INTRODUCTION

Chocolate (from the Nahuatl ‘xocolatl’) is a worldwide consumed confectionary product crafted from cocoa beans (De Vuyst & Leroy, 2020; Santander Muñoz et al., 2020; Watson et al., 2013). Cocoa beans are the seeds of the cocoa tree, *Theobroma cacao* L. (referring to the Greek for food ['broma'] of the gods ['theos'] and probably the Mixe-Zoquean ‘kakaw’ or ‘kakawa’) (Coe & Coe, 2019; Grivetti & Shapiro, 2009). The origin of the cocoa tree is thought to be in the Western Upper Amazon region, from where it has been widely distributed throughout the equatorial zone as a result of its domestication (Motamayor et al., 2002; Thomas et al., 2012). In the pre-Hispanic Mesoamerican civilizations, cocoa played an important role, not only as a food or beverage product but also as a religious entity; furthermore, the cocoa seeds were used as a currency and to depict a political and/or social status (Grivetti & Shapiro, 2009). The meaning of *xocolatl* is sour water, as the traditional preparations by the Mayans, and the Mexica/Aztecs later, primarily consisted of mixing the fermented and ground cocoa seeds with water for the preparation of (mostly medicinal and/or ritual) beverages (Coe & Coe, 2019; Grivetti & Shapiro, 2009; Watson et al., 2013). It is only after the Castilians introduced cocoa in Europe in

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the 16th century that cocoa products began to be associated with sweetness, as the ground cocoa beans were mixed with vanilla, cinnamon and sugar (Grivetti & Shapiro, 2009).

Nowadays, the gross of the cocoa production is located in Africa (mainly Ivory Coast, Ghana, Cameroon and Nigeria) counting for 76.2% of the worldwide production (ICCO, 2021a), followed by the Americas (17.6%; mainly Ecuador and Brazil), and Asia and Oceania (6.2%; mainly Indonesia). The worldwide chocolate trade is a growing market, valued at USD 130.6 billion (Grand View Research, 2020), with a yearly increasing demand of cocoa beans (from 4.1 million tonnes in 2011–2012 to 4.7 million tonnes in 2019–2020; ICCO, 2021b). However, cocoa yields are challenging, as the cocoa crop is suffering from aged plantations, diseases and climate change (Bekele & Philips-Mora, 2019; Gateau-Rey et al., 2018). Advances in the field to produce higher cocoa yields or cocoa hybrids with improved resistance to diseases have been made. At the same time, the development of microbial starter cultures to steer cocoa fermentation processes has been investigated as a response to an increasing interest to improve the quality and flavour of cured cocoa beans (De Vuyst & Leroy, 2020).

The present review aims at highlighting the effect of candidate yeast strains on starter culture-initiated (SCI) cocoa fermentation processes and at linking their use with the production of desirable flavour compounds in the cured cocoa beans and cocoa liquors and/or chocolates produced therefrom.

**COCOA FERMENTATION**

Cocoa beans need to be cured before chocolate manufacturing can begin (De Vuyst & Leroy, 2020; Santander Muñoz et al., 2020; Watson et al., 2013). Fermentation and drying of cocoa is a traditional process that is typically carried out on plantation by local farmers, whose methods may differ depending on the cocoa-producing region. The harvested cocoa pods are cut-opened and their contents, consisting of cocoa beans embedded in a mucilaginous pulp, are placed in heaps, wooden boxes, baskets or trays, all of which are covered with banana leaves, depending on the local practices applied (Crafack et al., 2014; De Vuyst & Weckx, 2016; Mota-Gutierrez et al., 2018; Papalexandratou, Camu, et al., 2011). Depending on the cocoa variety (Criollo, Forastero, Trinitario, Nacional and hybrids of these main four varieties), preconditioning of the cocoa pulp-bean mass is sometimes applied, mainly aiming at reducing the pulp mass and hence the acidity of the cured cocoa beans (De Vuyst & Leroy, 2020; Santander Muñoz et al., 2020). The fermentation time can also vary according to the cocoa variety and local practices, usually ranging from 2 to 10 days (De Vuyst & Leroy, 2020).

Fermentation of the cocoa pulp-bean mass, followed by drying, is a key step in the curing of cocoa beans, as it leads to the removal of the cocoa pulp, the killing of the embryo and the development of the typical cocoa flavour and colour (De Vuyst & Leroy, 2020; Santander Muñoz et al., 2020; Watson et al., 2013). Most of the biochemical changes that occur in the cocoa pulp and cocoa beans during the fermentation step are the result of the dynamics and activities of the micro-organisms involved. It is well known that cocoa fermentation is steered by three main microbial groups, namely yeasts, lactic acid bacteria (LAB) and acetic acid bacteria (AAB), each one contributing differently to and along the process (see below). However, other microbial groups, such as enterobacteria, filamentous fungi and/or bacilli can also occur, although with a minor contribution to the fermentation process (e.g. pectinolytic activity, citric acid metabolism, pyrazine production) or even undesired activities, given the time frames they are active in during the fermentation and/or drying step (Illeghems et al., 2015; Ouattara et al., 2020; Papalexandratou, Falony, et al., 2011; Pereira et al., 2020; Watson et al., 2013). The contribution of each microbial group is well established (De Vuyst & Leroy, 2020; Santander Muñoz et al., 2020; Watson et al., 2013). Yeasts initially perform an alcoholic fermentation, consuming the pulp carbohydrates to produce ethanol and carbon dioxide, and display pectinolytic activity to degrade the cocoa pulp pectin. Simultaneously, LAB consume pulp carbohydrates (initially mainly fructose and then glucose), generating lactic acid, acetic acid, ethanol, carbon dioxide and/or other metabolites (e.g. mannitol, acetoin, diacetyl and 2,3-butanediol). This microbial group is also the main contributor to citrate consumption, the main organic acid present in the cocoa pulp and responsible for its low pH, which causes the pH of the fermenting mass to increase slightly. The ethanol produced by the yeasts is used by the AAB in a later stage of the fermentation process to oxidize it to acetic acid, when oxygen penetrates into the fermenting mass. Furthermore, the AAB overoxidize the generated acetic acid to carbon dioxide and water. All these oxidation reactions are exothermic, which causes the temperature of the fermenting mass to increase considerably. At this stage, the high concentrations of bean-penetrating acetic acid, together with the high temperature, leads to the killing of the seed embryo by decreasing the bean pH and destruction of the subcellular cotyledon structure, which triggers a series of biochemical conversions in the cocoa beans that greatly impact the cocoa flavour and colour development (De Vuyst & Leroy, 2020). The destruction of the internal bean structure also causes proteolytic degradation of the seed proteins, hydrolysis
of sucrose, oxidation of polyphenols, and leakage of polyphenols and other phytochemical compounds, such as methylxanthines.

**CONTRIBUTION OF YEASTS TO COCOA FERMENTATION PROCESSES**

Always considered as a single microbial group because of their many common activities during food fermentation processes, yeasts appear in a relatively high species diversity during cocoa fermentation (Agyirifo et al., 2019; Almeida et al., 2019; Arana-Sánchez et al., 2015; Ardhana & Fleet, 2003; Crafa et al., 2013; Daniel et al., 2009; Delgado-Ospina et al., 2020; Díaz-Muñoz et al., 2021; Fernández-Niño et al., 2021; Hamdouche et al., 2015, 2019; Ho et al., 2014, 2015; Illeghems et al., 2012; Jespersen et al., 2005; Koné et al., 2016; Lagunes Gálvez et al., 2007; Lima et al., 2021; Maura et al., 2016; Meersman et al., 2013; Miguel et al., 2017; Moreira et al., 2013, 2017; Mota-Gutierrez et al., 2018; Nielsen et al., 2005, 2007; Ouattara & Niamké, 2021; Pacheco-Montealegre et al., 2020; Papalexandratou & De Vuyst, 2011; Serra et al., 2019; Verce et al., 2021; Visintin et al., 2016). In decreasing order of relative abundance, *Pichia, Hanseniaspora, Saccharomyces* and *Candida* are the most commonly found genera involved in cocoa fermentation, representing an even greater species diversity.

