peel. The peel and part of the mucilage were mechanically removed in the pulped process. In both processes, 40 L of coffee cherries were manually selected and transferred to cylindrical high-density polyethylene bioreactors with 50 L (Picillo, São Paulo, Brazil). The method used was self-induced anaerobiosis fermentation (SIAF), according to da Mota et al. (2022). Five treatments were performed: SIAF control (without inoculation), fresh *S. cerevisiae* CCMA0543 (FS), fresh *T. delbrueckii* CCMA0684 (FT), microencapsulated *S. cerevisiae* CCMA0543 (MS), and microencapsulated *T. delbrueckii* CCMA0684 (MT). The fresh yeast cells were recovered by centrifugation and resuspended in 500 mL distilled water. The microencapsulated yeasts were previously reactivated in water at 40 °C for 30 min. For this, distilled water was added to 250 g of microencapsulated yeast until a final volume of 500 mL. Then, the yeasts were inoculated into coffee at a final concentration of approximately 10^7 cells/g. The fermentation was carried out in duplicate. The coffee fermentation temperature (glass thermometer

153 standard, - 20 to + 110 ◦C) and soluble solids concentration (°Brix – refractometer, Lorben,
154 Brazil) were monitored once a day during all processing. Then, the coffee was dried in the sun
155 on suspended terraces until obtaining 11-12% moisture during 12 and 11 days for natural and
156 pulped coffee, respectively. Samples (100 g) were taken at 0, 96, and 180 h of fermentation
157 and the end of drying. Finally, the samples were stored at -20 °C for further analysis.
158
159 **2.4 DNA extraction from samples and real-time PCR (qPCR)**
160 The yeast populations were monitored by real-time PCR (qPCR). First, DNA from strains
161 and coffee samples collected at 0, 96, and 180 h of fermentation and at the final drying time
162 were extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the
163 "DNA Purification from Tissues." For the standard curves, the starter cultures were grown
164 separately on a YEPG medium at 28 °C for 24 h. Then, serial dilutions (1:10) from 10^3 to 10^8
165 cells/mL were performed. Cells were counted using the Neubauer chamber. Each point on the
166 curve was measured in triplicate.
167 The qPCR analysis was performed using the Rotor-Gene Q system (Qiagen,
168 Hombrechtikon, ZH, Switzerland). The primers used for *S. cerevisiae* CCMA 0543 were SC-
169 5fw 5´AGGAGTGCGGTTCTTTGTAAAG-3´ and SC-3bw 5´-
170 TGAAATGCGAGATTCCCCA-3´. In addition, the primers Tods L2 5´-
171 CAAAGTCATCCAAGCCAGC-3´ and Tods R2 5´-TTCTCAAACAATCATGTTTGGTAG-
172 3´ were used for *T. delbrueckii* CCMA 0684 amplification (Díaz et al., 2013; Zott et al.,
173 2010). The specificity of each pair of primers was confirmed by searching GenBank using
174 BLAST (http://www.ncbi.nlm.nih.gov/BLAST/).
175
176 **2.5 Sugars, alcohols, and organic acids by high-performance liquid**
177 **chromatography (HPLC)**

Sugars, alcohols, and organic acids were analyzed at 0, 96, and 180 h of fermentation and at the final drying time. The extraction was performed according to da Mota et al. (2022), with modifications. The coffee sample (10 g) was extracted twice with 10 mL of Mili-Q water and vortexed for 5 min at room temperature. Then, the tubes containing 20 mL of solution were centrifuged (12,745 ×*g* at 4 °C for 10 min). Only for acid analysis, the perchloric acid solution was used for adjusting the supernatant pH to 2.11 and then re-centrifuged under the same conditions. The supernatant was filtered through a 0.22 µm cellulose acetate membrane. The filtered extract was stored at -18 °C until analysis. The extracts were analyzed using an HPLC system (Shimadzu) according to da Mota et al. (2022). A Shim-pack SCR-101H (7.9 mm x 30 cm) column was used for acids with a 100 mM perchloric acid solution as the mobile phase and a 0.6 mL/min flow rate. A Shim-pack SCR-101C (7.9 mm x 30 cm) column was used for sugars and alcohols with ultra-pure water as the mobile phase and a flow rate of 0.5 mL/min. The oven temperature was maintained at 50°C for analysis of the acids, detected with a UV detector at 210 nm, and at 80°C for analysis of the sugars and alcohols, detected with a refractive index detector. The quantification of compounds was performed using calibration curves constructed with different concentrations of standard compounds [malic and citric acids were purchased from Merck (Darmstadt, Germany), sucrose, glucose, fructose, alcohol, lactic, acetic, succinic, and tartaric acids were purchased from Sigma-Aldrich (Saint Louis, MO, United States), butyric acid was purchased from Riedel-deHaen (Seelze, Germany)] (Evangelista et al., 2014). All analyses were performed in triplicate.

2.6 Volatile compounds

The volatile compounds were extracted by the headspace solid-phase microextraction technique (SPME-HS). The fiber (divinylbenzene/carboxy/polydimethylsiloxane HS-SPME 50/30 µm; Supelco Co., Bellefonte, PA, USA) was used to extract the volatile compounds.

First, the green and roasted coffee (2 g) were macerated with liquid nitrogen separately. Then, the samples were placed in a 15 mL hermetically sealed vail. The fiber was equilibrated for 15 min at 60 °C and then exposed to coffee for 30 min at the same temperature (Cassimiro et al., 2022). Then, the injections were performed by exposing the fibers for 2 min. Volatile compounds were analyzed by gas chromatography-mass spectrometry (GCMS-QP 2010, Shimadzu) equipped with a Carbo-Wax 20M column (30 m×0.25 mm×0.25 μm). The oven temperature was adjusted to 60 °C for 5 min, followed by an increase to 230 °C at 10 °C/min rate. Then, it was kept at 230 °C for 15 min. The carrier gas (He) was used at 1.9 mL/min (Martinez et al., 2021). Finally, the compounds were identified using the GC-MS software (version 2.6), and the mass spectra for each peak were evaluated in the NIST11 database. In addition, a series of alkanes (C10–C40) was used to calculate each compound's retention index (RI). Then, it was compared with the RI values from the literature. All samples were analyzed in triplicate.

2.7 Temporal dominance of sensations (TDS) with Q-grader tasters

Sensory analysis was performed after approval by the Ethics Committee of the Federal University of Lavras (CAAE: 63924722.9.0000.5148). According to the Specialty Coffee Association guidelines (SCA, 2018), the coffees were prepared. The samples (100 g) were roasted in a roaster (Probatino, Curitiba, BRA) and ground in an electric mill (Pinhalense, São Paulo, BRA). The proportion used was 8.25 g of coffee per 150 mL of water. Three trained coffee experts evaluated the samples with Q-Grader Coffee Certificates. The temperature used was between 50 and 55 °C. The attributes were defined in the first evaluation, and the eight most cited attributes were selected for evaluation in the TDS (Pineau et al., 2009). The samples were coded with three digits and evaluated in three repetitions. First, the tasters left the coffee in their mouths for 3 s, and then each sample was evaluated for 20 s. The panelists

were trained to use the SensoMaker software version 1.8 (Pinheiro et al., 2013) and the procedures for data acquisition.

2.8 Statistics analysis

In order to evaluate the best state for the use of yeast, the qPCR data, carbohydrates, alcohol, organic acids, and volatile compounds were evaluated by analysis of variance (ANOVA), adopting a significance level of 5% probability. ANOVA was used to compare means between the same yeast strain in the fresh and microencapsulated state at each processing time. The software used was Sisvar 5.6 (Ferreira, 2014). Volatile compounds were also evaluated by principal component analysis (PCA) using the Chemoface software (Nunes et al., 2012).

3. Results and discussion

3.1 Physicochemical characterization during SIAF

When performed correctly, coffee fermentation by self-induced anaerobiosis may standardize the beverage's quality and reduce the economic loss for producers (da Mota et al., 2022; Huch & Franz, 2015). In addition, the use of ADY as a starter culture contributes to the genetic stability of the strain at room temperature and, consequently, reduces the producers' costs with transport and storage (Rodriguez-Porrata et al., 2008).

The parameters measured during SIAF can be seen in Table 1. The coffee temperature ranged from 15.5 to 19.9 °C. This temperature increase is expected due to the exothermic reaction during the intense metabolic activity of starter cultures and epiphytic coffee microorganisms (Correa et al., 2014). In natural and pulped coffee, there was no statistical difference in coffee temperature between FS and MS after 180 h. However, regarding *T.*

delbrueckii, there was a difference between fresh and microencapsulated yeast in natural processing. The temperature was higher with fresh yeast, with an increase of 0.5 °C.

In both processes, a decrease or stabilization of the coffee temperature was observed after 180 h of fermentation, indicating the process's end. The concentration of soluble solids in the coffee beans started at 26 °Brix, and after 96 hours, it reached 15.2 °Brix. However, it was impossible to evaluate after 180 h of fermentation due to the lack of mucilage adhered to the beans.

3.2 Monitoring of starter cultures by qPCR

Yeast populations were monitored in natural and pulped coffee by qPCR (Fig. 1). This is a fast and reliable technique used to identify and quantify microorganisms in different foods (Zott et al., 2010). *S. cerevisiae* and *T. delbrueckii* are species found naturally in the coffee fruit. However, the inoculated coffees showed higher populations during the times evaluated in both processing.

In order to evaluate cell viability, fresh and microencapsulated yeast populations were statistically compared during fermentation and at the end of drying (Fig. 1). After 180h of fermentation, in the pulped processing, there was no statistical difference in the yeast populations. However, in natural processing, FS ($2.05x10^7$ cell/g) showed a higher population than MS ($1.09x10^7$ cell/g), while MT ($7.97x10^7$ cell/g) showed a higher population than FT ($1.76x10^7$ cell/g).

At the end of drying, in natural processing, FS ($5.16x10^7$ cell/g) reached a higher population than MS ($3.24x10^7$ cell/g). However, the opposite behavior was observed in pulped coffee. MS ($3.26x10^8$ cell/g) obtained a higher population than FS ($1.83x10^8$ cell/g). Finally, MT ($2.23x10^8$ cell/g) reached a higher population in natural processing than FT ($1.27x10^8$ cell/g), but there was no difference in pulped coffee.

Fresh yeast undergoes stress situations during biomass production in a molasses-based medium that may affect its fermentative capacity and technological performance (Gómez-Pastor et al., 2011). The same behavior can be observed with microencapsulated yeast. During spray drying, cells are mainly subjected to thermal and osmotic stress (Fu & Chen, 2011). Nevertheless, intact and injured cells can reproduce in a growth medium with appropriate nutrients, such as coffee fruit. However, due to the repair process, injured cells tend to have a more extended latency (Fu & Chen, 2011; Martins et al., 2022b), which probably caused these differences in cell viability.

Although differences were observed between fresh and microencapsulated yeast populations, both could adapt and compete with the epiphytic microbiota of coffee fruits. These factors are essential because the metabolic activity of each yeast may be related to differences detected in volatile and non-volatile compounds and, consequently, in the sensory characteristics of the beverage (Bressani et al., 2021).

3.3 Carbohydrates, alcohol, and organic acids

The coffee fruit has essential compounds that allow the growth of microorganisms, such as fructose, glucose, sucrose, citric, malic, and succinic acids (Silva, 2015). In this way, the starter cultures and the microorganisms in the fruit metabolize these compounds during fermentation, producing organic acids, alcohol, and other metabolic compounds that affect the sensory characteristics of the beverage (Cassimiro et al., 2022; Prakash et al., 2022; Shankar et al., 2022).

