

# Optimizing chocolate production through traceability: A review of the influence of farming practices on cocoa bean quality

Rolando Saltini <sup>a</sup>, Renzo Akkerman <sup>a\*</sup>, Stina Frosch <sup>b</sup>

<sup>a</sup> Department of Management Engineering, Technical University of Denmark, Kgs. Lyngby (Copenhagen), Denmark.

<sup>b</sup> National Food Institute, Technical University of Denmark, Kgs. Lyngby (Copenhagen), Denmark.

\* Corresponding author. Tel. +45 45254736; fax: +4545256005. E-mail address: [renzo@dtu.dk](mailto:renzo@dtu.dk) (R. Akkerman)

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## Abstract

Due to recent developments in traceability systems, it is now possible to exchange significant amounts of data through food supply chains. Farming practices applied by cocoa farmers at the beginning of the chocolate supply chain strongly influence several quality parameters of the finished chocolate. However, information regarding these practices does not normally reach the chocolate manufacturer. As a consequence, many specifications of the raw material cannot be taken into consideration in the operational decision making processes related to chocolate production. In recent years many studies have been investigating the influence of certain farming practices on cocoa beans and the subsequent chocolate quality parameters. However, no comprehensive analysis of the process variables in the chain and their effects on the quality can be found. In this paper we review and classify the available literature on the topic in terms of process variables throughout the chain, and their effects on quality and flavour aspects of cocoa beans and the eventual chocolate product. After analysing the literature, we are able to identify potential benefits of using data regarding the farming practices into the chocolate production process. These potential benefits especially concern product quality and production yield, giving directions for the future of chocolate production.

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## 1. Introduction

The chocolate supply chain is very long, complex and includes many different actors. It begins with the cocoa farmers, who grow, harvest, extract, ferment, dry, and pack the cocoa beans. Then, the cocoa beans from several farmers are collected and often mixed by local buyers, traders, local buying stations, and exporters until they reach the chocolate manufacturing plant. Several characteristics of chocolate strongly depend on the processes done at the very beginning of the supply chain. Flavour compounds like polyphenol and flavour precursors like free amino acids and reducing sugars, are formed during the fermentation that cocoa farmers do right after harvesting the cocoa pods. Due to the Maillard reaction, which takes place during the roasting process done by the chocolate manufacturer, flavour precursors are transformed into flavour compounds, like aldehydes and pyrazines. These flavour compounds are responsible for the flavour profile of the finished chocolate (de Brito, García, Gallão, Cortelazzo, Fevereiro & Braga, 2001).

Different studies and surveys show differences in the farming practices regarding growing, fermenting and drying the cocoa beans; not only between countries, but also between farmers within the same country. Most of the cocoa beans produced worldwide are produced by small-sized farmers, and then combined in larger and larger batches until the chocolate manufacturer is reached. As the farmers' activities are responsible for defining many of the qualitative characteristics of the cocoa beans, it is easy to imagine that the chocolate manufacturers often receive very heterogeneous batches of cocoa beans due to the various farming practices. Furthermore, the small economical resources of cocoa farmers do not permit them to make accurate analysis for identifying the cocoa cultivar, so, very often mislabelled cocoa beans are traded (Motamayor, Lachenaud, Wallace sa Silva e Mota, Loor, Kuhn, Brown & Schnell, 2008).

For these reasons chocolate manufacturers have only rough expectations of the qualitative parameters by country of origin. In order to avoid country or supplier reliance, often cocoa traders or chocolate manufacturers blend different batches of cocoa beans with the aim of having uniform and constant raw materials to produce chocolate. The processing parameters to produce chocolate are then adjusted on the expected characteristics of the blend of beans, quite often only based on simple indicators such as the origin of the cocoa.

In the literature many studies focus on how some specific cocoa farming practices or different conditions influence specific parameters of the cocoa beans. An example is how different fermentation methods influence the amino acids concentration of the cocoa beans. Afoakwa et al. (2008) for example, gives a good overview on the effect of different post-harvesting treatments on the flavour profile of chocolate. However, information about the farming practices rarely reaches the chocolate manufacturers, which is possibly due to the presence of many actors in the early stages of the supply chain. This means that the obtained knowledge from these studies cannot be used in the further production of chocolate.