The most predominant yeast species found in spontaneous cocoa fermentation processes are, in decreasing order of relative abundance, *Saccharomyces cerevisiae*, *Pichia kudriavzevii*, *Hanseniaspora opuntiae*, *Hanseniaspora uvarum*, *Hanseniaspora guilliermondii*, *Pichia manshurica*, *Pichia kluyveri* and *Candida tropicalis* (Figure 1). Other yeast genera that appear in spontaneous cocoa fermentation processes but at lower relative abundances are *Wickerhamomyces*, *Kluyveromyces*, *Torulaspora* and *Rhodotorula*. Yeasts find the fresh cocoa pulp a very suitable environment for their growth, as it consists of a sugar-rich, anaerobic environment with a low pH that restricts the growth of other micro-organisms (De Vuyst & Leroy, 2020; Roelofsen, 1958). As for every spontaneous fermentation process, the inoculation source of yeasts has to be reduced to the surrounding environment, namely surfaces of the cocoa pods (and other tropical fruits), banana leaves, insects, or worker hands and utensils (Camu et al., 2008; De Vuyst & Weckx, 2016; Maura et al., 2016; Roelofsen, 1958; Watson et al., 2013). Thus, the occurrence of one or the other yeast species may depend on the post-harvesting methods applied and even the ripening level of the cocoa beans and harvesting season (Figueroa-Hernández et al., 2019; Nielsen et al., 2005; Ouattara & Niamké, 2021; Pacheco-Montealegre et al., 2020; Roelofsen, 1958). Not only the fermentation method (wooden boxes, heaps or trays) but also the turning/mixing of the fermenting mass has an impact on the microbiota succession upon fermentation (Camu et al., 2008; Hamdouche et al., 2019; Nielsen et al., 2005; Pacheco-Montealegre et al., 2020; Ramos et al., 2020). Concerning yeast species adaptation, the tolerance

![Figure 1](image-url) 
**Figure 1**  Top 10 most found yeast species in spontaneous cocoa fermentation processes (a) and applied as (members of) starter cultures (b). The colours are according to the yeast genera, namely *Candida* (gold), *Hanseniaspora* (green), *Kluyveromyces* (purple), *Pichia* (blue) and *Saccharomyces* (orange). The x-axis represents the number of studies in which the yeast species occurs (a) or has been applied as starter culture (b).
towards stress conditions (e.g. high acidity, high temperature, high ethanol concentration, high osmolarity), use of available carbohydrates and/or production of antifungal compounds determine their prevalence throughout the fermentation process. In this way, *Hanseniaspora* species are often found at the beginning of the fermentation process but disappear in favour of more ethanol- and temperature-tolerant yeast species, such as *S. cerevisiae*, *P. kudriavzevii*, *P. manshurica* or *P. kluveri* (Daniel et al., 2009; De Vuyst & Leroy, 2020; De Vuyst & Weckx, 2016; Díaz-Muñoz et al., 2021; Ho et al., 2018; Koné et al., 2016; Lagunes Gálvez et al., 2007; Maura et al., 2016; Meersman et al., 2013; Nielsen et al., 2005; Papalexandratou et al., 2013).

**Pectinase activity**

One of the main purposes of the cocoa fermentation process is the removal of the mucilaginous pulp that surrounds the cocoa beans. The cocoa pulp, whose viscosity is high because of the presence of pectin, is liquified throughout the fermentation process by the activity of endogenous pectinases and microbial pectin-degrading enzymes (De Vuyst & Leroy, 2020; Schwan, 1998; Schwan et al., 1995). Some yeast species typically present in cocoa fermentation processes, especially *Candida norvegensis*, *Candida zeylanoides*, *Candida nitrativorans*, Kluveromyces fragilis, *Kluveromyces marxianus*, *P. kudriavzevii*, *P. kluveri* and *S. cerevisiae* possess a high pectinolytic activity, as tested either in vitro or in actual cocoa fermentation processes (Buamah et al., 1997; Crafack et al., 2013; Delgado-Ospina et al., 2020; Dzogbefia et al., 1999; Lagunes Gálvez et al., 2007; Samagaci et al., 2016; Sanchez et al., 1985; Schwan et al., 1997). The pectinolysis activity in yeasts is mediated by the expression of mainly (endo)polygalacturonase genes (Meersman et al., 1997; C. Díaz-Muñoz, M. Verce, L. De Vuyst, and S. Weckx, unpublished results). However, the presence of these genes has not always been related to pectin degradation, as their expression and activity can be influenced by the physicochemical conditions of the cocoa fermentation process (Blanco et al., 1999; Samagaci et al., 2014b; Schwan et al., 1997; C. Díaz-Muñoz, M. Verce, L. De Vuyst, and S. Weckx, unpublished results). The yeasts’ polygalacturonase enzyme degrades the primary chain of the pectin backbone by hydrolysis of the α-1,4-glycosidic linkages, resulting in the loss of most of the elastic, fibrous consistency of the cocoa pulp (Blanco et al., 1999; Meersman et al., 2017). The physicochemical conditions under which this enzyme is active vary very much, depending on the yeast species (and even strains), although it is usually active in ranges of pH from 3.5 to 6.0 and temperatures of 30–50°C and under rather anaerobic atmosphere (with certain species not being affected by oxygen levels), reflecting circumstances that are typically encountered during cocoa fermentation processes (Blanco et al., 1999; Buamah et al., 1997; Samagaci et al., 2014b). As the cocoa pulp liquifies, oxygen can enter the fermenting mass, AAB proliferate better and produce acetic acid, and acetic acid penetration into the cocoa beans allows the fermentation process to proceed. Furthermore, the cocoa sweetings, before considered as waste product, can be retained for the production of alcoholic beverages or marmalades (Buamah et al., 1997; Dzogbefia et al., 1999; Meersman et al., 2017).

**Invertase activity and fermentation of carbohydrates**

Immature cocoa pods are rich in sucrose, which is hydrolysed into glucose and fructose by the pulp invertase throughout the maturation process (De Vuyst & Leroy, 2020; Hansen et al., 1998; Schwan et al., 1995). However, sucrose can still be present upon cocoa pod opening and, therefore, yeast invertases (*SUC* genes in *S. cerevisiae*; Carlson & Botstein, 1983) are thought to play an important role in its conversion (De Vuyst & Weckx, 2016). Once the main fermentable monosaccharides become available, they are promptly used by highly fermentative yeasts to produce ethanol and carbon dioxide, sometimes leading to a slight temperature increase (Díaz-Muñoz et al., 2021; Ramos et al., 2014; Romanens et al., 2020). Additionally, the assimilation of glucose during anaerobic conditions leads to the production of glycerol to maintain the redox balance (Rodrigues et al., 2006). This metabolite can migrate into the cocoa beans and may influence the taste and mouthfeel properties of chocolate (Díaz-Muñoz et al., 2021; Ho et al., 2014, 2018). Although there is a preference for glucose, most yeast species are capable of fermenting both glucose and fructose. Although the alcoholic fermentation is less efficient in terms of energy production than the oxidation of pyruvate through mitochondrial respiration, it is part of the make-accumulate-consume strategy followed by some yeasts to produce high concentrations...
of ethanol and, thus, outcompete other micro-organisms present in the fermentation matrix (Pfeiffer & Morley, 2014). Indeed, *Hanseniaspora* species appear at early stages of the fermentation course, which later disappear in favour of ethanol-tolerant *Saccharomyces* or *Pichia* species once ethanol levels peak (Daniel et al., 2009; Koné et al., 2016; Lagunes Gálvez et al., 2007; Maura et al., 2016; Meersman et al., 2013; Papalexandratou, Falony, et al., 2011; Samagaci et al., 2016; Sanchez et al., 1985).

**Citrate metabolism**

Citrate assimilation during fermentation of the cocoa pulp-bean mass is typically assigned to LAB metabolism, although certain yeast species can also assimilate this organic acid, as has been demonstrated in vitro (Daniel et al., 2009; Samagaci et al., 2016). Some of these citrate-positive yeasts isolated from fermenting cocoa pulp-bean mass belong to the commonly occurring species *P. kudriavzevii*, *P. kluyveri* and *C. tropicalis*. Indeed, some yeasts can metabolize citrate through the tricarboxylic acid (TCA) or glyoxylate cycle (Casal et al., 2008). To this end, aerobic conditions are required (De Vuyst & Leroy, 2020). Furthermore, a suitable transporter must be present to take up pulp citric acid (Cásio & Leão, 1991; Xi et al., 2021). Yet, many yeast species may not consume citrate during cocoa fermentation, especially when carbohydrates are extensively available and aerobic conditions are not fulfilled, or simply because they are not competitive enough compared to the capacity of LAB to metabolize it. As some authors claim yeasts to be the only indispensable microbial group in cocoa fermentation processes (Ho et al., 2014, 2015), the presence of citrate-positive yeasts becomes necessary to obtain properly fermented cocoa beans. Moreover, an increase in the concentration of pulp citric acid at the end of the cocoa fermentation process is sometimes found, suggesting a microbial citric acid production (Camu et al., 2007; Díaz-Muñoz et al., 2021; Ho et al., 2018; Papalexandratou, Vrancken, et al., 2011). This could be ascribed to the yeast’s oxidation of ethanol to acetaldehyde and further to acetyl-CoA, generating an excess flux through the TCA cycle, leading to an accumulation of citrate and its subsequent secretion (Casal et al., 2008).

**Flavour production**

Besides fermenting the pulp substrates, the yeast metabolism is also responsible for the production of pyruvate metabolites and amino acid conversion products, which influence the flavour characteristics of the cocoa beans (Castro-Alayo et al., 2019; Cevallos-Cevallos et al., 2018; De Vuyst & Leroy, 2020; Dzialo et al., 2017; Ho et al., 2014; Koné et al., 2016; Meersman et al., 2016; Menezes et al., 2016; Mota-Gutierrez et al., 2019; Ramos et al., 2014). A big proportion of these metabolites consists of volatile organic compounds (VOCs), including higher aldehydes, higher alcohols and esters. Many of the VOCs are produced through the Ehrlich pathway, such as higher aldehydes, higher alcohols and acetate esters, whereas others derive from ethanol and lipid metabolism, such as ethyl esters. Organic acids (e.g. acetic acid, succinic acid and citric acid) are also produced through the oxidative metabolism of yeasts, resulting in increasing concentrations when the carbohydrates are almost depleted (Díaz-Muñoz et al., 2021; Dzialo et al., 2017; Mota-Gutierrez et al., 2018; Rodrigues et al., 2006). Finally, originating from the pyruvate metabolism, acetaldehyde, acetoic, diacetyl and 2,3-butanediol can be produced (De Vuyst & Leroy, 2020; Dzialo et al., 2017). Because of their correlation with flavour-active compounds, some yeast species have been graded as highly aromatic, namely *Pichia fermentans*, *P. kluyveri*, *P. kudriavzevii*, *P. manshurica* and *S. cerevisiae* (Crafack et al., 2013, 2014; Díaz-Muñoz et al., 2021; Koné et al., 2016; Moreira et al., 2021; Pereira et al., 2017; Ramos et al., 2014). Yet, many of the flavour compounds produced by yeasts show a high intraspecies diversity because of different enzymatic activities (Pereira et al., 2017). However, many flavour-active compounds or their precursors are already present in the fresh cocoa beans and their dynamics are a result of proper physicochemical conditions during the fermentation process (Díaz-Muñoz et al., 2021). Thus, yeasts can also play an indirect role in flavour formation, ensuring the fulfilment of the conditions to the proper development of cocoa flavour (Díaz-Muñoz et al., 2021; Ho et al., 2014; Leal et al., 2008).