Sucrose, glucose, fructose, and ethanol concentrations were statistically compared between coffees fermented with fresh and microencapsulated yeast, and some differences were observed (Table 2). After 180 h of fermentation, in the coffees inoculated with *S. cerevisiae*, there was a statistical difference only for the sucrose concentration in the natural

processing. Coffee fermented with FS (0.04 g/kg) showed a higher concentration than coffee inoculated with MS (not detected). Regarding coffees inoculated with *T. delbrueckii*, there was no difference in the concentration of carbohydrates and ethanol between FT and MT in both processes.

At the end of drying, there was no difference between the compound's concentration detected in coffees inoculated with FS and MS in natural processing. However, in pulped coffee, coffee fermented with FS showed a lower concentration of glucose, fructose, and ethanol and a higher concentration of sucrose, with differences of 0.07, 0.43, 2.05, and 0.05 g/kg. Regarding coffee fermented with *T. delbrueckii*, in natural processing, coffee with MT showed a lower concentration of sucrose, glucose, and ethanol, with differences of 0.54, 0.82, and 1.59 g/kg. On the other hand, in the pulped processing, coffee fermented with MT showed a lower concentration of glucose and fructose and a higher concentration of sucrose, with differences of 0.05, 0.08, and 0.13 g/kg.

These differences in the compound's concentrations were also found in other works. For example, Márquez-Montes et al. (2007) compared the metabolic activity of Mexican brewer's yeast dehydrated by spray drying with fresh yeast. There was a difference in reducing sugar concentration during fermentation, but the final residual concentration was similar. Regarding ethanol, fresh yeast (6.51%) had a higher concentration than dried yeast (4.73%).

Citric, malic, succinic, lactic, and acetic acids were detected in both processing after fermentation. However, oxalic and tartaric acids were detected only in pulped coffee (Table 3). These organic acids contribute to the beverage's acidity, an essential attribute of the coffee quality. The desirable acidity contributes to the coffee beverage's freshness. In addition, it may increase the perception of sweetness (Farah & de Lima, 2019).

The yeast behavior in the fresh and microencapsulated states was statistically compared. At the end of fermentation, coffee fermented with MS showed lower citric, malic, and

succinic acid consumption in both processes, with differences of up to 1.43 g/kg. On the other hand, coffee with MS showed higher production of lactic (4.72 g/kg) and acetic acid (0.30 g/kg) in natural coffee. However, coffee fermented with FS showed higher production of lactic and acetic acids in pulped coffee, with an increase of 3.09 and 0.18 g/kg.

Regarding coffee fermented with *T. delbrueckii*, in pulped processing, microencapsulated yeast showed lower consumption of citric and malic acids than fresh yeast, with differences of 0.63 and 0.04 g/kg. Coffee samples fermented with MT produced a higher concentration of acetic acid in both processing and tartaric acid in the pulped coffee, with an increase of up to 0.23 g/kg. However, in natural processing, coffee fermented with FT (0.68 g/kg) showed lower succinic acid consumption than coffee fermented with MT (0.49 g/kg).

During coffee fermentation, yeasts produce organic acids, such as acetic and succinic acids. These acids contribute to beverage acidity and are formed through pyruvate metabolism and tricarboxylic acid cycle activity (De Vuyst & Leroy, 2020). The low oxygen concentration during SIAF also favors the growth of lactic acid bacteria (Cassimiro et al., 2022). These bacteria are naturally present in the coffee fruit (Pereira et al., 2022). Based on this, lactic acid production may be related to the presence of lactic acid bacteria. In addition, some species also produce ethanol, CO_2, and acetic acid (Vandenberghe et al., 2018).

At the end of drying, acetic acid was not detected in any treatment, and lactic acid had the highest concentration (14.23 g/kg). In both processes, coffee fermented with MS reached the highest concentration of succinic acids, increasing up to 2.09 g/kg. In addition, it showed a higher concentration of oxalic, citric, and malic acids in pulped coffee, with differences of 0.02, 1.19, and 0.07 g/kg. Coffee samples fermented with FS showed higher concentrations of tartaric and lactic acids in pulped coffee, with differences of 0.13 and 0.90 g/kg.

Concerning coffee fermented with *T. delbrueckii*, the microencapsulated yeast showed a higher concentration of oxalic acid (0.03 g/kg) and malic acid (0.10 g/kg) in the pulped

coffee. However, FT showed the highest concentration of citric acid in pulped coffee and succinic acid in both processes, with an increase of up to 0.83 g/kg. In addition, it showed higher concentrations of citric (6.23 g/kg), malic (0.29 g/kg), and lactic (10.50 g/kg) acids in natural coffee, with increases of 2.00, 0.16, and 4.16 g/kg.

These organic acids detected at the end of drying may influence the beverage's sensory attributes. For example, citric acid has a fresh acidity, contributing notes of orange and lemon. Malic acid has a mild flavor and contributes to fruity notes like apples (Farah & de Lima, 2019). That said, fruity notes were mainly detected in natural coffee inoculated with *S. cerevisiae*. Finally, lactic acid may contribute to buttery notes (Farah & de Lima, 2019). However, this attribute was not perceived in any treatment in this study.

Thus, the yeast state (fresh and microencapsulated) influenced the concentration of the compounds. This result may be related to the differences in cell viability found during SIAF and at the drying end (Fig. 1). Martins et al. (2022b) also found differences between fresh and microencapsulated yeast in coffee peel and pulp fermentation. However, despite these differences, both microencapsulated yeasts were metabolically active. Like fresh yeasts, they could metabolize the sugars and acids of the coffee fruit during the SIAF, corroborating with the data found in this study.

3.4 Volatile compounds

Different molecules can be generated through yeasts' carbon and nitrogen metabolism during coffee fermentation, influencing the beverage's aroma (Bressani et al., 2021; Elhalis et al., 2021). Eighteen volatile compounds were identified in green coffee and 42 in roasted coffee (Supplementary material – Table S1 and Table S2). These compounds were grouped into 12 chemical classes: acids, alcohols, aldehydes, esters, furanone, furans, ketones, phenols, pyrazines, pyridines, pyrroles, and others.

The PCA was performed to evaluate the differences between fresh and microencapsulated yeasts since similar samples tend to be grouped (Fig. 2). In green coffee, more significant correlations can be observed between coffees inoculated with fresh yeasts in both processes. This result is due to their proximity and the acute angle between them. In natural processing, coffee inoculated with MS correlated with compounds belonging to the esters class. On the other hand, coffee inoculated with MT was not correlated with any other treatment. This behavior was probably observed because the coffee inoculated with MT had a lower concentration of several volatile compounds than the other treatments (Supplementary material – Table S1). Finally, coffee inoculated with MS and MT in pulped coffee correlated with compounds belonging to the ketone and alcohol classes, respectively (Fig. 2).

In roasted coffee, there was a separation between coffees inoculated with fresh and microencapsulated yeasts in both processes. Coffees inoculated with fresh yeasts were found on the left side of the PCA, while coffees inoculated with microencapsulated yeasts were found on the right side (Fig. 2). In natural and pulped coffee, only three and five volatile compounds were on the left side. Thus, all other volatile compounds correlated with coffees inoculated with microencapsulated yeasts. The volatile compounds detected in coffees inoculated with fresh and microencapsulated yeasts were the same. However, the areas of some compounds were statistically different, which probably contributed to this separation (Supplementary material – Table S2). The same results were found by Romano et al. (2014) in wine fermentation.

The most significant volatile compounds detected were 2-Furanmethanol, Furfural, 2-Furancarboxaldehyde, 5-methyl-, 2-Furanmethanol, acetate, and Ethanone, 1-(2-furanyl)- belonging to the furan class. Furthermore, Pyrazine, 2,6-dimethyl - and Butanoic acid, 4-hydroxy- belonging to pyrazines and acids were also detected. These volatile compounds

401 contribute sweet, caramel, chocolate, nuts, and fruity flavors (Flament, 2001). These attributes
402 were detected as dominant in the SIAF coffees by TDS.

403 In order to facilitate the identification of fermentation efficiency using fresh and
404 microencapsulated yeasts, the volatile compounds detected in roasted coffee were analyzed by
405 chemical classes (Fig. 3). The relation between the total area was statistically compared.
406 Concerning natural coffee, the coffee inoculated with FS had the highest pyrazine
407 concentration (57%). Pyrazines are essential to the coffee flavor and may contribute sensory
408 notes of roasted nuts, walnuts, cocoa, and caramel (Flament, 2001; Sunarharum et al., 2014).
409 However, only the caramel attribute was detected in TDS as dominant in coffee inoculated
410 with FS. Conversely, the coffee inoculated with MS had the highest pyrroles (53%) and
411 pyridines (60%) concentration. Pyrroles may contribute to fruity, sweet, and nutty notes
412 (Flament, 2001). These attributes were also detected as dominant in coffee inoculated with
413 MS.

414 However, in pulped coffee, the coffee inoculated with MS obtained the highest
415 concentration of alcohols (67%), ketones (60%), pyrazines (54%), pyrroles (58%), phenols
416 (54%), esters (57%) and others (63%). Concerning *T. delbrueckii*, the coffee inoculated with
417 the microencapsulated yeast obtained the highest concentration in 11 of the 12 chemical
418 classes in both processes. These differences ranged from 0.9 to 39.7%.

419 According to the literature, wines obtained with dried yeast also produced higher
420 concentrations of volatile compounds than those obtained from fresh yeast. In addition, wines
421 obtained with dried yeast showed a higher number of volatile compounds (Romano et al.,
422 2014). In another study, microencapsulated cells increased the fermentation efficiency of
423 *Hanseniaspora osmophila* compared to free cells (Alberico et al., 2021).

424 In this context, studies evaluated different genes involved in the yeast response to the
425 stress suffered during the drying process (Nakamura et al., 2008; Singh et al., 2005). These

426 authors concluded that desiccation tolerance is a complex process with contributions from
427 hundreds of genes. In addition, some of these genes may be related to metabolic pathways
428 involved in producing volatile compounds (Romano et al., 2014).

429 However, based on the results found in this work, both microencapsulated yeasts
430 maintained viability and vitality during coffee fermentation. Thus, these results are promising
431 because these are the main parameters that influence active dry yeasts' physiological stability
432 and fitness (Rodriguez-Porrata et al., 2008).

433

434 **3.5 Sensory analysis**

435 The temporal dominance of sensations is used to record multiple sensory attributes
436 simultaneously over time. Thus, the panelist identifies which attribute is dominant at each test
437 moment. This technique is an excellent alternative for evaluating complex products such as
438 coffee (Pineau et al., 2009). In this way, SIAF coffees' temporal dominance of sensations in
439 natural processing is described in Figure 4.

440 The SIAF control showed dominant notes of nuts (from 4.6 to 7.1 s) and caramel (from
441 6.5 to 7.2, from 8.5 to 11.5, and from 14.5 to 20.0 s) (Fig. 4A). The coffee inoculated with FS
442 showed fruity (from 4.8 to 5.2 s), and caramel (from 8.1 to 20 s) notes (Fig. 4B). Conversely,
443 coffee inoculated with MS showed fruity (from 4.5 to 5.5 s), caramel (from 7.5 to 9 s) and
444 nuts (from 9.0 to 10.9 s) notes (Fig. 4D). The coffee inoculated with FT showed dominant
445 notes of nuts (from 7.5 to 11.5 s) and caramel (from 8.9 to 20.0 s) (Fig. 4C), while coffee
446 inoculated with MT showed only notes of caramel (from 10.1 to 20.0 s) (Fig. 4E).

447 Thus, in natural coffee, the fruity attribute was dominant only in coffee inoculated with *S.*
448 *cerevisiae*. The volatile compound 2-Furanmethanol, acetate was detected in more significant
449 concentrations in these coffees and may have contributed to this sensory perception

(Supplementary material – Table S2). This compound contributes fruity, banana, and sweet notes (Flament, 2001).