In recent years, an increasing effort in the implementation of traceability systems can be seen, mainly as a result of a growing concern with food quality and food safety (van der Vorst, 2006). This has also lead to the introduction of legislation in parts of the world, such as the European Union (European Parliament and Council, 2002). As a consequence of the increased implementation of traceability systems in the food industry, detailed information on how products were handled and treated, could actually be transferred to the producer of the final product, and could be used to optimize production operations (Akkerman, Farahani & Grunow, 2010).

As most cocoa beans are produced in non-European countries, Ivory Coast being the worldwide leader producer, EU regulation does not apply to the actors in the beginning of the cocoa supply chain. In most cases, this results in no data exchange between farmers and chocolate manufactures (Saltini & Akkerman, 2012). As more and more traceability information is available, and more and more tools are available to support traceability (Miller, 2009), it is essential to collect knowledge on how to utilize that information for more than just the organization of recalls in case of food safety problems. This requires a comprehensive view on the chocolate supply chain and all the variables affecting product characteristics. By studying the effects of the different variables due to different farming practices, the processes done by the chocolate manufacturer could be optimized depending on the characteristics of the raw materials (see Figure 1). Therefore, this paper

aims to review all relevant studies in relation to cocoa beans and chocolate production, analyse the different variables investigated, and identify their influence on the characteristics of the cocoa beans and the resulting chocolate. Based on this overview, we contribute to the literature by proposing and discussing several potential benefits, mainly concerning product quality and production yield, so that chocolate producers can evaluate them in order to invest and develop traceability systems that can record and transfer data regarding the farmers' operations. Furthermore, our review also highlights which processes are well known, and which are lacking research, leading to suggestions for further research. As such, this paper contributes not only to the literature on chocolate production, but also on food traceability and operations management.

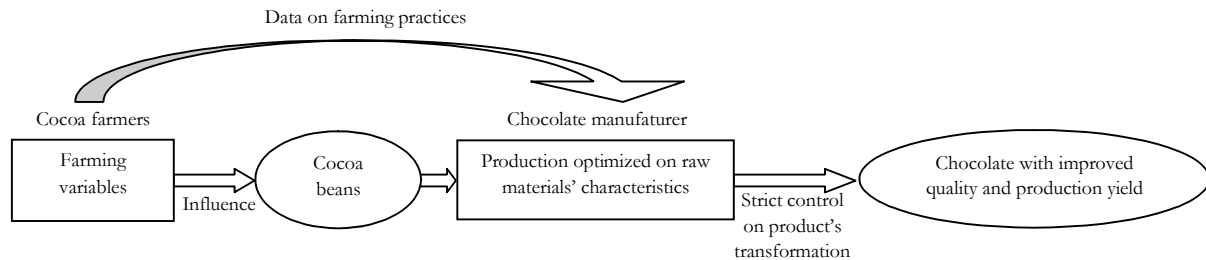


Figure 1. Improved chocolate production control on the basis of increased traceability information.

## 2. Variables in the chain

In this section we present our literature review of the variables related to the production of cocoa beans and their effects on cocoa beans as well as on chocolate. All the analysed literature is classified in Appendix A, which contains a large matrix that relates the variables related to the farming practises and their influence on the products from cocoa bean to chocolate. For each relation, we included the references to the analysed studies. Figure 2 summarizes the production processes to produce cocoa beans and the related variables. In each of the following sub-sections we review the literature on a specific variable.

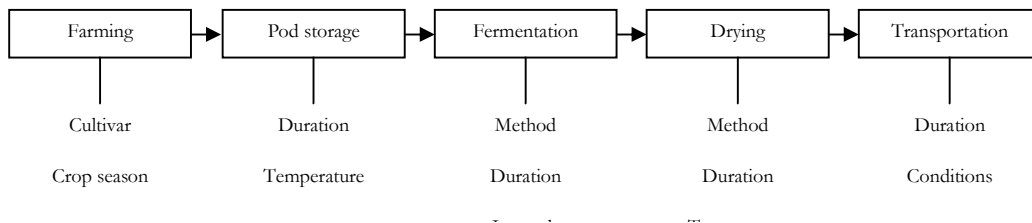


Figure 2. Production processes of cocoa beans and the corresponding variables.

### 2.1 Farming

All the literature related with the variables of the very first operations of the supply chain and their effects on cocoa beans and chocolate can be seen in Table 1.