**STARTER CULTURE-INITIATED COCOA FERMENTATION PROCESSES**

**Geographical distribution of yeast starter culture-initiated fermentation trials**

The production and export of cured cocoa beans is an important economic source for many countries located in the cocoa belt (approximately 20° north and south of the equator; Figure 2). Most of the cocoa is harvested, fermented and dried by local farmers or, at best, small cooperatives in a traditional, spontaneous fashion (De Vuyst & Weckx, 2016). The traditional post-harvesting techniques applied do not always result in cured cocoa beans of a suitable quality, especially when spontaneous...
fermentation processes are applied, which usually implies a lack of reproducibility between fermentation batches and an increased risk of contamination and growth of less desirable micro-organisms. Therefore, starter cultures have been introduced, albeit mainly at research level and to a very limited extent commercially, to improve the outcome of the cocoa fermentation process and the quality of the cured cocoa beans (Castro-Alayo et al., 2019; Chagas Junior, Ferreira, & Lopes, 2021; De Vuyst & Leroy, 2020; Figueroa-Hernández et al., 2019; Mota-Gutierrez et al., 2019; Pereira et al., 2016). Just a few companies have launched projects to implement starter cultures at industrial level (Figueroa-Hernández et al., 2019).

The quest to develop a performant starter culture mixture to be applied in cocoa fermentation processes started in the mid-20th century, although earlier reports from the beginning of the 20th century exist (Roelofsen, 1958; Watson et al., 2013). Since then, different microbial species have been proposed as candidate starter culture strains, mainly yeasts, LAB and AAB, but also other bacteria such as Bacillus have sometimes been tested (De Vuyst & Leroy, 2020). Yeasts are the most applied microbial group as members of starter culture mixtures, whether alone or in combination with other microbial groups, typically LAB (Figure 3; Tables 1–3). In the last decades, an increasing number of yeast SCI fermentation trials have been performed, perhaps as an answer to the increasing demand of high-quality cured cocoa beans and flavourful chocolates. Most of the yeast SCI fermentation trials have been performed in Brazil (Battista et al., 2015, 2016; Chagas Junior, Ferreira, Andrade, et al., 2021; Chagas Junior, Ferreira, Gloria, et al., 2021; Leal et al., 2008; Menezes et al., 2016; Miguel et al., 2017; Moreira et al., 2017, 2021; Pereira et al., 2017; Ramos et al., 2014; Santos et al., 2020; Schwan, 1998; Viesser, Pereira, Neto, Rogez, et al., 2021; Visintin et al., 2017; Figure 2).

In the past, the cocoa trees in this country were gravely affected by witches’ broom disease (caused by the fungus Moniliophthora perniciosa), which led different research institutes to develop new cocoa hybrids from the traditional local varieties, mostly Amelonado (Aime & Phillips-Mora, 2005; Motamayor et al., 2008; Purdy & Schmidt, 1996). As a result, several starter cultures have been tested with a wide variety of local cocoa hybrids (Tables 1–3). West Africa, the Indian subcontinent, and Southeast Asia have also witnessed the use of yeast SCI fermentation trials with their local cocoa varieties, consisting mostly of Forastero or hybrids thereof in Cameroon (Mota-Gutierrez et al., 2018), Ghana (Buamah et al., 1997; Crafack et al., 2013, 2014; Dzogbefia et al., 1999; Lefeber et al., 2012), India (Sandhya et al., 2016; Saunshi et al., 2019; Saunshia et al., 2018), and Ivory Coast (Assi-Clair et al., 2019; Sanchez et al., 1985) and local hybrids in Indonesia (Apriyanto et al., 2017; Cempaka et al., 2014; Roelofsen, 1958) and Malaysia (Mahazar et al., 2015; Meersman et al., 2015, 2016; Samah et al., 1992). The Trinitario cocoa variety appears in a few studies performed in Australia (Ho et al., 2018) and Central America (Costa et al., 2016; Costa et al., 2017; Costa et al., 2018; Costa et al., 2019).
Rica [Díaz-Muñoz et al., 2021], Honduras [Romanens et al., 2020] and Mexico [Sanchez, 1989]). Finally, a yeast SCI fermentation trial was performed in a country located completely outside of the cocoa belt, namely Taiwan (23° north), albeit that the authors did not report the cocoa variety used (Lin & Choong, 2021).

**Fermentation methods**

Different fermentation scales and methods have been applied during the various yeast SCI fermentation trials examined (Tables 1–3 and references herein). Most of them have been applied at farm scale (more than 50 kg of fermenting cocoa pulp-bean mass), making use of wooden boxes in most cases. These large-scale fermentation trials are very suitable to study the effect of starter culture inoculation in traditional, on-farm, commercial cocoa fermentation processes. However, downscaling to small-scale fermentation processes (between 10 and 50 kg of fermenting cocoa pulp-bean mass) is more suitable to assess different yeast strains, cocoa varieties or conditions during a single field experiment. In general, the physicochemical conditions (pH and temperature), microbial succession and biochemical transformations that occur during small-scale cocoa fermentation processes are very reproducible compared to those on farm scale. Moreover, chocolates produced with cured cocoa beans from these processes can be successfully manufactured, achieving satisfactory quality standards. Some of these small-scale fermentation processes are still carried out in wooden boxes. Nevertheless, plastic boxes, trays, buckets, baskets or bags are mostly used, to obtain a more controlled and sterile environment at the start of the fermentation process. Finally, micro-fermentation processes (less than 10 kg of fermenting cocoa pulp-bean mass) have been applied. Typically, this strategy is followed to investigate many different yeast strains or to have a better control of the fermentation conditions (e.g. sterility and measurement of cocoa pulp sweatings). Although the microbial communities found are still very similar to those usually found at farm scale, some physicochemical conditions are not fulfilled, such as the increase in temperature, due to an insufficient fermenting mass. These trials are usually performed using laboratory equipment, such as measuring cylinders, Erlenmeyer flasks or glass jars, or other containers that also ensure sterile and controlled conditions, such as plastic boxes or stainless-steel bowls.