Regarding pulped coffee, the TDS is described in Figure 5. The SIAF control showed dominant notes of fruity (from 3.8 to 4.7 s) and caramel (from 9.5 to 12.5 s) (Fig. 5A). Both coffees inoculated with *S. cerevisiae* showed only dominant notes of caramel and honey. Conversely, coffee inoculated with FT showed dominant notes of nuts (from 4.5 to 5.9 s), caramel (5.9 to 8.0 s), chocolate (12.5 to 15.5 s), and floral (17.5 to 18.5 s) (Fig. 5C). The coffee inoculated with MT showed notes of caramel (from 6.5 to 7.5 and 8.3 to 9.6 s), honey (from 9.5 to 11.3 s) and nuts (from 12.1 to 20.0 s) (Fig. 5E).

Thus, coffees inoculated with *T. delbrueckii* showed a greater diversity of dominant attributes in pulped coffee. The nuts attribute was detected as dominant only in this treatment and may be related to the volatile compounds Ethanone, 1-(2-furanyl), Pyrazine, 3-ethyl-2,5-dimethyl-, Ethanone, 1-(1H-pyrrol-2-yl)- (Supplementary material – Table S2). The caramel attribute was detected as dominant in all treatments and may be characteristic of the variety used. The volatile compounds 2-Furanmethanol, Furfural, 2-Furancarboxaldehyde, 5-methyl-, Ethanone, 1-(2-furanyl)- and Butanoic acid, 4-hydroxy- probably contributed to this sensory perception (Supplementary material – Table S2).

Therefore, there were some differences in the dominant attributes between coffees inoculated with fresh and microencapsulated yeast. This result was expected since there were significant differences in the concentration of organic (Table 3) and volatile compounds (Supplementary material – Table S2) in the SIAF coffee beans. These differences probably influenced the sensory perception of the panelists.

Coffees inoculated with FS and MS in pulped coffee were the only ones that showed no differences between the dominant attributes. On the other hand, coffees inoculated with FT and MT in pulped coffee showed more significant differences between the attributes.

However, despite the differences, both microencapsulated yeasts proved efficient and can be used as a starter culture in the coffee fermentation process.

4. Conclusion

Some differences were found between fresh and microencapsulated yeast behavior during and after SIAF. However, despite the differences, both microencapsulated yeasts could adapt and compete with the microorganisms naturally present in the coffee fruit. In addition, the microencapsulated yeasts were metabolically active, contributing important sensory attributes to the coffees. Therefore, both microencapsulated yeasts may be considered with commercial potential, particularly for coffee producers interested in using starter cultures during SIAF. Finally, considering the parameters analyzed, the most suitable yeast for natural and pulped processing would be MS and MT, respectively.

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Declarations

Conflict of Interest

The authors declare no competing interests.

References

Alberico, G., Capece, A., Mauriello, G., Pietrafesa, R., Siesto, G., Garde-Cerdán, T., Maresca, D., Romano, R., & Romano, P. (2021). Influence of Microencapsulation on Fermentative Behavior of *Hanseniaspora osmophila* in Wine Mixed Starter Fermentation. *Fermentation*, *7*(3), 112. https://doi.org/10.3390/fermentation7030112

Andrade, D. P., Ramos, C. L., Botrel, D. A., Borges, S. V., Schwan, R. F., & Ribeiro Dias, D. (2019). Stability of microencapsulated lactic acid bacteria under acidic and bile juice conditions. *International Journal of Food Science & Technology*, *54*(7), 2355–2362. https://doi.org/10.1111/ijfs.14114

Aponte, M., Troianiello, G. D., Di Capua, M., Romano, R., & Blaiotta, G. (2016). Impact of different spray-drying conditions on the viability of wine *Saccharomyces cerevisiae* strains. *World Journal of Microbiology and Biotechnology*, *32*(1), 13. https://doi.org/10.1007/s11274-015-1956-5

Bressani, A. P. P., Martinez, S. J., Batista, N. N., Simão, J. B. P., Dias, D. R., & Schwan, R. F. (2021). Co-inoculation of yeasts starters: A strategy to improve quality of low altitude Arabica coffee. *Food Chemistry*, *361*, 130133. https://doi.org/10.1016/j.foodchem.2021.130133

Bressani, A. P. P., Martinez, S. J., Sarmento, A. B. I., Borém, F. M., & Schwan, R. F. (2021). Influence of yeast inoculation on the quality of fermented coffee (*Coffea arabica* var. Mundo Novo) processed by natural and pulped natural processes. *International Journal of Food Microbiology*, *343*, 109107. https://doi.org/10.1016/j.ijfoodmicro.2021.109107

Cassimiro, D. M. de J., Batista, N. N., Fonseca, H. C., Naves, J. A. O., Dias, D. R., &

Schwan, R. F. (2022). Coinoculation of lactic acid bacteria and yeasts increases the quality of wet fermented Arabica coffee. *International Journal of Food Microbiology*, *369*, 109627. https://doi.org/10.1016/j.ijfoodmicro.2022.109627

Correa, E. C., Jiménez-Ariza, T., Díaz-Barcos, V., Barreiro, P., Diezma, B., Oteros, R., Echeverri, C., Arranz, F. J., & Ruiz-Altisent, M. (2014). Advanced Characterisation of a Coffee Fermenting Tank by Multi-distributed Wireless Sensors: Spatial Interpolation and Phase Space Graphs. *Food and Bioprocess Technology*, *7*(11), 3166–3174. https://doi.org/10.1007/s11947-014-1328-4

da Mota, M. C. B., Batista, N. N., Dias, D. R., & Schwan, R. F. (2022). Impact of microbial self-induced anaerobiosis fermentation (SIAF) on coffee quality. *Food Bioscience*, *47*, 101640. https://doi.org/10.1016/j.fbio.2022.101640

Da Mota, M. C. B., Batista, N. N., Rabelo, M. H. S., Ribeiro, D. E., Borém, F. M., & Schwan, R. F. (2020). Influence of fermentation conditions on the sensorial quality of coffee inoculated with yeast. *Food Research International*, *136*, 109482. https://doi.org/10.1016/j.foodres.2020.109482

da Silva, B. L., Pereira, P. V., Bertoli, L. D., Silveira, D. L., Batista, N. N., Pinheiro, P. F., de Souza Carneiro, J., Schwan, R. F., de Assis Silva, S., Coelho, J. M., & Bernardes, P. C. (2021). Fermentation of *Coffea canephora* inoculated with yeasts: Microbiological, chemical, and sensory characteristics. *Food Microbiology*, *98*, 103786. https://doi.org/10.1016/j.fm.2021.103786

da Silva, M. C. S., da Luz, J. M. R., Veloso, T. G. R., Gomes, W. dos S., Oliveira, E. C. da S., Anastácio, L. M., Cunha Neto, A., Moreli, A. P., Guarçoni, R. C., Kasuya, M. C. M., & Pereira, L. L. (2022). Processing techniques and microbial fermentation on microbial profile and chemical and sensory quality of the coffee beverage. *European Food Research and Technology*, *248*(6), 1499–1512. https://doi.org/10.1007/s00217-022-

550 03980-6
551 De Vuyst, L., & Leroy, F. (2020). Functional role of yeasts, lactic acid bacteria and acetic
552 acid bacteria in cocoa fermentation processes. *FEMS Microbiology Reviews*, *44*(4), 432–
553 453. https://doi.org/10.1093/femsre/fuaa014
554 Desai, K. G. H., & Park, H. J. (2005). Recent Developments in Microencapsulation of Food
555 Ingredients. *Drying Technology*, *23*(7), 1361–1394. https://doi.org/10.1081/DRT-
556 200063478
557 Díaz, C., Molina, A. M., Nähring, J., & Fischer, R. (2013). Characterization and Dynamic
558 Behavior of Wild Yeast during Spontaneous Wine Fermentation in Steel Tanks and
559 Amphorae. *BioMed Research International*, *2013*, 1–13.
560 https://doi.org/10.1155/2013/540465
561 Elhalis, H., Cox, J., Frank, D., & Zhao, J. (2020). The role of wet fermentation in enhancing
562 coffee flavor, aroma and sensory quality. *European Food Research and Technology*.
563 https://doi.org/10.1007/s00217-020-03641-6
564 Elhalis, H., Cox, J., Frank, D., & Zhao, J. (2021). Microbiological and Chemical
565 Characteristics of Wet Coffee Fermentation Inoculated With *Hansinaspora uvarum* and
566 *Pichia kudriavzevii* and Their Impact on Coffee Sensory Quality. *Frontiers in*
567 *Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.713969
568 Evangelista, S. R., Silva, C. F., Miguel, M. G. P. da C., Cordeiro, C. de S., Pinheiro, A. C. M.,
569 Duarte, W. F., & Schwan, R. F. (2014). Improvement of coffee beverage quality by
570 using selected yeasts strains during the fermentation in dry process. *Food Research*
571 *International*, *61*, 183–195. https://doi.org/10.1016/j.foodres.2013.11.033
572 Farah, A., & de Lima, Â. G. (2019). Organic Acids. In *Coffee: production, quality and*
573 *chemistry* (pp. 517–536). The Royal Society of Chemistry.
574 Ferreira, D. F. (2014). Sisvar: a Guide for its Bootstrap procedures in multiple comparisons.

575 *Ciência e Agrotecnologia*, *38*(2), 109–112. https://doi.org/10.1590/S1413-
576 70542014000200001
577 Flament, I. (2001). *Coffee flavor chemistry* (1st ed.). John Wiley & Sons.
578 Fu, N., & Chen, X. D. (2011). Towards a maximal cell survival in convective thermal drying
579 processes. *Food Research International*, *44*(5), 1127–1149.
580 https://doi.org/10.1016/j.foodres.2011.03.053
581 Gómez-Pastor, R., Pérez-Torrado, R., Garre, E., & Matallana, E. (2011). Recent advances in
582 yeast biomass production. In *Biomass—Detection, Production and Usage*. InTech.
583 Huch, M., & Franz, C. M. A. P. (2015). Coffee: fermentation and microbiota. In *Advances in*
584 *Fermented Foods and Beverages* (pp. 501–513). Elsevier. https://doi.org/10.1016/B978-
585 1-78242-015-6.00021-9
586 Liu, H., Cui, S. W., Chen, M., Li, Y., Liang, R., Xu, F., & Zhong, F. (2019). Protective
587 approaches and mechanisms of microencapsulation to the survival of probiotic bacteria
588 during processing, storage and gastrointestinal digestion: A review. *Critical Reviews in*
589 *Food Science and Nutrition*, *59*(17), 2863–2878.
590 https://doi.org/10.1080/10408398.2017.1377684
591 López-Cruz, R., Ragazzo-Sánchez, J. A., & Calderón-Santoyo, M. (2020).
592 Microencapsulation of *Meyerozyma guilliermondii* by spray drying using sodium
593 alginate and soy protein isolate as wall materials: a biocontrol formulation for
594 anthracnose disease of mango. *Biocontrol Science and Technology*, *30*(10), 1116–1132.
595 https://doi.org/10.1080/09583157.2020.1793910
596 Márquez-Montes, R., Robles-Olvera, V., Luna-Solano, G., & Salgado-Cervantes, M. A.
597 (2007). Metabolic Activity of the Dehydrated Yeast by Spray Drying. *Drying*
598 *Technology*, *25*(7–8), 1281–1285. https://doi.org/10.1080/07373930701438824
599 Martinez, S. J., Rabelo, M. H. S., Bressani, A. P. P., Da Mota, M. C. B., Borém, F. M., &

Schwan, R. F. (2021). Novel stainless steel tanks enhances coffee fermentation quality. *Food Research International*, *139*, 109921. https://doi.org/10.1016/j.foodres.2020.109921