#### 2.1.1 Cultivar

The cocoa tree belongs to the genus *Theobroma cacao*. Within this genus different subspecies can be identified. According to the literature, these subspecies can be classified within four cultivars: Criollo, Forastero, Trinitario and Nacional (e.g. Counet, Ouwerx, Rosoux & Collin, 2004). However, in the literature cocoa beans might be named differently, depending on origin, commercial names, habits and so on. Below we sum up the different names used in the literature into the four main cultivars according to de Muijnck (2005), Baker, Tomlins and Gay (1994), Lainé (2001) and Sukha, Butler, Umaharan and Boulton (2008).

- Criollo: the most commonly farmed in south-central America. A niche variety called Sanchez is produced in the Dominican Republic. This variety is specially known to be produced with a very short or absent fermentation step. Other names: Bahia, Sanchez.
- Forastero: typically from the Amazon region. The Forastero cultivar includes two subgroups, Amelonado and Amazon, these latter might be divided further into Lower Amazon and Upper Amazon, depending on its origin. However, according to Tomlins, Baker, Daplyn and Adomako (1993), there are no significant chemical differences between these subgroups of the Forastero cultivar. Other names: Amelonado, Amazon, Lower Amazon, Upper Amazon, bulk cocoa, Accra.
- Trinitario: is a hybrid cultivar originated by Criollo and Amelonado Forastero. Other names: fine cocoa, flavour cocoa, hybrid.
- Nacional: is a niche cultivar which grows in Ecuador. Low genetic differences with Criollo.

As already illustrated by the existence of the Trinitario cultivar, hybridisation between cultivars sometimes happens, and it should also be noted that some genetic diversity exists within the cultivars, often due to locally adapted varieties (Sounigo, Umaharan, Christopher, Sankar and Ramdahin, 2005). Recent studies show that misidentification of the cocoa cultivar happens in between 15% and 44% of the cases (Motamayor et al., 2008), which could significantly affect final product quality. Different cocoa cultivars have significantly quality differences, which are reflected in the characteristics of the final product. These differences have been extensively studied and are well known by chocolate producers. However, no publication that clearly defines these differences was found. Here, we clarify them by summing up the most important characteristics for each of the previously mentioned cultivars.

*Criollo*. Studies found that chocolates produced with Criollo cultivar have high levels of procyanidins, which are the main class of polyphenols in cocoa products. Procyanidins contributes to the astringency and bitterness taste of the chocolate, even when the cocoa beans come from different countries, implying that the high levels do not depend on different farming practices but on genetic characterization (Counet et al., 2004). Sensorial studies confirm the influence of procyanidins on the flavour profile, as chocolates produced with this cultivar are characterized by intense bitterness and astringency tones (Clapperton, Yow, chan, lim, Lockwood, Romanczyk & Hammerstone, 1994).

Criollo beans also resulted to be the cultivar with a very high amount of pyrazines (Reineccius, Kenney & Weissberger, 1972). Pyrazines comprise over 40% of the cocoa powder essence and can be used as tracers for the cocoa flavour (Serra Bonvehí, 2005). As is well known, pyrazines and other aromatic compounds are formed during the Maillard reaction which takes place during the roasting process between reducing sugars and amino acids. Thus, as the previously mentioned studies show high levels of aromatic compounds in chocolates produced with Criollo variety, there must also be a high concentration of flavour precursors (reducing sugars and amino acids) before the roasting process. The literature confirms this logic theory, at least concerning reducing sugars (Keeney, 1972). Regarding amino acids, controversial data is found in the literature. The level of amino acids however might depend on proteolysis during fermentation, which might vary due to different fermentations applied (methods, duration and microflora) (Ortiz de Bertorelli et al., 2009; Kirchoff, Biehl & Crone, 1989a).

Another important difference is acidity; the low pH in Criollo might easily affect the flavour profile (Ortiz de Bertorelli et al., 2009). Indeed, sensorial analysis found the chocolate produced with this cultivar to be characterized by acidic tones (Clapperton et al., 1994). Summing up, the cocoa beans of Criollo cultivar have a very strong flavour potential, but in order to exploit it, the cocoa beans need to be handled properly.