**Physicochemical, microbiological and chemical investigations**

To assess the effect of yeast starter culture inoculation on cocoa fermentation, cocoa liquors and/or chocolates, physicochemical, microbiological and/or chemical
| SCI trial | Cocoa variety (amount, kg) | Fermentation method | Yeast species (original name between brackets) | Effect on fermentation course | Effect on chocolate | References |
|-----------|---------------------------|---------------------|-----------------------------------------------|-------------------------------|--------------------|------------|
| 1         | NA (1000)                 | NA                  | Kluyveromyces marxianus (Zygosaccharomyces marxianus) | Faster rise of temperature   | No significant differences | Roelofsen (1958) |
|           |                           |                     | Saccharomyces cerevisiae (Saccharomyces fragilis) | Faster rise of temperature   | No significant differences |            |
| 2         | Amazonian hybrid (70)     | Plastic boxes       | Pectinolysis activity | Lower proportion of brown beans | No significant differences | Sanchez et al. (1985) |
|           |                           |                     | Candida zeylanoides | Lower proportion of brown beans | Significant differences. Lower quality |            |
|           |                           |                     | Kluyveromyces fragilis | Slower fermentation process | Significant differences. Lower quality |            |
| 3         | NA (100)                  | NA                  | Brettanomyces claussenii | NA                           | Significant differences in the sensory analysis. Successful triangle test | Sanchez (1989) |
|           |                           |                     | Candida famata | NA                           | Significant differences in the sensory analysis. Successful triangle test |            |
| 4         | Comum hybrid (200)        | Wooden boxes        | Pectinolysis activity | Phased inoculation leads to less fermented beans. Initial inoculation leads to a slightly faster fermentation process | No significant differences in the sensory analysis | Schwan (1998) |
|           |                           |                     | Ethanol tolerance | NA                           |                             |            |
| 5         | Forastero hybrid (500)    | Wooden boxes Heaps  | Consistent quality | Consistent ethanol and acetic acid production | Significant differences in the sensory analysis. Standardization of the end product | Lefeber et al. (2012) |
| 6         | Local hybrids (PH16, PS3030, FA13, PS239; 60) | Wooden boxes | Flavour | Faster consumption of carbohydrates. Enhanced ethanol production | NA | Ramos et al. (2014) |
| 7         | Local hybrid (PS1319; 100) | Wooden boxes | Stress tolerance | Faster consumption of carbohydrates. Enhanced ethanol production | Differences in the sensory analysis. Stronger coffee and sour notes. No significant differences in the acceptance | Batista et al. (2015) |
|           |                           |                     | Saccharomyces cerevisiae + Hanseniaspora uvarum + Pichia kluyveri | Differences in the sensory analysis. Stronger coffee and sour notes. No significant differences in the acceptance | More intense fruity notes |            |
| 8         | Local hybrid (50) Baskets | Thermo-tolerance Killer activity Pectinolytic activity | Saccharomyces cerevisiae | Reduced yeast diversity | Different aromatic profiles (cocoa liquors). Higher production of ethyl acetate and acetate esters (cocoa liquors). Significant differences in the sensory analysis. Preference for the inoculated ones | Meersman et al. (2015) |
| 9         | Local hybrid (PS1319; 100) | Wooden boxes | Stress tolerance | Different VOC profiles | Different VOC profiles. Higher isoamyl acetate and ethyl acetate | Batista et al. (2016) |
|           |                           |                     | Saccharomyces cerevisiae + Hanseniaspora uvarum + Pichia kluyveri | Different VOC profiles | Differences in the sensory analysis. More intense fruity notes |            |
| 10        | Local hybrid (50) Baskets | Thermo-tolerance Flavour | Saccharomyces cerevisiae | Reduced yeast diversity | Different VOC profiles (cocoa liquors). Higher production of ethyl acetate and acetate esters (cocoa liquors). Higher concentrations of volatiles (chocolates). Significant differences in the sensory analysis (chocolates) | Meersman et al. (2016) |
|           |                           |                     | Pichia kluyveri | No prevalence of the inoculated strain | Significant differences in the sensory analysis (chocolates). Lower concentrations of volatiles (chocolates) |            |
|           |                           |                     | Hanseniaspora uvarum | No prevalence of the inoculated strain | Significant differences in the sensory analysis (chocolates). Lower concentrations of volatiles (chocolates) |            |
| SCI trial | Cocoa variety (amount, kg) | Fermentation method | Starter culture selection | Yeast species (original name between brackets) | Effect on fermentation course | Effect on chocolate | References |
|-----------|---------------------------|---------------------|--------------------------|-----------------------------------------------|-----------------------------|---------------------|-------------|
| 11        | Local hybrids (CCN51, PS3030, FA13, CEPEC 2004; 100) | Wooden boxes | Improved quality | Saccharomyces cerevisiae | NA | Differences ascribed to cocoa hybrids (no comparison with spontaneous fermentation) | Menezes et al. (2016) |
| 12        | Local hybrid (PH16; 100) | Wooden boxes | Flavour | Saccharomyces cerevisiae<sup>a</sup> | Reduced microbial diversity. | Differences in the sensory analysis. Lower dominance of cocoa flavour, bitterness, astringency and acidity | Miguel et al. (2017) |
| 13        | Local hybrid (PH15; 100) | Wooden boxes | Flavour | Saccharomyces cerevisiae<sup>a,b</sup> | Faster consumption of carbohydrates. Enhanced ethanol production. Greater increase of pH and temperature. No clear differences in the VOC profiles | Significant differences in the sensory analysis. Dominance of bitterness, cocoa flavour and sweetness | Moreira et al. (2017) |
| 14        | Local hybrids (PS1319, S30; 300) | Wooden boxes | Pectinolysis activity Thermo-tolerance Stress tolerance | Saccharomyces cerevisiae Torulaspora delbrueckii | Faster consumption of carbohydrates. Enhanced ethanol production No prevalence of the inoculated strain | Significant differences in the sensory analysis. Different VOC profiles | Visintin et al. (2017) |
| 15        | Forastero hybrids (heap, 100: box, 200) | Heaps Wooden boxes | Pectinolysis activity Thermo-tolerance Stress tolerance | Saccharomyces cerevisiae Torulaspora delbrueckii | No significant differences in the VOC profiles | Significant differences in the sensory analysis. No differences in the VOCs profiles | Mota-Gutierrez et al. (2018) |
| 16        | Forastero (50) | Wooden boxes | Consistent quality | Saccharomyces cerevisiae<sup>a,b</sup> | No significant differences in the metabolites measured according to the inoculation, in contrast to the fermentation method | NA | Saunshi et al. (2019) |
| 17        | Trinitario Forastero (180) | Wooden boxes | Anti-fungal activity | Saccharomyces cerevisiae<sup>a</sup> | No significant differences in the metabolites measured according to the inoculation. Slower fermentation process Lower amount of well-fermented beans | No differences in the sensory analysis | Romanens et al. (2020) |
| SCI trial | Cocoa variety (amount, kg) | Fermentation method | Starter culture selection | Yeast species (original name between brackets) | Effect on fermentation course | Effect on chocolate | References |
|-----------|---------------------------|---------------------|--------------------------|-----------------------------------------------|-----------------------------|-------------------|------------|
| 18        | Forastero (Scavina; 70)   | Wooden boxes        | Improved quality         | *Rhodotorula mucilaginosa*                    | Increased concentrations of free amino acids. Higher percentage of brown beans | NA                 | Santos et al. (2020) |
|           |                           |                     |                          | *Torulaspora delbrueckii*                     | Retarded consumption of carbohydrates. Increased concentrations of free amino acids. Higher percentage of brown beans | NA                 |                        |
|           |                           |                     |                          | *Candida parapsilosis*                        | Retarded consumption of carbohydrates. Increased concentrations of free amino acids. Higher percentage of brown beans | NA                 |                        |
|           |                           |                     |                          | *Pichia galeformis*                           | Increased concentrations of free amino acids. Higher percentage of brown beans | NA                 |                        |
|           |                           |                     |                          | *Pichia klyveri*                              | Increased concentrations of free amino acids. Higher percentage of brown beans | NA                 |                        |
|           |                           |                     |                          | *Pichia kudriavzevii*                         | Enhanced consumption of carbohydrates. Higher percentage of brown beans | NA                 |                        |
|           |                           |                     |                          | *Saccharomyces cerevisiae*                    | Enhanced consumption of carbohydrates. Higher percentage of brown beans | NA                 |                        |
|           |                           |                     |                          | *Pichia membraniﬁcens*                       | Enhanced consumption of carbohydrates. Higher percentage of brown beans | NA                 |                        |
| 19        | Forastero (90)            | Wooden boxes        | Flavour                  | *Saccharomyces cerevisiae*                    | Higher organic acid concentrations. Higher pyrazine concentrations. | NA                 | Chagas Junior, Ferreira, Andrade, et al. (2021) |
|           |                           |                     |                          | *Pichia kudriavzevii*                         | Enhanced higher alcohol and aldehyde productions. | NA                 |                        |
|           |                           |                     |                          | *Saccharomyces cerevisiae + Pichia kudriavzevii* | Higher concentrations of esters. Higher pyrazine concentrations. | NA                 |                        |
TABLE 1 (Continued)

| SCI trial | Cocoa variety (amount, kg) | Fermentation method | Starter culture selection | Yeast species (original name between brackets) | Effect on fermentation course | Effect on chocolate | References |
|-----------|---------------------------|---------------------|---------------------------|-----------------------------------------------|----------------------------|-------------------|------------|
| 20        | Forastero (50)            | Wooden boxes        | Improved quality          | Saccharomyces cerevisiae                      | Faster increase of temperature. Highest acidity. Higher biogenic amine concentrations | NA                | Chagas Junior, Ferreira, Gloria, et al. (2021) |
|           |                           |                     |                           | Pichia kudriavzevii                          | Faster increase of temperature. Higher concentration of phenolic compounds. Higher phenylethylamine concentration | NA                |           |
|           |                           |                     |                           | Saccharomyces cerevisiae + Pichia kudriavzevii | Faster fermentation process. Faster increase of temperature. Higher concentrations of phenolic compounds. Higher concentrations of methylxanthines. Higher phenylethylamine concentration | NA                |           |
| 21        | Local hybrids (CEPFC 2002, FA13, 100) | Wooden boxes | Flavour                   | Saccharomyces cerevisiae                      | Differences in the VOC profiles. Differences in the protein profiles. | Differences in the VOC profiles. Higher number of organic acids. No significant differences in the sensory analysis. Described as sourer, fruitier, sweeter and less astringent | Moreira et al. (2021) |
|           |                           |                     |                           | Saccharomyces cerevisiae + Pichia kluyveri    | Differences in the protein profiles. | Differences in the VOC profiles. No significant differences in the sensory analysis. Described as bitter and sweeter but less sour |           |

Abbreviation: NA, not available.

aLactic acid bacterial strain used as part of the inoculum cocktail.
bAcetic acid bacterial strain used as part of the inoculum cocktail.
**TABLE 2** Overview of the main effects of the application of yeasts during small-scale starter culture-initiated (SCI) fermentation trials on the fermentation course and the chocolates produced from the concomitant cured cocoa beans. Comparisons are made with spontaneous fermentation processes, unless stated otherwise.