Martinez, S. J., Simão, J. B. P., Pylro, V. S., & Schwan, R. F. (2021). The Altitude of Coffee Cultivation Causes Shifts in the Microbial Community Assembly and Biochemical Compounds in Natural Induced Anaerobic Fermentations. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.671395

Martins, P. M. M., Batista, N. N., Santos, L. D., Dias, D. R., & Schwan, R. F. (2022a). Microencapsulation by spray drying of coffee epiphytic yeasts *Saccharomyces cerevisiae* CCMA 0543 and *Torulaspora delbrueckii* CCMA 0684. *Brazilian Journal of Microbiology*. https://doi.org/10.1007/s42770-022-00776-4

Martins, P. M. M., Batista, N. N., Santos, L. D., Dias, D. R., & Schwan, R. F. (2022b). Microencapsulation of epiphytic coffee yeasts by spray drying using different wall materials: Implementation in coffee medium. *International Journal of Food Microbiology*, *379*, 109839. https://doi.org/10.1016/j.ijfoodmicro.2022.109839

Nakamura, T., Mizukami-Murata, S., Ando, A., Murata, Y., Takagi, H., & Shima, J. (2008). Changes in Gene Expression of Commercial Baker's Yeast during an Air-Drying Process that Simulates Dried Yeast Production. *Journal of Bioscience and Bioengineering*, *106*(4), 405–408. https://doi.org/10.1263/jbb.106.405

Nunes, C. A., Freitas, M. P., Pinheiro, A. C. M., & Bastos, S. C. (2012). Chemoface: a novel free user-friendly interface for chemometrics. *Journal of the Brazilian Chemical Society*, *23*(11), 2003–2010. https://doi.org/10.1590/S0103-50532012005000073

Pereira, T. S., Batista, N. N., Santos Pimenta, L. P., Martinez, S. J., Ribeiro, L. S., Oliveira Naves, J. A., & Schwan, R. F. (2022). Self-induced anaerobiosis coffee fermentation: Impact on microbial communities, chemical composition and sensory quality of coffee.

Food Microbiology, *103*, 103962. https://doi.org/10.1016/j.fm.2021.103962

Pineau, N., Schlich, P., Cordelle, S., Mathonnière, C., Issanchou, S., Imbert, A., Rogeaux, M., Etiévant, P., & Köster, E. (2009). Temporal Dominance of Sensations: Construction of the TDS curves and comparison with time–intensity. *Food Quality and Preference*, *20*(6), 450–455. https://doi.org/10.1016/j.foodqual.2009.04.005

Pinheiro, A. C. M., Nunes, C. A., & Vietoris, V. (2013). SensoMaker: a tool for sensorial characterization of food products. *Ciência e Agrotecnologia*, *37*(3), 199–201. https://doi.org/10.1590/S1413-70542013000300001

Prakash, I., R, S. S., P, S. H., Kumar, P., Om, H., Basavaraj, K., & Murthy, P. S. (2022). Metabolomics and volatile fingerprint of yeast fermented robusta coffee: A value added coffee. *LWT*, *154*, 112717. https://doi.org/10.1016/j.lwt.2021.112717

Rapoport, A., Golovina, E. A., Gervais, P., Dupont, S., & Beney, L. (2019). Anhydrobiosis: Inside yeast cells. *Biotechnology Advances*, *37*(1), 51–67. https://doi.org/10.1016/j.biotechadv.2018.11.003

Rodriguez-Porrata, B., Novo, M., Guillamón, J., Rozès, N., Mas, A., & Cordero Otero, R. (2008). Vitality enhancement of the rehydrated active dry wine yeast. *International Journal of Food Microbiology*, *126*(1–2), 116–122. https://doi.org/10.1016/j.ijfoodmicro.2008.05.016

Rokka, S., & Rantamäki, P. (2010). Protecting probiotic bacteria by microencapsulation: challenges for industrial applications. *European Food Research and Technology*, *231*(1), 1–12. https://doi.org/10.1007/s00217-010-1246-2

Romano, P., Pietrafesa, R., Romaniello, R., Zambuto, M., Calabretti, A., & Capece, A. (2014). Impact of yeast starter formulations on the production of volatile compounds during wine fermentation. *Yeast*, n/a-n/a. https://doi.org/10.1002/yea.3034

SCA, S. C. A. of A. (2018). *Cupping Protocols*. https://sca.coffee/research/protocols-best-

650 practices. Accessed July 15, 2022
651 Shankar, R. S., Sneha, H. P., Prakash, I., Khan, M., H N, P. K., Om, H., Basavaraj, K., &
652 Murthy, P. S. (2022). Microbial ecology and functional coffee fermentation dynamics
653 with *Pichia kudriavzevii*. *Food Microbiology*, *105*, 104012.
654 https://doi.org/10.1016/j.fm.2022.104012
655 Silva, C. F. (2015). Microbial activity during coffee fermentation. In *Cocoa and Coffee*
656 *Fermentation* (pp. 368–423). CRC Taylor & Francis, Boca Raton, FL.
657 Silva, C. F., Schwan, R. F., Sousa Dias, Ë., & Wheals, A. E. (2000). Microbial diversity
658 during maturation and natural processing of coffee cherries of *Coffea arabica* in Brazil.
659 *International Journal of Food Microbiology*, *60*(2–3), 251–260.
660 https://doi.org/10.1016/S0168-1605(00)00315-9
661 Singh, J., Kumar, D., Ramakrishnan, N., Singhal, V., Jervis, J., Garst, J. F., Slaughter, S. M.,
662 DeSantis, A. M., Potts, M., & Helm, R. F. (2005). Transcriptional Response of
663 *Saccharomyces cerevisiae* to Desiccation and Rehydration. *Applied and Environmental*
664 *Microbiology*, *71*(12), 8752–8763. https://doi.org/10.1128/AEM.71.12.8752-8763.2005
665 Sunarharum, W. B., Williams, D. J., & Smyth, H. E. (2014). Complexity of coffee flavor: A
666 compositional and sensory perspective. *Food Research International*, *62*, 315–325.
667 https://doi.org/10.1016/j.foodres.2014.02.030
668 Suryabhan, P., Lohith, K., & Anu-Appaiah, K. A. (2019). Sucrose and sorbitol
669 supplementation on maltodextrin encapsulation enhance the potential probiotic yeast
670 survival by spray drying. *LWT*, *107*, 243–248. https://doi.org/10.1016/j.lwt.2019.03.002
671 Tan, D. T., Poh, P. E., & Chin, S. K. (2018). Microorganism preservation by convective air-
672 drying—A review. *Drying Technology*, *36*(7), 764–779.
673 https://doi.org/10.1080/07373937.2017.1354876
674 Vanden Braber, N. L., Díaz Vergara, L. I., Rossi, Y. E., Aminahuel, C. A., Mauri, A. N.,

675 Cavaglieri, L. R., & Montenegro, M. A. (2020). Effect of microencapsulation in whey
676 protein and water-soluble chitosan derivative on the viability of the probiotic
677 *Kluyveromyces marxianus* VM004 during storage and in simulated gastrointestinal
678 conditions. *LWT*, *118*, 108844. https://doi.org/10.1016/j.lwt.2019.108844
679 Vandenberghe, L. P. S., Karp, S. G., de Oliveira, P. Z., de Carvalho, J. C., Rodrigues, C., &
680 Soccol, C. R. (2018). Solid-State Fermentation for the Production of Organic Acids. In
681 *Current Developments in Biotechnology and Bioengineering* (pp. 415–434). Elsevier.
682 https://doi.org/10.1016/B978-0-444-63990-5.00018-9
683 Vilela, D. M., Pereira, G. V. de M., Silva, C. F., Batista, L. R., & Schwan, R. F. (2010).
684 Molecular ecology and polyphasic characterization of the microbiota associated with
685 semi-dry processed coffee (*Coffea arabica* L.). *Food Microbiology*, *27*(8), 1128–1135.
686 https://doi.org/10.1016/j.fm.2010.07.024
687 Zott, K., Claisse, O., Lucas, P., Coulon, J., Lonvaud-Funel, A., & Masneuf-Pomarede, I.
688 (2010). Characterization of the yeast ecosystem in grape must and wine using real-time
689 PCR. *Food Microbiology*, *27*(5), 559–567. https://doi.org/10.1016/j.fm.2010.01.006
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691 **Figures caption**

692 **Fig. 1.** Dynamic behavior of *Saccharomyces cerevisiae* CCMA 0543 (a,b) and *Torulaspora*
693 *delbrueckii* CCMA 0684 (c,d) in natural (a,c) and pulped processing (b,d) measured by qPCR
694 at 0, 96, and 180 h of fermentation and at the end of drying. Data are presented as mean. The
695 statistic was used to compare the averages between fresh and microencapsulated yeast in each
696 fermentation time. Therefore, different lowercase letters are significant at $p \leq 0.05$ by analysis
697 of variance.