		Effect of the variables											
		Proteins/ Amino acids	Sugars	Polyphenols	Pyrazynes	pH	Flavour profile	Microflora	Beans quality	Colour	Moisture	Prod. yield	Health benefits
Cocoa cultivar	Ortiz de Bertorelli et al. (2009) Kirchhoff et al. (1989a) Keeney (1972)			Counet et al. (2004)	Counet et al. (2004); Reineccius et al. (1972)	Tomlins et al. (1993); Ortiz de Bertorelli et al. (2009)	Sukha et al. (2008); Keeney (1972); Reineccius et al. (1972); Bailey et al. (1962); Clapperton et al. (1994); Frauendorfer and Schieberle (2006); Afoakwa et al. (2008)	Not relevant			Not relevant	Motamayor et al. (2003); Motamayor et al. (2008)	
Origin / crop year	Kirchhoff et al. (1989a); Rohsius et al. (2006); Sukha et al. (2004); Serra Bonvehí and Ventura Coll (2002); Caligiani et al. (2007)			Counet et al. (2004); Arlorio et al. (2008); Caligiani et al. (2007)	Counet et al. (2004)		Sukha et al. (2004); Jinap (1995)		Chaiseri and Dimick (1989); Caligiani et al. (2007)	Arlorio et al. (2008); Counet et al. (2004)	Not relevant		Arlorio et al. (2008); Counet et al. (2004)
Storing	Ortiz de Bertorelli et al. (2009); Tomlins et al. (1993)	Tomlins et al. (1993); Meyer et al. (1989)				Ortiz de Bertorelli et al. (2009); Meyer et al. (1989); Faborode et al. (1995); Tomlins et al. (1993)	Nazaruddin et al. (2006); Howat et al. (1957); Samah et al. (1993); Ardhana (2003); Meyer et al. (1989)	Meyer et al. (1989)	Tomlins et al. (1993)		Ortiz de Bertorelli et al. (2009)		

Table 1. Literature analyzed related with the variables of cocoa farming and their effects on cocoa beans and chocolate.

*Forastero*. The forastero variety supplies over the 95% of the world's cocoa. Forastero cocoa trees are very productive and are considered to be moderately resistant to pests and diseases (Lima, Almeida, Nout, & Zwietering, 2011a). When compared to Criollo, the chocolate produced with Forastero beans showed lower procyanidins levels and an almost similar amount of pyrazines as Criollo (Counet et al., 2004; Reineccius et al., 1972). Reineccius et al. (1972) also found that the pyrazine content varied considerably even among lots of the same type of beans. As the concentration of aromatic compounds is a little lower than for Criollo, the flavour precursors before roasting are also expected to be slightly lower. Medium concentration of reducing sugars is found in Forastero beans (Keeney, 1972). After fermentation and drying the Forastero beans showed higher pH when compared with Criollo beans (Ortiz De Bertorelli et al., 2009). The chemical differences are subsequently reflected in the sensorial profile of the produced chocolate; chocolate produced from the Forastero beans resulted to be less bitter, less astringent and less acid than chocolate produced on either Criollo beans or Trinitario beans (Clapperton et al., 1994; Sukha et al., 2008; de Muijnck, 2005).

*Trinitario*. The Trinitario cultivar is known to be the most productive and resistant to diseases (Motamayor, Risterucci, Heath & Lanaud, 2003). Chocolate produced with Trinitario cocoa beans showed similar levels of procyanidins to the one produced with Forastero beans (Counet et al., 2004). Only small differences were found by sensorial studies (Sukha et al., 2008). Historically, this cultivar is known to have strong basic chocolate characters and some typical winery type of aroma that are not found in other varieties (Afoakwa, Paterson, Fowler & Ryan, 2008, Bailey et al., 1962).

*Nacional*. This cultivar is known to have very low concentration of reducing sugars after fermentation (Keeney, 1972). Indeed, low levels of pyrazines are found after roasting (Reineccius et al., 1972). With this information it is reasonable to assume that chocolate produced with this cultivar would probably have very soft flavour attributes.

#### 2.1.2 Crop season and country of origin

Most of the world's cocoa is produced in West African countries, with Ivory Coast counting for the 37% of the worldwide production and Ghana for the 20%. Also other tropical areas, like Central and South America or Southern Asia contribute significantly to the global production of cocoa beans (Anonymous, 2009). Even though that more than 95% of the cocoa cultivated worldwide is of Forastero cultivar (Doyle, Beuchat & Montville, 2001), cocoa batches from different countries might show significant differences in several attributes.