| SCI trial | Cocoa variety (amount, kg) | Fermentation method | Starter culture selection | Yeast species (original name between brackets) | Effect on fermentation course | Effect on chocolate | References |
|-----------|---------------------------|---------------------|---------------------------|-----------------------------------------------|-------------------------------|---------------------|------------|
| 1         | Local hybrid (Sabah; 10)  | Plastic bags        | Flavour potential         | *Saccharomyces cerevisiae* *(Saccharomyces chevalieri)* | Slower consumption of carbohydrates. Enhanced ethanol production. Enhanced acetic acid production | Differences in the sensory analysis | Samah et al. (1992) |
| 2         | NA (45)                   | Plastic baskets     | Pectinolysis activity     | *Kluyveromyces marxianus*                      | Increased protein degradation. Reduced acidity. Increased fermentation degree | Significant differences in the sensory analysis | Leal et al. (2008) |
| 3         | Forastero (20)            | Plastic trays       | Pectinolysis activity     | *Kluyveromyces marxianus* ^a,b^                 | Faster consumption of carbohydrates | Differences in the sensory analysis. Most bitter, sour and astringent. Lowest sweetness and general liking | Crafack et al. (2013) |
|           |                           |                     | Flavour                   | *Pichia kluyveri* ^a,b^                        |                              |                     |            |
| 4         | Forastero (NA)            | Plastic bags        | Improved quality          | *Saccharomyces cerevisiae*                     | Enhanced production of ethanol | NA                  | Cempaka et al. (2014) |
| SCI trial | Cocoa variety (amount, kg) | Fermentation method | Starter culture selection | Yeast species (original name between brackets) | Effect on fermentation course | Effect on chocolate | References |
|-----------|---------------------------|---------------------|--------------------------|-----------------------------------------------|--------------------------------|---------------------|------------|
| 5         | Forastero (20)            | Plastic trays       | Flavour                  | *Kluyveromyces marxianus*<sup>a,b</sup>         | Minor differences in the concentration of flavour precursors | Different VOC profiles (cocoa liquors and chocolates). Higher concentrations of higher alcohols and esters (cocoa liquors). Significant differences in the sensory analysis. Unsuccessful triangle test | Crafack et al. (2014) |
| 6         | Forastero (10)            | Wooden boxes        | Consistent quality       | *Pichia kluyveri*<sup>a,b</sup>               | Minor differences in the concentration of flavour precursors | Different VOC profiles (cocoa liquors and chocolates). Higher concentrations of higher alcohols and esters (cocoa liquors). Significant differences in the sensory analysis. Unsuccessful triangle test | Sandhya et al. (2016) |

*Notes:*
- **a** indicates differences in the concentration of flavour precursors.
- **b** indicates different VOC profiles (cocoa liquors and chocolates).
- **c** indicates higher concentrations of higher alcohols and esters (cocoa liquors).
- **d** indicates significant differences in the sensory analysis.
- **e** indicates unsuccessful triangle test.

(Continues)
| SCI trial | Cocoa variety (amount, kg) | Fermentation method | Starter culture selection | Yeast species (original name between brackets) | Effect on fermentation course | Effect on chocolate | References |
|----------|---------------------------|---------------------|---------------------------|-----------------------------------------------|-----------------------------|-------------------|------------|
| 7        | Forastero (10)            | Wooden boxes        | Improved quality          | *Saccharomyces cerevisiae*<sup>a,b</sup>       | Differences ascribed to the soaking treatment of the cocoa beans (no comparison with spontaneous fermentation) | NA                | Saunshia et al. (2018) |
| 8        | Forastero x Trinitario hybrid (30) | Plastic boxes | Flavour | *Saccharomyces cerevisiae*<sup>a,b</sup> | Enhanced production of VOCs | Differences in the sensory analysis. Unsuccessful triangle test | Assi-Clair et al. (2019) |
| 9        | Trinitario (20)           | Plastic boxes       | Consistent quality Flavour | *Saccharomyces cerevisiae*<sup>a,b</sup> | Faster fermentation process. Greater increase of temperature. Faster consumption of carbohydrates. Enhanced production of ethanol. Enhanced production of VOCs | NA                | Díaz-Muñoz et al. (2021) |
|          |                           |                     |                           | *Pichia kudriavzevii*<sup>a,b</sup> | Enhanced production of VOCs | NA                |            |
|          |                           |                     |                           | *Saccharomyces cerevisiae* + *Pichia kudriavzevii*<sup>a,b</sup> | Faster consumption of carbohydrates. Enhanced production of ethanol. Enhanced production of VOCs | NA                |            |
| 10       | NA (20)                   | Wooden boxes        | Improved quality          | *Saccharomyces cerevisiae*<sup>a,b</sup>       | No significant differences in the pH and total acidity between spontaneous and yeast starter culture inoculations | Similar aroma profile between spontaneous and yeast starter culture inoculations. No significant differences in the sensory analysis | Lin and Choong (2021) |
analyses have to be performed through the application of different techniques (Figure 4 and references herein). During most of the yeast SCI fermentation trials, the pH and temperature of the fermenting mass (pulp) have been analysed and for only some the inner pH of the cocoa beans has also been checked. Cocoa pulp sweatings have only been analysed in three fermentation trials, whereas the measurement of their total titratable acidity has become a tendency in recent years as a measurement of the organic acid dynamics during cocoa fermentation.

The physicochemical changes that occur throughout cocoa fermentation processes depend on the microbial activities and, therefore, the yeast starter culture strain(s) used. Thus, culture-dependent and/or culture-independent microbiological techniques have been applied to monitor the growth and prevalence of the starter culture strain(s) used. In what follows, both yeasts and bacteria will be considered, as the starter cultures used for cocoa fermentation are usually composed of strains of species of both microbial groups (Tables 1–3). Culture-dependent techniques rely on the incubation of the microbiota plated on selective agar media and the identification of picked isolates, either through morphological and phenotypical characterization or through the application of molecular techniques. Concerning the latter, protein-based techniques, such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), and genomic DNA-based techniques, such as rep-polymerase chain reaction (PCR) fingerprinting, pulsed-field gel electrophoresis, restriction fragment length polymorphism and interdelta analysis, can be distinguished. These techniques are usually coupled to DNA sequencing of phylogenetic markers of cluster-representative isolates or electrophoresis bands. Sanger sequencing of long DNA fragments of the 16S rRNA gene for bacteria and the D1/D2 domain of the 26S rRNA gene, the ACT1 gene and/or the internal transcribed spacer (ITS) region for yeasts is usually performed, achieving a species-level taxonomic identification. The biggest disadvantages of culture-dependent techniques are the identification of only culturable micro-organisms and their differential growing capacities, which may disrupt their relative abundances. Consequently, culture-independent techniques have been applied by targeting whole-community DNA extracted and purified directly from the cocoa pulp-bean mass. Thus, quantitative PCR (qPCR), PCR-denaturing gradient gel electrophoresis (DGGE) and amplicon-based sequencing have been applied to monitor starter cultures used in cocoa fermentation processes. However, differences in the taxonomic resolution of these techniques have to be taken into account. qPCR has been applied to monitor the cocoa microbiota at species level, as it relies on the use of species-specific primers designed
Table 3 Overview of the main effects of the application of yeasts during micro-scale starter culture-initiated (SCI) fermentation trials on the fermentation course and the chocolates produced from the concomitant cured cocoa beans. Comparisons are made with spontaneous fermentation processes.

| SCI trial | Cocoa variety (amount, kg) | Fermentation method | Starter culture selection | Yeast species (original name between brackets) | Effect on fermentation course | Effect on chocolate | References |
|-----------|----------------------------|---------------------|---------------------------|-----------------------------------------------|-------------------------------|---------------------|------------|
| 1         | Forastero hybrid (1)       | Measuring cylinder  | Pectinolysis activity    | *Saccharomyces cerevisiae*                   | Increased sweating yields. Faster pulp liquefaction | NA                  | Buamah et al. (1997) |
|           |                            |                     |                           | *Kluyveromyces fragilis*                      | Increased sweating yields. Faster pulp liquefaction | NA                  |            |
|           |                            |                     |                           | *Candida famata* (Torulopsis candida)         | Increased sweating yields   | NA                  |            |
|           |                            |                     |                           | *Candida norvegensis*                         | Increased sweating yields   | NA                  |            |
| 2         | Forastero hybrid (1)       | Measuring cylinder  | Pectinolysis activity    | *Saccharomyces cerevisiae*                   | Faster consumption of carbohydrates | NA                  | Dzogbefia et al. (1999) |
|           |                            |                     |                           | *Saccharomyces chevalieri*                    | Faster consumption of carbohydrates | NA                  |            |
|           |                            |                     |                           | *Kluyveromyces fragilis*                      | Faster consumption of carbohydrates | NA                  |            |
|           |                            |                     |                           | *Candida famata*                              | Faster consumption of carbohydrates | NA                  |            |
|           |                            |                     |                           | *Candida norvegensis*                         | Faster consumption of carbohydrates | NA                  |            |
| 3         | NA (5)                     | Stainless steel bowl| Anti-fungal activity     | *Candida sp.*                                 | No differences              | NA                  | Mahazar et al. (2015) |
| 4         | NA (0.1)                   | Glass jar           | NA                        | *Saccharomyces cerevisiae*                    | No differences              | NA                  | Apriyanto et al. (2017) |
| 5         | NA (0.4)                   | Erlenmeyer flasks   | Flavour                   | *Pichia kudriavzevii*                        | No differences              | NA                  | Pereira et al. (2017) |
| 6         | Trinitario (3.5)           | Plastic boxes       | Functional role of microbial groups | *Hanseniaspora guilliermondii + Pichia kudriavzevii + Kluyveromyces marxianus + Saccharomyces cerevisiae* | Faster fermentation process. Enhanced ethanol production. Higher number of VOCs detected | No significant differences in the sensory analysis. Unsuccessful triangle test | Ho et al. (2018) |

Abbreviation: NA, not available.

*a*Lactic acid bacterial strain used as part of the inoculum cocktail.