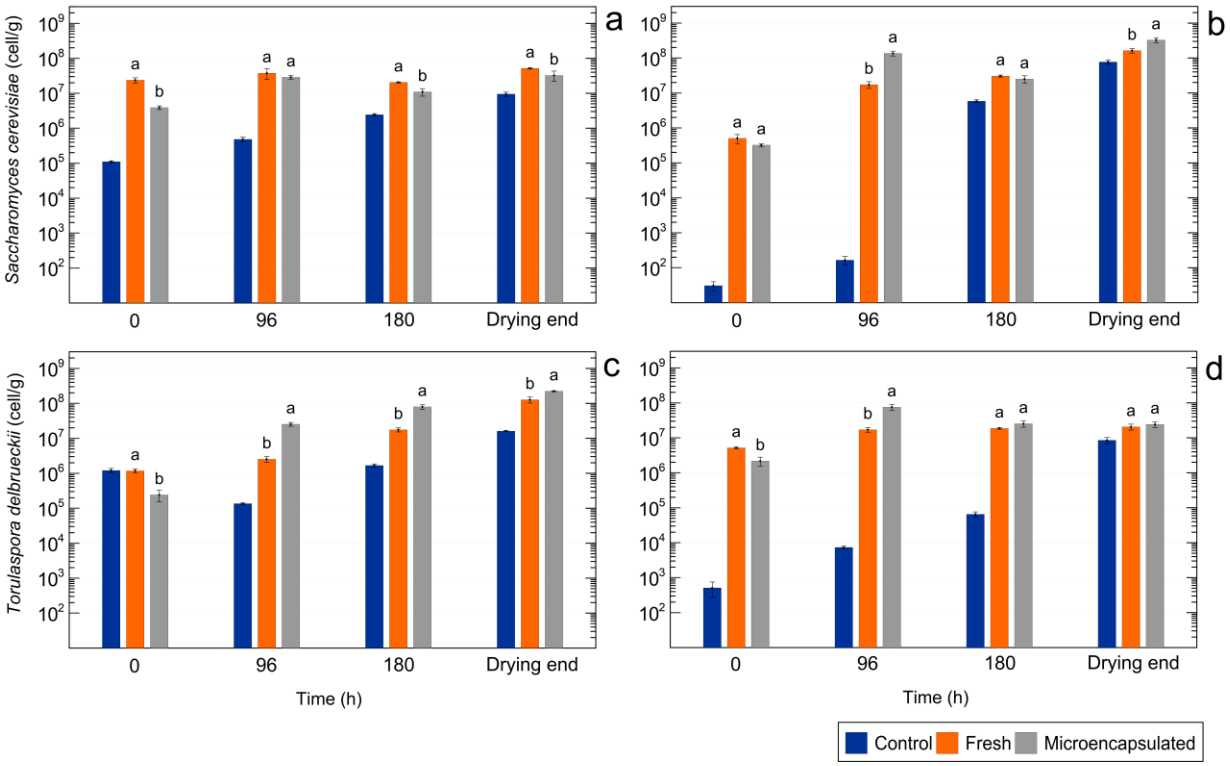

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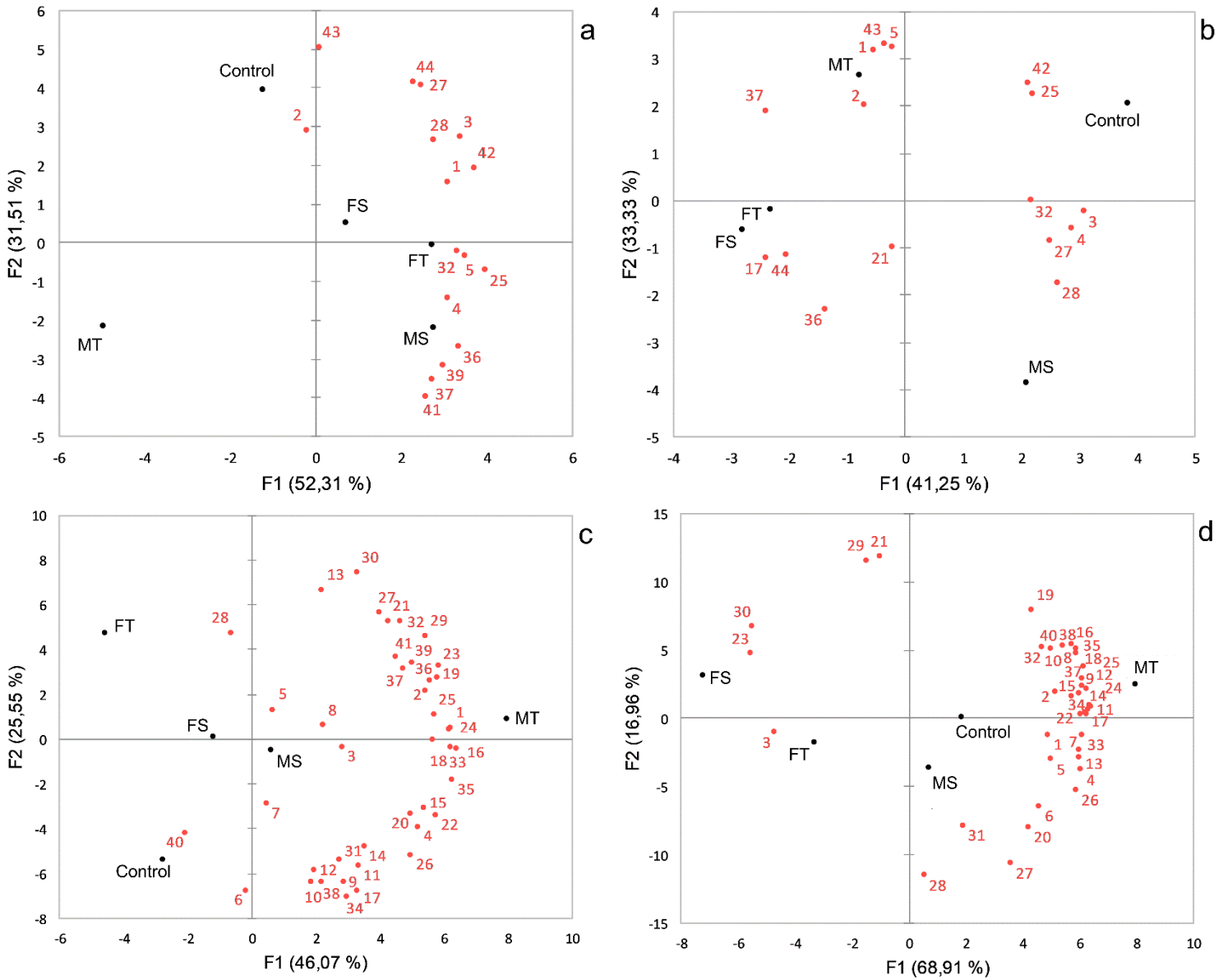
Fig. 2. Principal component analysis of the main compounds detected in green (a,b) and roasted coffee (c,d) by the natural (a,c) and pulped (b,d) processing. Control: without inoculation; FS: fresh *S. cerevisiae* CCMA0543; FT: fresh *T. delbrueckii* CCMA0684; MS: microencapsulated *S. cerevisiae* CCMA0543; MT: microencapsulated *T. delbrueckii* CCMA0684. (1) Benzyl alcohol; (2) Phenylethyl Alcohol; (3) Hexadecanal-; (4) 2-Pentadecanone, 6,10,14-trimethyl-; (5) Caffeine; (6) Pyrazine, 3-ethyl-2,5-dimethyl-; (7) Ethanone, 1-(2-furanyl)-; (8) 2-Furanmethanol, acetate; (9) 2-Furancarboxaldehyde, 5-methyl-; (10) Butanoic acid, 4-hydroxy-; (11) 2-Furanmethanol; (12) 1H-Pyrrole, 1-(2-furanylmethyl)-; (13) 3-Methyl-2-pyrazinylmethanol; (14) 2-Thiophenemethanol; (15) Ethanone, 1-(1H-pyrrol-2-yl)-; (16) 1H-Pyrrole-2-carboxaldehyde; (17) 2-Pyrrolidinone; (18) 3-Pyridinol, 2-methyl-; (19) 3-Pyridinol; (20) 5-Hydroxymethylfurfural; (21) n-Hexadecanoic acid; (22) Acetamide; (23) Octanoic acid; (24) 4-Hydroxy-2-methylacetophenone; (25) Hexadecanoic acid, methyl ester; (26) Indole; (27) 2-Pentadecanone; (28) 2-Heptadecanone; (29) Maltol; (30) n-Decanoic acid; (31) Furfural; (32) Phenol; (33) 2(5H)-Furanone; (34) 2-Pyrrolidinone, 1-butyl-; (35) 1H-Pyrrole-2-carboxaldehyde, 1-methyl-; (36) Tetradecanoic acid, ethyl ester; (37) 9,12-Octadecadienoic acid, ethyl ester; (38) 4-Methyl-5H-furan-2-one; (39) Ethyl Oleate; (40) Pyrazine, 2,6-dimethyl-; (41) Octadecanoic acid, ethyl ester; (42) Hexanoic acid, 2-methylbutyl ester; (43) Eicosane; (44) Pentadecanal-.


a
F2 (31,51 %)
F1 (52,31 %)
Control
FS
FT
MT
MS
b
F2 (33,33 %)
F1 (41,25 %)
MT
Control
FT
FS
MS
c
F2 (25,55 %)
F1 (46,07 %)
FT
FS
MS
MT
Control
d
F2 (16,96 %)
F1 (68,91 %)
FS
MT
Control
FT
MS

712

Fig. 3. Volatile compounds classes identified in coffees roasted and fermented with *S. cerevisiae* CCMA 0543 (a,b) and *T. delbrueckii* CCMA 0684 (c,d) by natural (a,c) and pulped (b,d) processing. Data are presented as mean. The statistic was used to compare the averages between fresh and microencapsulated yeast in each chemical class. Therefore, different lowercase letters are significant at $p \leq 0.05$ by analysis of variance.

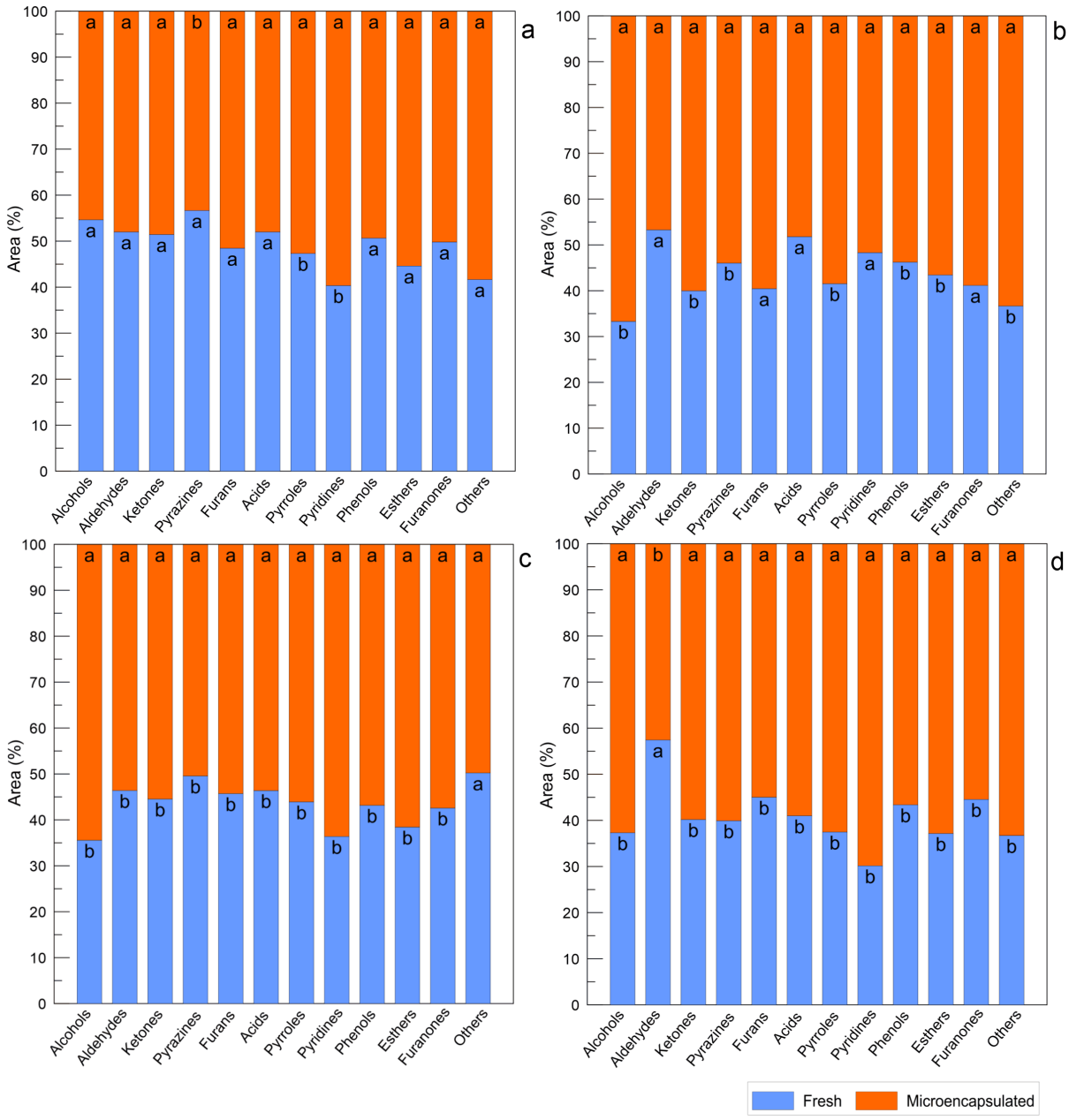


Fig. 4. Temporal dominance of sensations (TDS) curves in natural coffee. a) Control (without inoculation), b) inoculated with fresh *S. cerevisiae* CCMA0543, c) inoculated with fresh *T. delbrueckii* CCMA0684, d) inoculated with microencapsulated *S. cerevisiae* CCMA0543, e) inoculated with microencapsulated *T. delbrueckii* CCMA0684.