When analysing samples of cocoa beans from different West African countries it was found that several attributes vary significantly, e.g. the amino acids content could have a variation up to 20% (Serra Bonvehí & Ventura Coll, 2002). Even if the cultivar of the beans was not mentioned this result represents a very important finding especially when taking into consideration West African countries. These countries are supposed to farm the same cultivar (Forastero) and, according to Rohsius, Matissek and Lieberei (2006), are considered countries producing reliable and constant average quality cocoa beans. Thus, if in these countries there were such a big heterogeneity, what would be the variation in smaller cocoa producing countries? We do believe that such big variation is caused by the different farming practices in use.

In West Africa cocoa is farmed in small-sized plantations with an average size of only 3 or 4 hectares (Anonymous, 2009). As most of the farmers' activities are not standardized, the quality of the beans might differ significantly between them. Lainé (2001) and Lima et al (2011a) confirm this hypothesis, pointing out the use of very different farming practices, such as harvesting method and time. Overall, if we compare the cocoa beans produced in West African countries with the ones produced in other parts of the continent, we found that the latter ones have lower amino acids content (Rohsius et al., 2006). This difference could be caused by the fact that countries like Ghana and Ivory Coast are not only the biggest producers, but also the countries where cocoa farming has the biggest impact on local economies. Because of this, governmental agencies and big cocoa traders are assisting and educating local farmers in order to have a product with the highest quality (Rosenberg, Eckstein & Brett, 2009).

Differences are however not only country-dependent, but also farmer-dependent, as significant differences can also be found in samples of cocoa beans from the same country (Rohsius et al., 2006). This study confirms the theory previously mentioned: that higher heterogeneity is present in samples from countries where cocoa farming is not the most important economical source, meaning that very different practices are used and an urgent need of standardization is present. These data also suggest that the efforts done by public and private organization in the last years in countries like Ghana or Ivory Coast are giving their first results.

In this section we have discussed the origin-dependent variation in amino acids content as an example because many studies focus on this topic. Obviously also many other attributes, like sugar, polyphenols or fat content are origin dependent, and several publications investigating these variations are available in the literature (Kirchhoff et al., 1989a; Rohsius et al., 2006; Arlorio, Locatelli, Travaglia, Coisson, Del grosso, Minassi, Appendino & Martelli, 2008; Counet et al.,

2004; Caligiani, Cirlini, Palla, Ravaglia & Arlorio, 2007; Lima et al., 2011a; Afoakwa, Paterson, Fowler & Ryan, 2008). We have included them in the classification in Appendix A.

As a result of all these differences in chemical composition of cocoa beans we expect that such differences are reflected in the flavour profile of the cocoa derived products. Indeed, studies confirm this: significant differences are clearly seen in the flavour profiles of cocoa derived products produced of cocoa beans from different origins (Jinap, 1995; Sukha et al., 2008).

## 2.2 Pod storage

Pod storage prior to splitting of the beans has been recommended for cocoa beans which are difficult to ferment or to give chocolate with a strong acid flavour. It is clearly shown in the literature, that pod storage prior to splitting would reduce the sucrose, glucose, fructose, ethanol and acetic acid content, and increase the pH in fermented cocoa beans, improving the flavour of the final chocolate. For this reason, pod storage might be beneficial for beans that tend to develop low pH and acidic flavours, such as of Criollo. (Meyer, Biehl, Said & Samarakoddy 1989; Tomlins et al., 1993) However, pod storage does not only have benefits. The amount of mouldy beans significantly increases with pod storage, and as a consequence it increases the production waste (Meyer et al., 1989; Tomlins et al., 1993; Ortiz de Bertorelli et al., 2009). To summarise the literature on pod storage; pod storage might be very useful, but only in some cases as mentioned above and if well controlled.

## 2.3 Fermentation

After harvesting and storing, the cocoa beans are extracted from the pods and prepared for fermentation (Lopez & Dimick, 1995; Doyle et al., 2001). In this section, the principal variables of the fermentation process and their influence on the product are reviewed. The literature related with the variables of cocoa fermentation and their effects on cocoa beans and chocolate is classified in Table 2.