*b*Acetic acid bacterial strain used as part of the inoculum cocktail.
for the yeast species of interest, typically those of the inoculated strains. Also DGGE relies on the use of primers that target only pre-defined microbial groups or species. The DNA regions targeted are usually parts of the 26S rRNA gene for yeasts and the 16S rRNA gene for LAB and AAB. Albeit that rRNA-PCR-DGGE can be a strategy to achieve species-level identification, it does not characterize the entire microbial communities, especially when the bacterial 16S rRNA regions are targeted. Therefore, direct amplicon-based sequencing of the V4 hypervariable region of the bacterial 16S rRNA gene and of the ITS1 region of yeast DNA in whole-community DNA is more suitable to obtain a more complete picture of the microbial communities present during fermentation. However, the fragment length of the amplified region is limited by the capacities of second-generation sequencing technologies (250–300 bp), which diminishes the taxonomic resolution. Thus, the amplification of this region leads to the obtainment of short reads, which only allows genus-level identification, as the resulting amplicons used to be clustered into operational taxonomic units (OTUs) using a dissimilarity threshold. The application of more advanced bioinformatic pipelines, such as those to obtain amplicon sequence variants (ASVs) and oligotypes, has allowed to obtain a finer taxonomic resolution to analyse whole-community DNA from spontaneous cocoa fermentation processes and a more accurate monitoring when starter cultures are applied (Díaz-Muñoz et al., 2021; Pacheco-Montealegre et al., 2020). Although following different approaches, both methodologies rely on a sequencing error-aware model to curate amplicon sequences and unravel variants contained within OTUs, as differences as little as one nucleotide can be potentially resolved (Callahan et al., 2016; Eren et al., 2013). Another strategy to approach a strain-level monitoring of yeast starter cultures is an interdelta analysis, albeit culture dependently, which relies on the amplification of delta elements from the S. cerevisiae retrotransposons TY1 and TY2 (Meersman et al., 2015, 2016). However, the latter strategy can only be used to monitor S. cerevisiae strains. In contrast, it

**FIGURE 4** Overview of the physicochemical parameters measured (pink), the culture-dependent (light green) and/or culture-independent (dark green) microbiological analysis carried out, and the metabolite target analysis performed (beige) for each of the starter culture-initiated fermentation trials listed. MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; MPC, morphological and phenotypical characterization; PCR-DGGE, polymerase chain reaction-denaturing gradient gel electrophoresis; PFGE, pulsed-field gel electrophoresis; RFLP, restriction fragment length polymorphism; qPCR, quantitative polymerase chain reaction; VOCs, volatile organic compounds. Metabolites have been analysed during the fermentation step, drying step or in cured cocoa beans, unless stated otherwise (L, cocoa liquors; C, chocolate)
has been demonstrated recently that ASV analysis can be suitable to differentiate inoculated strains of various bacterial and yeast species from environmental variants belonging to the same taxa and thus fine-monitoring starter cultures (Díaz-Muñoz et al., 2021). The future is, nevertheless, promising for the development of new strategies to achieve an accurate strain-level monitoring of the starter cultures used. Novel long-read amplicon-based sequencing methods are becoming available, which can improve the resolution of the ASV analysis (Callahan et al., 2019). Also, shotgun metagenomics of whole-community DNA from spontaneous cocoa fermentation processes, encompassing metagenomic recruitment plotting and metagenome-assembled genomes, have demonstrated to be a valuable technique to study the cocoa microbiota in a precise way (Agyirifo et al., 2019; Almeida & De Martinis, 2021; Illeghems et al., 2015; Lima et al., 2021; Serra et al., 2019; Verce et al., 2021; Viesser, Pereira, Neto, Favero, et al., 2021). Moreover, various bioinformatic strategies to use shotgun metagenomics for strain identification have been developed (Yan et al., 2020). Although such studies have been mostly applied to analyse the human microbiome, they open up the possibility to be used for strain-level monitoring of starter cultures during fermentation of the cocoa pulp-bean mass.

Once the microbiological strategy to follow the starter culture prevalence(s) during fermentation is determined, several chemical techniques (mostly based on chromatographic separation coupled to various detection techniques, including mass spectrometry) are applied to assess the influence of the starter culture(s) on the substrate consumption and metabolite production dynamics (Figure 4 and references herein). Saccharides, organic acids, sugar alcohols and ethanol are the most quantified compounds, since they provide the most essential information about the fermentation course, such as the consumption of sucrose, glucose, fructose and citric acid, and the production of ethanol, glycerol, mannitol, lactic acid and acetic acid. The production of these metabolites has a primary impact on the proper development of the fermentation process and, therefore, their targeted analysis is of paramount importance to assess the contribution of starter culture(s) to this fermentation process and the curing of cocoa beans. To better study the effect of starter culture(s) on flavour development during fermentation, in particular VOCs are targeted in a qualitative (Moreira et al., 2017, 2021; Pereira et al., 2017), semi-quantitative (Batista et al., 2016; Chagas Junior, Ferreira, Andrade, et al., 2021; Crafack et al., 2014; Ho et al., 2018; Lin & Choong, 2021; Meersman et al., 2015, 2016; Menezes et al., 2016; Mota-Gutierrez et al., 2018; Ramos et al., 2014; Saunshia et al., 2018; Visintin et al., 2017) or quantitative way (Assi-Clair et al., 2019; Díaz-Muñoz et al., 2021). The semi-quantitative approach provides a fingerprint of the VOC profiles of fermenting and/or cured cocoa beans, whereby the latter ones in combination with a quantitative approach are most suitable to obtain a precise view of the VOCs produced or transformed during fermentation. The VOC profiles of cocoa liquors and/or chocolates are also sometimes targeted, as the influence of the starter culture(s) can also be noticed in the end products (Figure 4). Sensory analyses, including triangle tests, further contribute to the determination of significant differences between inoculated and non-inoculated fermentation batches (Tables 1–3 and references herein). Regarding phytochemical compounds, the alkaloids, polyphenols, amino acids and derivatives, and/or biogenic amines are sometimes targeted, although no clear influence of the starter culture(s) applied is usually found (Figure 4; Tables 1–3; and references herein). Other compounds targeted during yeast SCI fermentation trials are proteins and/or metals, among others.

**Screening of candidate yeast starter culture strains**

Most of the yeasts used as candidate starter culture strains are selected because of their proven prevalence and performance in spontaneous cocoa fermentation processes (Figure 1). Nevertheless, a screening is sometimes performed to ensure the use of the best performing strains regarding pectin degradation, saccharide consumption and ethanol production, tolerance to stress conditions, and/or VOC production (Figueroa-Hernández et al., 2019; Koffi et al., 2018; Pereira et al., 2016, 2020). Furthermore, besides selecting natural yeast strains with improved qualities, researchers have also developed breeding strategies to generate hybrid yeast strains with increased pectinolytic activity, thermostolerance and/or aromatic potential (Leal et al., 2008; Meersman et al., 2015, 2016). Most of the earlier yeast SCI fermentation trials have focused on the selection of yeast strains with an increased pectinolytic activity, since this could favour the desirable course of the fermentation process and even accelerate it (Buamah et al., 1997; Crafack et al., 2013; Dzogbefia et al., 1999; Leal et al., 2008; Meersman et al., 2015; Mota-Gutierrez et al., 2018; Sanchez et al., 1985; Schwan, 1998; Visintin et al., 2017). Therefore, several screening experiments to select candidate yeast strains with the highest polygalacturonase activity have been performed (Crafack et al., 2013; Daniel et al., 2009; Delgado-Ospina et al., 2020; Leal et al., 2008; Meersman et al., 2015, 2017; Samagaci et al., 2014a,b, 2016; Schwan et al., 1997). Alternatively, the activity of underreported yeast enzymes that could play a role in cocoa fermentation processes, including cellulase, β-glucosidase, laccase, lipase and xylanase, has...
been screened for (Delgado-Ospina et al., 2020). Hence, it turned out that a high enzyme diversity is found, both at interspecies and intraspecies level, with barely any strain displaying high activity for more than one of those enzymes. The tolerance towards different stress conditions typically encountered in cocoa fermentation processes has often been tackled so that robust isolates are selected as candidate yeast starter culture strains, ensuring their prevalence throughout the entire process (Daniel et al., 2009; Delgado-Ospina et al., 2020; Koffi et al., 2018; Meersman et al., 2015, 2016; Pereira et al., 2012; Romanens et al., 2019; Samagaci et al., 2014a, 2016; Visintin et al., 2016). At the initial stages of cocoa fermentation processes, yeasts must grow in a sugar-rich environment with a high-osmolarity pressure (Pereira et al., 2012; Visintin et al., 2016). It has been recently found that \textit{S. cerevisiae} strains isolated from cocoa contain a genetic adaptation that makes them more resistant to high-osmolarity environments, just as it happens with \textit{S. cerevisiae} strains involved in wine fermentation processes (Gonçalves et al., 2016; C. Díaz-Muñoz, M. Verce, L. De Vuyst, and S. Weckx, unpublished results). Furthermore, yeasts must be tolerant to increasing ethanol and lactic acid concentrations in the fermenting mass. Again, \textit{S. cerevisiae} is known to be an ethanol-tolerant yeast, a reason why it has been the focus of studies aiming at elucidating the main factors behind yeasts’ tolerance and stress responses (Snoek et al., 2016; Stanley et al., 2010; Vamvakas & Kapolos, 2020). Whereas the ethanol stress response consists of a reprogramming of the cell’s activities to survive until a recovery is possible, ethanol tolerance refers to the capacity to grow during prolonged ethanol exposure. However, as there are many different factors influencing ethanol tolerance in yeasts, it is difficult to identify the exact mechanisms present in \textit{S. cerevisiae} and other non-\textit{Saccharomyces} ethanol-tolerant yeast species representative for cocoa fermentation processes (e.g. \textit{P. kudriavzevii}, \textit{P. kluyveri}, \textit{Kazachstania humilis}, \textit{C. nitrativorans}) and absent in ethanol-sensitive yeasts, such as \textit{Hanseniaspora} spp. Finally, towards the late stages of the cocoa fermentation course, acetic acid concentrations and high temperatures are reached. Strains of \textit{S. cerevisiae} and \textit{P. kudriavzevii} are usually well adapted to these stressing conditions and these species are the best performing at the highest temperatures and/or acetic acid concentrations of fermenting cocoa pulp-bean mass, which are the biggest driving forces behind the die-off of yeasts upon fermentation (Daniel et al., 2009; Delgado-Ospina et al., 2020; Koffi et al., 2018; Meersman et al., 2015; Pereira et al., 2012; Romanens et al., 2019; Samagaci et al., 2016; Visintin et al., 2016). An analysis of the metatranscriptome of spontaneous cocoa fermentation processes has further identified different expression levels of stress response genes in \textit{S. cerevisiae} compared to \textit{H. opuntiae}, as well as a shift of gene expression from the early to late stages of cocoa fermentation (Verce et al., 2021). Also, the production of trehalose under varying conditions has been examined to understand the stress response in yeast strains of cocoa origin, since changes in the cell membrane composition and carbohydrate metabolism are part of the yeast response towards stress conditions (Delgado-Ospina et al., 2020; Pereira et al., 2020). These studies support the idea that different yeast species and genera cope with stress conditions in very different ways. Some researchers have selected candidate yeast strains (mainly \textit{S. cerevisiae}) according to their fermentative capacity and, therefore, their ethanol production (Koffi et al., 2018; Pereira et al., 2012; Samagaci et al., 2016). The ability to generate high quantities of ethanol, which can be oxidized to acetic acid by AAB upon fermentation, is directly linked to a highly fermentative metabolism, which ensures a fast consumption of carbohydrates and, hence, a fast fermentation process. Some authors also emphasize the importance of ethanol in the colour development of the beans, through the activation of glycosidases and polyphenol oxidases (Koffi et al., 2018). Indeed, a high production of ethanol ensures an optimal development of the fermentation process through its oxidation to acetic acid, in turn causing the death of the seed embryo and the activation of endogenous enzymes (Andersson et al., 2006; De Vuyst & Leroy, 2020; Kadow et al., 2015). The ability of many yeast species to produce killer toxins to outcompete other yeasts has also been examined (Meersman et al., 2016; Romanens et al., 2019; Ruggirello et al., 2019; Samagaci et al., 2016). Although antifungal activity may be of importance for a candidate yeast starter culture strain to prevail over the background microbiota and avoid the growth of undesired filamentous fungi, it has not been the focus of most researchers, since other parameters, such as fast fermentation and tolerance towards stress conditions are thought to be more relevant.