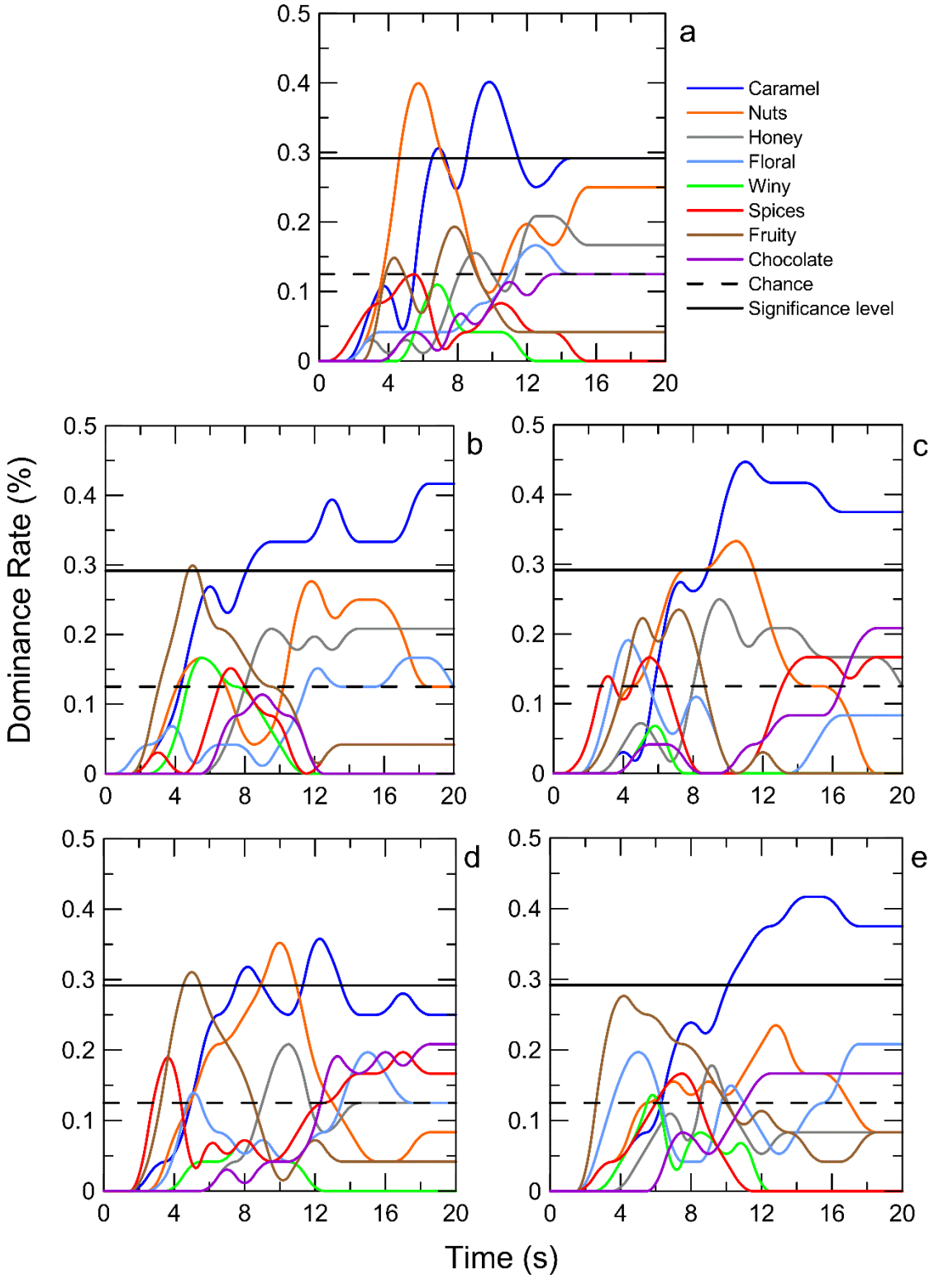
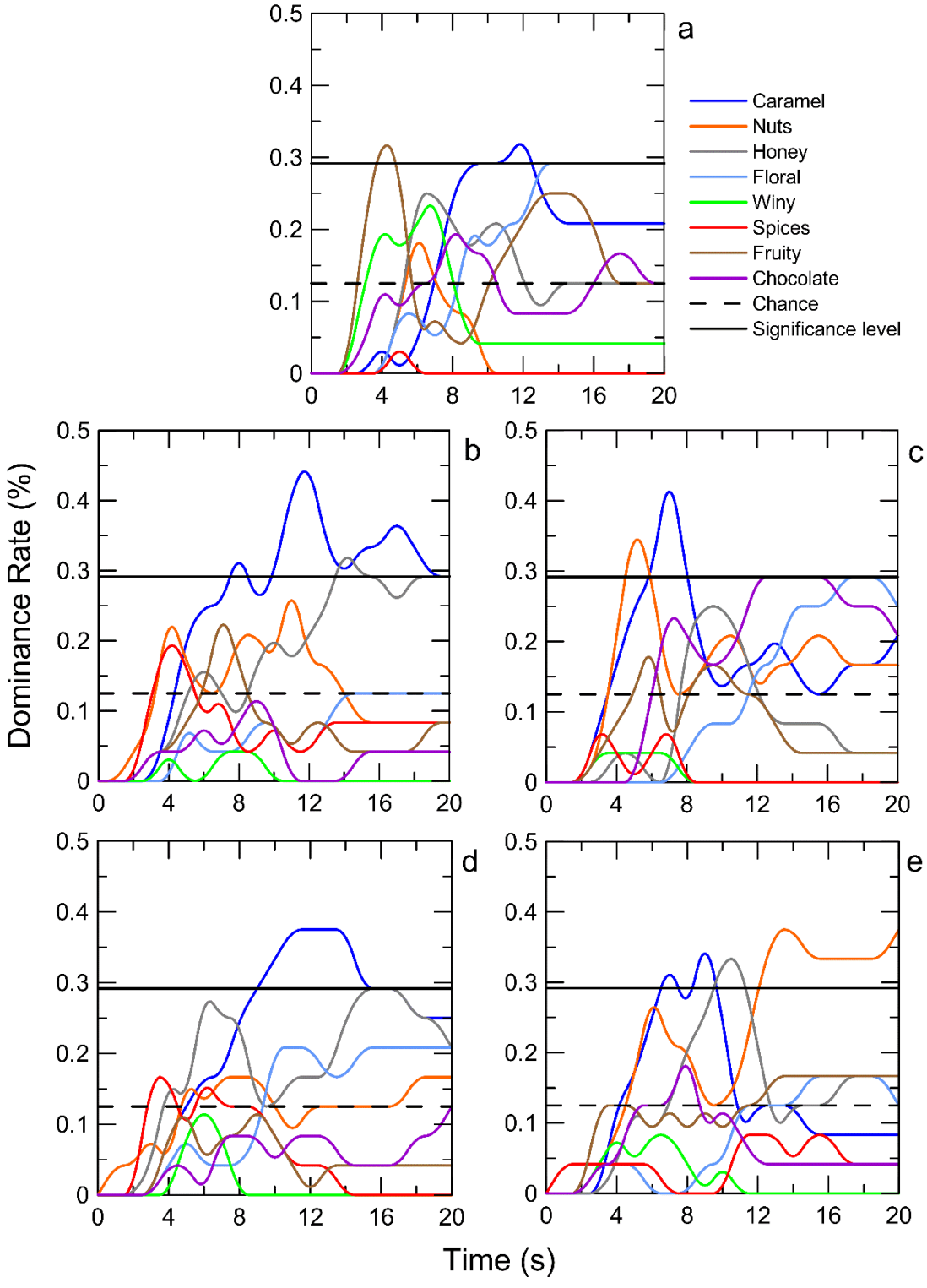


Fig. 5. Temporal dominance of sensations (TDS) curves in pulped coffee. a) Control (without inoculation), b) inoculated with fresh *S. cerevisiae* CCMA0543, c) inoculated with fresh *T. delbrueckii* CCMA0684, d) inoculated with microencapsulated *S. cerevisiae* CCMA0543, e) inoculated with microencapsulated *T. delbrueckii* CCMA0684.



730 **Tables**

731 **Table 1.** Coffee fermentation temperature and °Brix measurements at sampling times: 0, 96, and 180 h.

Fermentation time (h)	Treatments	Temperature (°C)		°Brix	
		Nat[a]	PC[b]	Nat[a]	PC[b]
0	Control	16.1±0.2	15.7±0.1	26.0±0.6	26.0±0.6
	Fresh *S. cerevisiae* CCMA 0543	16.1±0.2[a]	16.0±0.2[a]	26.0±0.6[a]	26.0±0.6[a]
	Microencapsulated *S. cerevisiae* CCMA 0543	16.5±0.4[a]	15.5±0.4[a]	26.0±0.6[a]	26.0±0.6[a]
	Fresh *T. delbrueckii* CCMA 0684	16.0±0.4[a]	16.1±0.1[a]	26.0±0.6[a]	26.0±0.6[a]
	Microencapsulated *T. delbrueckii* CCMA 0684	15.9±0.2[a]	15.9±0.1[a]	26.0±0.6[a]	26.0±0.6[a]
96	Control	18.7±0.2	18.5±0.3	20.0±0.7	19.0±0.7
	Fresh *S. cerevisiae* CCMA 0543	19.2±0.2[b]	17.9±0.3[b]	18.3±0.4[b]	15.2±0.4[b]
	Microencapsulated *S. cerevisiae* CCMA 0543	19.7±0.2[a]	18.8±0.1[a]	19.5±0.7[a]	16.1±0.2[a]
	Fresh *T. delbrueckii* CCMA 0684	19.0±0.4[b]	18.2±0.7[b]	19.5±0.7[a]	17.1±0.1[a]
	Microencapsulated *T. delbrueckii* CCMA 0684	19.9±0.1[a]	19.1±0.5[a]	18.2±0.2[b]	17.2±0.4[a]
180	Control	17.8±0.5	18.5±0.4	nd	nd
	Fresh *S. cerevisiae* CCMA 0543	17.9±0.1[a]	17.9±0.2[a]	nd	nd
	Microencapsulated *S. cerevisiae* CCMA 0543	17.6±0.4[a]	18.1±0.7[a]	nd	nd
	Fresh *T. delbrueckii* CCMA 0684	17.8±0.1[a]	17.6±0.2[a]	nd	nd
	Microencapsulated *T. delbrueckii* CCMA 0684	17.3±0.1[b]	18.0±0.4[a]	nd	nd

732 Data are presented as mean. The statistic was used to compare the averages between the same yeast strain in the fresh and
733 microencapsulated state at each fermentation time. a–b for each column, mean values with different letters are significant
734 at $p \leq 0.05$ by analysis of variance.
735 nd = not detected.
736 [a]Natural coffee
737 [b]Pulped coffee

738 **Table 2.** Carbohydrates and alcohol detected during self-induced anaerobic fermentation (SIAF) and at the end of drying
739 by high-performance liquid chromatography (HPLC).

Fermentation time (h)	Treatments	Carbohydrates (g/kg)						Alcohol (g/kg)	
		Sucrose		Glucose		Fructose		Ethanol	
		Nat[a]	PC[b]	Nat[a]	PC[b]	Nat[a]	PC[b]	Nat[a]	PC[b]
0	Coffee fruits	1.64	2.12	6.55	0.40	8.60	22.71	3.29	8.31
96	Control	0.06	0.34	5.90	0.11	7.77	14.55	8.33	12.21
	Fresh *S. cerevisiae* CCMA 0543	0.04[a]	0.28[a]	1.69[a]	0.08[a]	2.58[a]	0.34[a]	6.41[a]	17.41[a]
	Microencapsulated *S. cerevisiae* CCMA 0543	0.04[a]	1.15[a]	3.21[a]	0.04[a]	4.52[a]	0.04[a]	7.98[a]	15.37[a]
	Fresh *T. delbrueckii* CCMA 0684	0.06[a]	0.19[b]	2.23[b]	0.08[a]	3.39[b]	4.93[a]	6.40[b]	11.44[a]
	Microencapsulated *T. delbrueckii* CCMA 0684	0.06[a]	0.41[a]	6.46[a]	0.03[b]	8.18[a]	5.04[a]	13.11[a]	12.58[a]
180	Control	0.03	0.03	1.70	0.21	3.60	0.02	16.85	24.64
	Fresh *S. cerevisiae* CCMA 0543	0.04[a]	0.08[a]	0.68[a]	0.05[a]	1.04[a]	0.37[a]	11.00[a]	11.28[a]
	Microencapsulated *S. cerevisiae* CCMA 0543	nd[b]	0.22[a]	1.97[a]	nd[a]	2.41[a]	0.88[a]	14.35[a]	14.11[a]
	Fresh *T. delbrueckii* CCMA 0684	0.05[a]	0.04[a]	1.11[a]	0.12[a]	1.61[a]	0.43[a]	11.23[a]	16.04[a]
	Microencapsulated *T. delbrueckii* CCMA 0684	0.06[a]	0.05[a]	1.05[a]	0.15[a]	1.58[a]	0.62[a]	12.12[a]	17.64[a]
Drying end	Control	0.34	0.06	4.86	0.14	7.97	0.14	26.24	22.96
	Fresh *S. cerevisiae* CCMA 0543	0.12[a]	0.05[a]	1.22[a]	0.04[b]	0.89[a]	0.17[b]	10.82[a]	4.49[b]
	Microencapsulated *S. cerevisiae* CCMA 0543	0.05[a]	nd[b]	0.53[a]	0.11[a]	0.63[a]	0.60[a]	14.36[a]	6.54[a]
	Fresh *T. delbrueckii* CCMA 0684	0.58[a]	0.02[b]	1.17[a]	0.10[a]	0.73[a]	0.59[a]	17.95[a]	6.10[a]
	Microencapsulated *T. delbrueckii* CCMA 0684	0.04[b]	0.15[a]	0.35[b]	0.05[b]	0.67[a]	0.51[b]	16.36[b]	6.14[a]

740 Data are presented as mean. The statistic was used to compare the averages between the same yeast strain in the fresh and
741 microencapsulated state at each fermentation and drying time. a–b for each column, mean values with different letters are
742 significant at $p \leq 0.05$ by analysis of variance.
743 nd = not detected.
744 [a]Natural coffee
745 [b]Pulped coffee

746 **Table 3.** Organic acids detected during self-induced anaerobic fermentation (SIAF) and at the end of drying by high-
747 performance liquid chromatography (HPLC).

Fermentation time (h)	Treatments	Organic acids (g/kg)													
		Oxalic		Citric		Tartaric		Malic		Succinic		Lactic		Acetic	
		Nat	PC	Nat	PC	Nat	PC	Nat	PC	Nat	PC	Nat	PC	Nat	PC
0	Coffee fruits	nd	nd	4.85	3.01	nd	nd	1.13	1.74	0.76	1.17	nd	nd	nd	nd
96	Control	nd	nd	4.85	2.51	nd	0.13	0.72	0.49	0.64	1.23	1.07	1.38	nd	0.31
	Fresh *S. cerevisiae* CCMA 0543	nd^{a}	nd^{a}	1.93^{a}	2.63^{b}	nd^{a}	0.17^{a}	0.25^{a}	0.05^{b}	0.41^{a}	0.72^{a}	0.65^{b}	2.49^{a}	nd^{a}	0.06^{a}
	Microencapsulated *S. cerevisiae* CCMA 0543	nd^{a}	nd^{a}	2.37^{a}	6.34^{a}	nd^{a}	nd^{b}	0.34^{a}	0.37^{a}	0.40^{a}	0.63^{a}	1.45^{a}	0.53^{b}	nd^{a}	nd^{b}
	Fresh *T. delbrueckii* CCMA 0684	nd^{a}	nd^{a}	2.19^{b}	1.93^{b}	nd^{a}	nd^{a}	0.25^{b}	0.23^{b}	0.53^{b}	0.69^{b}	0.65^{b}	1.47^{a}	nd^{a}	nd^{b}
	Microencapsulated *T. delbrueckii* CCMA 0684	nd^{a}	nd^{a}	3.11^{a}	6.08^{a}	nd^{a}	nd^{a}	0.55^{a}	0.46^{a}	0.84^{a}	1.04^{a}	2.09^{a}	1.30^{a}	nd^{a}	0.09^{a}
180	Control	nd	nd	2.40	3.05	nd	0.25	0.13	0.15	0.99	0.89	3.95	8.33	0.31	0.52
	Fresh *S. cerevisiae* CCMA 0543	nd^{a}	nd^{a}	1.67^{b}	2.57^{b}	nd^{a}	0.13^{a}	0.03^{b}	nd^{b}	0.42^{b}	0.35^{b}	2.55^{b}	5.91^{a}	0.10^{b}	0.39^{a}
	Microencapsulated *S. cerevisiae* CCMA 0543	nd^{a}	nd^{a}	3.10^{a}	3.89^{a}	nd^{a}	0.20^{a}	0.15^{a}	0.12^{a}	0.70^{a}	0.74^{a}	4.72^{a}	2.82^{b}	0.30^{a}	0.21^{b}
	Fresh *T. delbrueckii* CCMA 0684	nd^{a}	nd^{a}	2.39^{a}	2.46^{b}	nd^{a}	0.19^{b}	0.10^{a}	0.07^{b}	0.68^{a}	0.82^{a}	2.99^{a}	2.79^{a}	0.13^{b}	0.27^{b}
	Microencapsulated	nd^{a}	nd^{a}	2.07^{a}	3.09^{a}	nd^{a}	0.27^{a}	0.12^{a}	0.11^{a}	0.49^{b}	0.85^{a}	2.64^{a}	2.25^{a}	0.24^{a}	0.50^{a}

	T. delbrueckii CCMA 0684														
Drying end	Control	nd	0.03	5.46	3.95	nd	nd	0.33	0.09	2.38	1.60	13.20	3.50	nd	nd
	Fresh *S. cerevisiae* CCMA 0543	nd[a]	0.01[b]	4.71[a]	2.52[b]	nd[a]	0.13[a]	0.14[a]	nd[b]	1.49[b]	0.48[b]	12.15[a]	4.89[a]	nd[a]	nd[a]
	Microencapsulated *S. cerevisiae* CCMA 0543	nd[a]	0.03[a]	5.97[a]	3.71[a]	nd[a]	nd[b]	0.29[a]	0.07[a]	2.09[a]	1.52[a]	14.23[a]	3.99[b]	nd[a]	nd[a]
	Fresh *T. delbrueckii* CCMA 0684	nd[a]	0.02[b]	6.23[a]	3.00[a]	nd[a]	nd[a]	0.29[a]	nd[b]	1.74[a]	1.21[a]	10.50[a]	2.00[a]	nd[a]	nd[a]
	Microencapsulated *T. delbrueckii* CCMA 0684	nd[a]	0.03[a]	4.23[b]	2.63[b]	nd[a]	nd[a]	0.13[b]	0.10[a]	0.91[b]	0.90[b]	6.34[b]	2.03[a]	nd[a]	nd[a]

Data are presented as mean. The statistics analysis was applied in order to compare the averages between the same yeast strain in the fresh and microencapsulated state at each fermentation and drying time. a–b for each column, mean values with different letters are significant at $p \leq 0.05$ by analysis of variance.

nd = not detected.

[a]Natural coffee

[b]Pulped coffee

754 **Supporting Information - Supplementary material**

755 **Table S1.** Identification and sensory perception of volatile compounds (peak areas of GC-MS) detected in green coffee

756 fermented by natural and pulped process.

Compounds	PCA number	LRI	GC-MS peak area (10^4)										Sensory perception*
			Nat					PC					
			1	2	3	4	5	1	2	3	4	5	
Acids													
n-Hexadecanoic acid	21	2866	n.d	n.d	n.d	n.d	n.d	0.47	0.36^{b}	0.53^{a}	0.73^{a}	0.38^{b}	Rancid and acid flavor
Alcohols													
Benzyl alcohol	1	1878	2.78	2.17^{b}	3.15^{a}	2.40^{a}	1.27^{b}	1.20	1.10^{a}	0.99^{a}	1.21^{a}	1.26^{a}	Floral, roasted bread, fruity with chemical nuances
Phenylethyl Alcohol	2	1939	2.84	2.65^{a}	2.36^{a}	3.00^{a}	2.66^{a}	2.17	1.37^{a}	1.71^{a}	3.36^{a}	3.83^{a}	Sweet, chocolate, fruity and floral flavor (apple and rose)
Aldehydes													
Hexadecanal	3	2108	1.60	1.33^{a}	1.45^{a}	1.73^{a}	0.54^{b}	1.70	1.15^{a}	1.61^{a}	1.14^{a}	1.33^{a}	--
Pentadecanal-	44	2162	0.42	0.29^{a}	0.29^{a}	0.35^{a}	0.14^{b}	0.20	0.25^{a}	0.20^{a}	0.22^{a}	0.18^{a}	--
Esters													
Hexadecanoic acid,	25	2202	0.69	0.83^{a}	1.01^{a}	0.96^{a}	0.52^{b}	0.59	0.47^{a}	0.47^{a}	0.44^{a}	0.53^{a}	--

methyl ester													
Tetradecanoic acid, ethyl ester	36	2117	0.50	1.01^{b}	1.28^{a}	1.30^{a}	0.66^{b}	n.d	0.38^{a}	0.51^{a}	0.28^{b}	0.36^{a}	--
9,12-Octadecadienoic acid, ethyl ester	37	2509	0.57	1.02^{b}	1.98^{a}	1.33^{a}	0.90^{b}	0.52	0.64^{a}	0.46^{b}	0.57^{a}	0.64^{a}	--
Ethyl Oleate	39	2465	0.10	0.23^{b}	0.35^{a}	0.38^{a}	0.19^{a}	n.d	n.d	n.d	n.d	n.d	Fatty, buttery
Octadecanoic acid, ethyl ester	41	2448	n.d	0.33^{b}	0.62^{a}	0.49^{a}	0.27^{b}	n.d	n.d	n.d	n.d	n.d	Mild, waxy
Hexanoic acid, 2-methylbutyl ester	42	1784	1.30	1.43^{a}	1.38^{a}	1.70^{a}	0.38^{b}	1.34	0.42^{a}	0.49^{a}	0.35^{a}	1.01^{a}	--
Ketones													
2-Pentadecanone, 6,10,14-trimethyl-	4	2159	0.88	0.99^{a}	1.13^{a}	1.37^{a}	0.90^{b}	1.55	1.00^{b}	1.54^{a}	1.25^{a}	1.23^{a}	Oily, jasmine, herbs, woody
2-Pentadecanone	27	2105	1.02	0.92^{a}	0.76^{a}	0.88^{a}	0.40^{b}	0.63	0.54^{a}	0.68^{a}	0.52^{b}	0.61^{a}	Fresh, jasmine
2-Heptadecanone	28	2226	0.67	0.83^{a}	0.57^{a}	0.74^{a}	0.36^{b}	0.59	0.46^{a}	0.67^{a}	0.46^{a}	0.48^{a}	--
Phenol													
Phenol	32	2066	0.23	0.42^{a}	0.34^{a}	0.35^{a}	0.17^{b}	0.45	0.30^{a}	0.36^{a}	0.37^{a}	0.27^{b}	Rubbery, plastic-like
Others													
Caffeine	5	3379	23.77	25.50^{b}	43.94^{a}	26.37^{a}	7.34^{b}	43.44	38.05^{a}	33.66^{a}	41.10^{b}	50.24^{a}	--
Eicosane	43	1799	4.05	1.53^{a}	0.81^{b}	1.24^{a}	0.28^{b}	0.75	0.57^{a}	n.d^{b}	0.48^{a}	0.71^{a}	--
2-Pyrrolidinone	17	2146	n.d	n.d	n.d	n.d	n.d	n.d	0.23^{b}	0.25^{a}	0.32^{a}	0.27^{a}	--

Data are presented as mean. The statistic was used to compare the averages between fresh and microencapsulated yeast.
a–b for each row, mean values with different letters are significant at $p \leq 0.05$ by Scott–Knott test.
nd = not detected.