Many SCI fermentation trials rely on a single yeast strain as the sole micro-organism inoculated, considering the use of citrate-positive LAB and/or ethanol- and acetic acid-oxidizing AAB strains unnecessary (Tables 1–3). Therefore, the selection of yeast strains capable to assimilate citrate becomes pertinent. Accordingly, a few screening studies have analysed the ability of yeast strains isolated from fermenting cocoa pulp-bean mass to assimilate citrate and/or grow in media with citric acid as the sole carbon source (Daniel et al., 2009; Samagaci et al., 2016). Isolates of \textit{Candida} species, \textit{P. kudriavzevii} and \textit{P. kluyveri} are often reported as citrate positive (Daniel et al., 2009; Samagaci et al., 2016; Visintin et al., 2016). Nevertheless, opposite results have also been reported (Jespersen et al., 2005; Romanens et al., 2019).

Finally, an increasing interest in cocoa flavour enhancement by yeasts has resulted in screening studies assessing
flavour production by candidate strains (Koné et al., 2016; Meersman et al., 2016; Mota-Gutierrez et al., 2019; Pereira et al., 2017, 2020). Either the ability to produce isoamyl acetate and ethyl acetate (Meersman et al., 2016) or the production of acetaldehyde and ethyl acetate (Pereira et al., 2017) has been the focus of such investigations. Moreover, a VOC fingerprinting is also used to obtain a more general view of the flavour production capacity of yeast isolates (Koné et al., 2016).

**Effects on cocoa fermentation and chocolates produced thereof**

Advantages that can be obtained with SCI fermentation processes are a fast fermentation progress, a reproducible end product ensuring a standard quality of cured cocoa beans and chocolates produced thereof, an enhanced VOC production upon fermentation, and different flavour notes in the chocolates produced from the concomitant cured cocoa beans (Tables 1–3 and references herein). Nevertheless, the effect of the starter culture strain(s) inoculated may depend on the cocoa variety, the fermentation method and the inoculation titre, besides the yeast strain(s) used. Strains of 22 different yeast species have been proposed as candidate yeast starter cultures, with strains of *S. cerevisiae* being, by far, the most studied ones, as they appear in 30 yeast SCI fermentation trials (Figure 1 and Tables 1–3). Indeed, *S. cerevisiae* is one of the most extensively used yeast species as starter culture in the production of many fermented foods, such as beer, wine or (sourdough) bakery products (Parapouli et al., 2020; Sicard & Legras, 2011; Steensels & Verstrepen, 2014). The ability of this budding yeast to ferment a wide variety of carbohydrates, together with its tolerance to several environmental conditions, may explain both its wide appearance in spontaneous cocoa fermentation processes and its success when used as starter culture (Meersman et al., 2015; Papalexandratou et al., 2013; Steensels & Verstrepen, 2014). The inoculation of *S. cerevisiae* strains very often leads to faster fermentation processes, as a result of a faster consumption of carbohydrates, and an enhanced production of ethanol, although the opposite has also been reported (Romanens et al., 2020; Samah et al., 1992). *Saccharomyces cerevisiae* is very well adapted to cocoa fermentation conditions, prevailing over the background microbiota, which leads to a reduced yeast diversity in fermentation processes initiated with strains of this yeast species (Díaz-Muñoz et al., 2021; Meersman et al., 2015, 2016; Miguel et al., 2017). Finally, rich VOC profiles in cured cocoa beans, cocoa liquors and/or chocolates have been reported to be a consequence of its inoculation (Figure 5 and Tables 1–3). Being encountered in spontaneous cocoa fermentation processes, strains of *P. kluyveri* (seven trials) and *P. kudriavzevii* (six trials) have also been applied extensively as yeast starter culture species (Figure 1). The production of a wide variety of flavour-active compounds usually justifies the application of strains of these yeast species and, therefore, an impact of their inoculation on the VOC profiles of cured cocoa beans, cocoa liquors and/or chocolates. Similarly to *S. cerevisiae*, the inoculation of strains of *Pichia* species can result in a fast consumption of carbohydrates and an enhanced ethanol production, a consequence of their highly fermentative metabolism. Nevertheless, an unsuccessful prevalence of the inoculated strains throughout the fermentation course has been reported in a few cases (Batista et al., 2015; Meersman et al., 2016; Visintin et al., 2017). Regarding *Hanseniaspora* species, which is a yeast highly encountered in the initial phases of spontaneous cocoa fermentation processes, only a few trials have been performed (Batista et al., 2015, 2016; Ho et al., 2018; C. Díaz-Muñoz, D. Van de Voorde, E. Tuenter, V. Lemaarcq, D. Vandewalle, J. Pedro Maio, A. Mencia, C. Hernandez-Aguirre, A. Comasio, E. Sioriki, S. Weckx, L. Pieters, K. Dewettinck, and L. De Vuyst; unpublished results). Moreover, as they were always inoculated together with other yeast strains, it is unclear to determine their impact on the fermentation process and/or the chocolates produced therefrom. Furthermore, the microbiological analyses performed in these cases have demonstrated the lack of growth (Batista et al., 2016) or a fast die-off (Ho et al., 2018). Oppositely, strains of *K. marxianus* (five trials) and *K. fragilis* (three trials) have been tested in SCI fermentation processes, although these yeast species are not often reported in spontaneous ones (Ho et al., 2014, 2015; Mota-Gutierrez et al., 2018). However, their use as starter culture strains can be justified by their high pectinolytic activity (Buamah et al., 1997; Crafack et al., 2013, 2014; Dzogbefia et al., 1999; Leal et al., 2008; Roelofs, 1958; Sanchez et al., 1985). Yet, only two trials have reported cocoa fermentation processes with increased sweating yields as a consequence of their inoculation (Buamah et al., 1997; Leal et al., 2008). Other reported yeast starter culture strains belong to species of the genus *Candida*, namely *Candida famata* (three trials), *C. norvegensis* (two trials), *Candida parapsilosis* (one trial) and *C. tropicalis* (one trial). This yeast genus is often found in spontaneous cocoa fermentation processes, although rarely as the prevailing yeast throughout the entire course. Its contribution to fermentation of the cocoa pulp-bean mass has not been studied extensively, partly due to its common appearance only in the background microbiota. Nevertheless, strains of species of this genus have been tested to increase the pectinolytic activity or inhibit the growth of undesired micro-organisms (Buamah et al., 1997; Dzogbefia et al., 1999; Mahazar et al., 2015; Sanchez
et al., 1985). Finally, strains of *Torulaspora delbrueckii* (three trials) have been tested as members of inoculation mixtures (Mota-Gutierrez et al., 2018; Santos et al., 2020; Visintin et al., 2017). *Torulaspora delbrueckii* is sometimes found at the initial stages of spontaneous cocoa fermentation processes and it has already been used as starter culture in wine production because of its tolerance to high saccharide concentrations (Parapouli et al., 2020; Visintin et al., 2016). However, the prevalence of the inoculated strains throughout the fermentation course is not always successful. Furthermore, they contribute poorly to the flavour profile of cured cocoa beans.