Nat = natural coffee
PC = pulped coffee
1 = Control (without inoculation)
2 = Inoculated with fresh *Saccharomyces cerevisiae* CCMA 0543
3 = Inoculated with microencapsulated *Saccharomyces cerevisiae* CCMA 0543
4 = Inoculated with fresh *Torulaspora delbrueckii* CCMA 0684
5 = Inoculated with microencapsulated *Torulaspora delbrueckii* CCMA 0684
*Sensory attributes are taken from: Flament & Bessière-Thomas (2001); Lee et al. (2015); Cassimiro et al. (2022); da Mota et al. (2020).

769 **Table S2.** Identification and sensory perception of volatile compounds (peak areas of GC-MS) detected in roasted coffee

770 fermented by natural and pulped process.

Compounds	PCA	LRI	GC-MS peak area (10^4)										Sensory perception*
			Nat					PC					
			1	2	3	4	5	1	2	3	4	5	
Acids													
n-Hexadecanoic acid	21	2866	1.19	2.10^a	1.50^a	1.96^b	2.65^a	2.87	3.74^a	2.40^b	3.17^a	3.57^a	Rancid and acid flavor
Butanoic acid, 4-hydroxy-	10	1684	29.02	27.28^a	25.33^a	25.12^b	27.99^a	27.76	17.31^a	17.22^a	21.70^b	35.11^a	Sweet, caramel and creamy flavor
Octanoic acid	23	2122	1.27	1.92^a	1.97^a	1.64^b	2.61^a	2.86	3.36^a	2.30^b	2.84^a	2.16^a	Fatty, waxy, cheese-like, acid, rancid
n-Decanoic acid	30	2263	0.23	0.77^a	0.76^a	1.07^a	1.22^a	1.44	1.95^a	1.24^b	1.57^a	1.27^a	Dust, fat, rancid, sweat, sour
Alcohols													
Benzyl alcohol	1	1878	1.80	2.04^a	2.10^a	1.76^b	2.37^a	0.41	0.37^b	0.72^a	0.64^b	1.00^a	Floral, roasted bread, fruity with chemical nuances
Phenylethyl Alcohol	2	1939	2.07	2.79^a	1.84^a	2.46^b	5.17^a	1.12	0.69^b	1.27^a	1.72^b	2.97^a	Sweet, chocolate, fruity and floral flavor (apple and rose)

Aldehydes													
Hexadecanal	3	2108	0.71	0.61[a]	0.56[a]	0.68[b]	0.78[a]	0.55	0.66[a]	0.57[a]	0.74[a]	0.55[b]	--
Esters													
Hexadecanoic acid, methyl ester	25	2202	1.41	1.45[a]	1.95[a]	1.46[a]	2.11[a]	1.56	1.07[a]	1.31[b]	1.09[b]	1.64[a]	--
Tetradecanoic acid, ethyl ester	36	2117	0.36	0.50[a]	0.67[a]	0.46[b]	0.75[a]	n.d	n.d	n.d	n.d	n.d	--
9,12-Octadecadienoic acid, ethyl ester	37	2509	1.04	2.49[a]	2.75[a]	1.69[a]	2.91[a]	0.98	0.79[a]	1.10[a]	0.67b	1.33a	--
Ethyl Oleate	39	2465	0.19	0.31[a]	0.57[a]	0.36[a]	0.62[a]	n.d	n.d	n.d	n.d	n.d	Fatty, buttery
Octadecanoic acid, ethyl ester	41	2448	0.32	0.85[b]	1.02[a]	0.63[a]	1.04[a]	n.d	n.d	n.d	n.d	n.d	Mild, waxy
Furanones													
2(5H)-Furanone	33	1757	3.55	3.52[a]	3.55[a]	3.32[a]	4.47[b]	5.33	3.49[a]	4.64[a]	4.54[b]	5.68[a]	Buttery
Furans													
Ethanone, 1-(2-furanyl)-	7	1491	20.13	19.01[b]	24.12[a]	18.42[a]	19.06[a]	27.52	12.81[a]	22.84[a]	19.30[b]	27.86[a]	Caramel, cocoa, almond, peanut and sweet
2-Furanmethanol, acetate	8	1531	24.71	29.03[a]	32.29[a]	25.41[a]	27.74[a]	27.62	19.45[a]	20.37[a]	21.69[b]	36.17[a]	Soft, floral, fruity, banana and sweet
2-Furancarboxaldehyde, 5-methyl-	9	1603	98.62	87.11[b]	99.92[a]	82.67[b]	95.78[a]	112.20	71.67[a]	87.90[a]	83.73[b]	124.58[a]	Spicy and slightly caramelized
2-Furanmethanol	11	1710	390.81	392.08[a]	361.38[a]	338.01[b]	394.42[a]	389.97	276.57[a]	358.31[a]	361.87[b]	476.89[a]	Mild, sweet and slightly caramelized flavor
5-	20	2503	2.62	2.90[a]	2.66[a]	2.15[a]	3.02[a]	5.16	2.97[b]	6.79[a]	3.79[a]	5.21[a]	Sweet flavor,

Hydroxymethylfurfural													herbaceous
Furfural	31	1409	153.06	113.38^{b}	162.68^{a}	112.08^{a}	147.15^{b}	164.36	108.89^{a}	147.61^{a}	181.18^{a}	145.24^{a}	Caramel, sweet, bread-like, toasted odor
4-Methyl-5H-furan-2-one	38	1909	1.95	1.99^{a}	1.94^{a}	1.69^{b}	1.90^{a}	2.12	1.51^{a}	1.63^{a}	1.60^{b}	2.60^{a}	Pungent, caramel and sweet flavor
Ketones													
2-Pentadecanone, 6,10,14-trimethyl-	4	2159	0.92	0.81^{a}	0.76^{a}	0.65^{b}	1.09^{a}	1.02	0.60^{b}	1.20^{a}	0.84^{b}	1.31^{a}	Oily, jasmine, herbs, woody
2-Pentadecanone	27	2105	0.48	0.48^{a}	0.54^{a}	0.58^{a}	0.63^{a}	0.58	0.54^{a}	0.61^{a}	0.59^{a}	0.58^{a}	Fresh, jasmine
2-Heptadecanone	28	2226	0.50	0.56^{a}	0.38^{a}	0.63^{a}	0.57^{a}	0.70	0.51^{a}	0.74^{b}	0.63^{a}	0.52^{b}	--
Ethanone, 1-(1H-pyrrol-2-yl)-	15	2026	7.51	7.32^{a}	6.98^{a}	6.64^{b}	8.29^{a}	8.48	5.76^{a}	8.32^{a}	7.27^{b}	11.48^{a}	Nuts, floral and fruity flavor
Phenols													
Phenol	32	2066	1.64	1.94^{a}	1.78^{a}	1.96^{b}	2.27^{a}	2.58	1.95^{a}	1.86^{a}	2.16^{a}	2.65^{a}	Rubbery, plastic-like
4-Hydroxy-2-methylacetophenone	24	2188	3.92	3.99^{a}	3.96^{a}	3.76^{b}	5.25^{a}	6.25	4.72^{b}	5.86^{a}	5.61^{b}	7.51^{a}	--
Pyrazines													
Pyrazine, 3-ethyl-2,5-dimethyl-	6	1447	10.97	11.06^{a}	10.98^{a}	9.36^{a}	10.01^{a}	6.72	4.36^{a}	9.07^{a}	8.84^{a}	9.77^{a}	Cocoa and walnut flavor
3-Methyl-2-pyrazinylmethanol	13	1957	0.59	0.78^{a}	0.69^{a}	0.74^{a}	0.76^{a}	0.73	0.46^{a}	0.95^{b}	0.68^{b}	1.11^{a}	--
Pyrazine, 2,6-dimethyl-	40	1213	40.17	43.96^{a}	32.04^{a}	32.82^{a}	32.92^{a}	43.22	31.58^{b}	32.56^{a}	26.43^{b}	43.25^{a}	Sweet, cocoa, roasted nut
Pyridines													
3-Pyridinol, 2-methyl-	18	2322	1.06	1.15^{a}	1.70^{a}	0.97^{b}	1.79^{a}	2.32	1.56^{b}	2.50^{a}	1.54^{b}	4.04^{a}	--

3-Pyridinol	19	2445	1.81	2.29[a]	3.23[a]	2.37[b]	4.03[a]	6.58	5.82[a]	5.22[a]	3.55[b]	7.74[a]	--
Pyrroles													
1H-Pyrrole, 1-(2-furanylmethyl)-	12	1791	9.35	8.21[a]	9.81[a]	7.89[b]	8.89[a]	10.32	6.02[a]	8.19[a]	7.87[b]	12.69[a]	Coffee and fruity
1H-Pyrrole-2-carboxaldehyde	16	2095	5.68	5.79[a]	5.91[a]	5.32[b]	7.10[a]	8.28	5.93[a]	6.48[a]	6.57[b]	10.46[a]	Pungent flavor and green grain
Indole	26	2424	2.41	2.16[a]	2.19[a]	1.85[b]	2.60[a]	2.68	1.59[b]	2.86[a]	2.41[b]	3.07[a]	Floral notes, sweet, warm, nutty
2-Pyrrolidinone, 1-butyl-	34	2284	3.11	2.15[a]	2.66[a]	1.88[a]	2.87[a]	3.03	1.45[b]	3.15[a]	1.70[b]	4.54[a]	--
1H-Pyrrole-2-carboxaldehyde, 1-methyl-	35	2149	1.86	1.85[a]	1.91[a]	1.65[a]	2.27[b]	2.57	1.82[a]	2.24[a]	1.98[b]	3.50[a]	Mild flavor
Others													
Caffeine	5	3379	117.42	95.13[a]	127.94[a]	124.49[a]	120.59[a]	196.29	99.61[b]	178.53[a]	101.18[b]	172.40[a]	--
2-Thiophenemethanol	14	1976	1.41	1.37[a]	1.24[a]	1.22[a]	1.45[a]	1.39	0.98[a]	1.35[a]	1.22[b]	1.82[a]	--
2-Pyrrolidinone	17	2146	11.48	8.58[a]	9.53[a]	7.69[a]	10.98[a]	9.65	5.84[b]	10.73[a]	6.69[b]	14.36[a]	--
Acetamide	22	1786	2.99	2.88[a]	2.85[a]	2.45[b]	3.46[a]	3.50	2.54[b]	4.00[a]	2.54[b]	4.87[a]	--
Maltol	29	2047	1.67	2.28[a]	2.44[a]	2.43[b]	3.47[a]	4.32	7.70[a]	3.80[a]	3.50[b]	5.91[a]	Fruit and caramel

771 Data are presented as mean. The statistic was used to compare the averages between fresh and microencapsulated yeast.
772 a–b for each row, mean values with different letters are significant at $p \leq 0.05$ by Scott–Knott test.
773 nd = not detected.
774 Nat = natural coffee
775 PC = pulped coffee
776 1 = Control (without inoculation)
777 2 = Inoculated with fresh *Saccharomyces cerevisiae* CCMA 0543
778 3 = Inoculated with microencapsulated *Saccharomyces cerevisiae* CCMA 0543
779 4 = Inoculated with fresh *Torulaspora delbrueckii* CCMA 0684

780 5 = Inoculated with microencapsulated *Torulaspora delbrueckii* CCMA 0684
781 *Sensory attributes are taken from: Flament & Bessière-Thomas (2001); Lee et al. (2015); Cassimiro et al. (2022); da
782 Mota et al. (2020).