Besides their positive effects on the fermentation progress, the influence of SCI fermentation processes on the chocolates produced is often evaluated by comparing their sensory characteristics with chocolates from cured cocoa beans of spontaneously fermented ones. In general, a dichotomy in the results is found (Tables 1–3). Whereas many fermentation trials have shown a difference in the sensory analysis of chocolates from cured cocoa beans of SCI and spontaneous fermentation processes, suggesting an impact of the inoculated strain(s) on the flavour characteristics of the end product, others failed to achieve such output. In particular, triangle tests have never proved a significant

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**FIGURE 5** Network of volatile organic compounds (VOCs) produced and linked with the starter culture yeast species inoculated during starter culture-initiated fermentation trials. The 20 most occurring VOCs reported in at least two trials were used to construct the network. The edge width is proportional to the number of times a VOC was linked with a yeast species. The colours are representative for the group of chemical compounds, namely alcohols (blue), aldehydes (green), esters (red) and ketones (purple). The yeast species involved are indicated in orange.
difference between the chocolates produced from SCI fermentation processes and spontaneous ones (Assi-Clair et al., 2019; Crafack et al., 2014; Ho et al., 2018), except for one study (Sanchez, 1989). Consequently, a systematic analysis of the microbiology and (bio)chemistry of the whole cocoa chain (from fresh cocoa beans to fermented cocoa beans, dried cocoa beans, roasted cocoa, cocoa liquors and the final chocolates), aiming at linking the fermentation, drying and further cocoa processing steps with a sensory analysis is necessary. As such, the importance of the selection of an appropriate yeast starter culture strain, the inoculation titre, and fermentation and drying methods and durations, as well as the roasting and consecutive steps of chocolate manufacturing can be estimated. Indeed, some studies have analysed the VOCs present in cured cocoa beans and in roasted and unroasted cocoa liquors and/or chocolates, reporting a significant influence of chocolate manufacturing (Batista et al., 2016; Crafack et al., 2014; Meersman et al., 2016; Moreira et al., 2017, 2021; Mota-Gutierrez et al., 2018; Pereira et al., 2017; Ramos et al., 2014; Viessler, Pereira, Neto, Rogez, et al., 2021; Visintin et al., 2017). In addition, the 20 VOCs that were most reported in the above-mentioned studies were used for this network construction.

Most occurring VOCs could be linked with different strains of yeast species that have been applied, suggesting that many yeast species are able to contribute to VOC production during cocoa fermentation processes. The yeast species linked with the greatest number of VOCs was S. cerevisiae, which could be expected, since strains of this yeast species have been linked with the production of many flavour compounds not only in cocoa but also in other food fermentation processes, and all necessary genes to produce these compounds are well documented in this yeast species (De Vuyst & Leroy, 2020; Dzialo et al., 2017). Moreover, strains of S. cerevisiae are the most applied to steer cocoa fermentation processes, which undoubtedly influenced the statistical approach. Regarding non- Saccharomyces yeast species, three Pichia species were also linked with a high number of VOCs, in this case demonstrating a different potential of VOC production among them. Whereas P. kluveri could be linked with acetoin (creamy), ethyl benzoate (flowery), ethyl dodecanoate (floral), ethyl octanoate (apricot), 2-pentanone (fruity) and phenyl acetalddehyde (floral/honey), P. kudriavzevii was linked with benzaldehyde (bitter/almond), 2,3-butanediol (creamy), ethyl acetate (solvent/fruity), 3-methyl butanal (chocolate), 2-methyl-1-butanol (winy), 3-methyl-1-butanol (malty) and 2-nonanone (fruity). Interestingly, the VOCs linked with P. fermentans were shared with those linked with P. kudriavzevii but not with P. kluveri. Torulaspora delbrueckii and K. marxianus could also be linked to a few VOCs, although to a much less extent, which was expected since the former yeast species usually occurs only at the initial stages of the fermentation process and the latter one is associated with a high pectinolytic activity and not with flavour production. Since most of these VOCs share common pathways for their production, the origin of the variability in VOC production may be explained at gene expression level or substrate specificity of the enzymes involved. All yeast species were linked with the production of 2-phenyl ethanol (rosy) and 2-phenyl acetate (fruity), except for P. fermentans, suggesting an active conversion of phenylalanine by yeasts during cocoa fermentation processes. The metabolism of amino acids through the Ehrlich pathway counts for the production of many of the VOCs examined, namely 2-methyl butanal (chocolate)

FLAVOUR-ACTIVE COMPOUND PRODUCTION BY INOCULATED YEASTS

Since spontaneous cocoa fermentation processes consist of a complex ecosystem that contains a wide yeast species diversity, it becomes difficult to link the production of specific VOCs to a single yeast strain or species, albeit that the LAB or AAB species involved should not be neglected. Nonetheless, an attempt to link the production of six VOCs of special interest with specific micro-organisms has recently been performed, showing that many yeast species are responsible for their production (Mota-Gutierrez et al., 2019). Indeed, by steering the cocoa fermentation process through the inoculation of starter cultures the microbial diversity decreases, enabling to establish correlations between the strains inoculated and the VOCs produced (Díaz-Muñoz et al., 2021; Mota-Gutierrez et al., 2018). For instance, whereas S. cerevisiae IMDO 050523 has been linked with isoamyl acetate, P. kudriavzevii IMDO 020508 has been linked with 3-methyl-butanal, 2-phenylethanol and ethyl decanoate (Díaz-Muñoz et al., 2021). Therefore, in the present review, a network was constructed by performing a meta-analysis of the VOCs detected in yeast SCI fermentation processes (albeit that in many of them also LAB and AAB species were present; Tables 1–3) and their absence in spontaneous ones and/or produced in higher concentrations when a starter culture was applied (Figure 5). Only SCI fermentation trials that measured VOCs (qualitatively, semi-quantitatively or quantitatively) were taken into account for this analysis (Assi-Clair et al., 2019; Batista et al., 2016; Chagas Junior, Ferreira, Andrade, et al., 2021; Crafack et al., 2014; Díaz-Muñoz et al., 2021; Meersman et al., 2015, 2016; Menezes et al., 2016; Moreira et al., 2017, 2021; Mota-Gutierrez et al., 2018; Pereira et al., 2017; Ramos et al., 2014; Viessler, Pereira, Neto, Rogez, et al., 2021; Visintin et al., 2017).
and 2-methyl-1-butanol from isoleucine, 3-methyl butanol, 3-methyl-1-butanol, and isoamyl acetate (banana) from leucine, and phenyl acetaldehyde, 2-phenyl ethanol and 2-phenyl acetate from phenylalanine (Dzialo et al., 2017). Interestingly, the acetate ester derived from isoleucine (2-methylbutyl acetate; fruity) could not be linked with any of the yeast starter cultures applied, whereas 1-butanol (from threonine; fruity) and isobutyl acetate (from valine; herby) were linked with S. cerevisiae and/or P. kluyveri, although in isolated cases (Batista et al., 2016; Díaz-Muñoz et al., 2021). Moreover, many ethyl esters were linked with the starter cultures applied. Except for ethyl acetate that originates from the condensation of ethanol and acetyl-CoA, long-chain ethyl esters derive from lipid metabolism through the condensation of long-chain fatty acids and ethanol. They may survive the chocolate manufacturing process better than short-chain esters (Meersman et al., 2016). Acetoin and 2,3-butanediol are typical fermentation products originating from pyruvate metabolism (Dzialo et al., 2017). Interestingly, S. cerevisiae could be linked to both compounds, whereas P. kluyveri could only be linked with acetoin and P. kudriavzevii and P. fermentans with 2,3-butanediol. Benzaldehyde has been reported to be present in fresh cocoa pulp (Cevallos-Cevallos et al., 2018), although it can also be produced through yeast metabolism by an additional decarboxylation of phenyl acetaldehyde, which would explain the presence of benzyl alcohol, its reduced product. The ketones 2-nonanone and 2-pentanone could also be linked with S. cerevisiae, P. kluyveri and P. kudriavzevii, although they probably originate from the plant, since these compounds have been detected in fresh cocoa pulp (Cevallos-Cevallos et al., 2018; Ho et al., 2015; Kadow et al., 2013; Moreira et al., 2017). A reason behind the link between these compounds and the yeast strains involved, based on the higher concentration of these ketones found in inoculated cocoa pulp-bean mass, could rather be an indirect role of the yeasts by contributing to fermentation conditions that avoid these ketones to be converted throughout the fermentation process.

**CONCLUSION**

The use of yeast starter cultures has shown to improve the quality and organoleptic properties of cured cocoa beans and chocolates produced thereof. However, a thorough screening as to select the best performing candidate yeast starter culture strains, their accurate monitoring during fermentation, and a multiphasic approach to assess their metabolic contribution to the fermentation and chocolate manufacturing processes, is of paramount importance to ensure their influence on the final chocolates. Future research is further needed to accomplish an accurate strain-level monitoring of the starter cultures used as well as to follow key flavour-active compounds from the fresh cocoa beans throughout their fermentation and drying process and each step of the chocolate manufacturing process.

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**CONFLICT OF INTEREST**

No conflict of interest declared.

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