### 2.3.1 Fermentation method

It is well known that different fermentation methods are used for fermenting cocoa beans depending on farmers, areas and countries (Lainé, 2001). Doyle et al. (2001) give a good description of the four most used fermentation methods; platforms, heaps, baskets and boxes. In this section we sum up the findings of recent research in relation to these four fermentation methods.

In general, cocoa beans fermented in boxes show relatively low concentrations of sugars, ethanol and acetic acid, and a high pH. At the beginning of the fermentation process the increase in temperature is slower than in the other fermentation methods. In some cases the box method has been categorized as a method with low uniformity, which may cause incomplete usage of sugars or high presence of defective beans (Tomlins et al., 1993; Guehi, Dabonne, Ban-Koffi, Kedjebo and Zahouli, 2010; Howat et al., 1957). Additionally, for this fermentation method it has been found that the size, shape and construction material of the box also significantly influences pH, tannins, sugar content and presence of purple beans (Portillo et al., 2007; Guehi et al., 2010).

When fermenting by using the heap method the temperature increases faster at the beginning of the process than in box fermentation, and a more uniform fermentation can be reached (Tomlins et al., 1993). This is probably the reason why less purple beans and more brown beans are found with the heap method in respect to box method (Guehi et al., 2010). However, other authors found no relevant differences between the two mentioned methods (Carr, Davies & Dougan, 1979).

The platform method is believed to be an obsolete method (Doyle et al., 2001), but due to its low costs it is still widely used, for example in West Africa (Lainé, 2001). Different ways to ferment the beans when using a platform method might be used. However, only minor differences were found between them by Lainé (2001). It can be generalized that fermenting on platforms would have quite a low fermentation rate. Probably for this reason, it was historically used for Criollo beans, which require short fermentation (about 2 or 3 days), but is considered inappropriate for Forastero, as it requires longer fermentation (5 to 8 days). For this latter cultivar, the platform method induces the growth of unwanted moulds and the consequent development of off flavours (Doyle et al., 2001).

The last method presented by Doyle et al. (2001) is the basket method. Unlike the methods previously mentioned, no literature was found on this method, which suggests that its use is very limited.

	Effect of the variables									
	Proteins/ amino acids	Sugars	Polyphenols	Pyrazynes	pH	Flavour profile	Microflora	Beans quality	Colour	Water content
Conditions of fermentation	Rohsius et al. (2006); Hashim et al. (1998); Tomlins et al. (1993)	Portillo et al. (2007); Hashim et al. (1998); Tomlins et al. (1993)	Portillo et al. (2007); Wollgast and Anklam (2000)		Guehi et al. (2010); Howat et al. (1957); Biehl et al. (1985); Tomlins et al. (1993)	Sukha et al. (2008); Tomlins et al. (1993); Doyle et al. (2001); Lainé (2001); Biehl et al. (1985)	Schwan and Wheals (2004)	Guehi et al. (2010); Carr et al. (1979); Tomlins et al. (1993); Doyle et al. (2001)	Wollgast and Anklam (2000)	Howat et al. (1957)
Duration of intervals	Hashim et al. (1998); Senanayake (1997)	Portillo et al. (2007); Hashim et al. (1998); Senanayake (1997)	Portillo et al. (2007); Wollgast and Anklam (2000)	Hashim et al. (1998)	Portillo et al. (2007); Senanayake (1997); Guehi et al. (2010)	Leal et al. (2008)	Camu et al. (2008b)	Tomlins et al. (1993); Guehi et al. (2010)	Wollgast and Anklam (2000)	
Fermentation grade	Kirchhoff et al., (1989a; 1989b); Adeyeye et al. (2010); Sukha et al. (2004); de Brito et al. (2001); de Muijnck (2005); Hashim et al. (1998); Rohan and Stewart (1967a; 1967b); Reineccius et al. (1972); Rohan (1964); Aremu et al. (1995); Aminet al. (1998)	Reineccius et al. (1972); Rohan and Stewart (1967a); Hashim et al. (1998)	Arlorio et al. (2008); Portillo et al. (2007); Camu et al. (2008b); Caligiani et al. (2007); Biehl et al. (1985); Wollgast and Anklam (2000)	Sukha et al. (2004); Reineccius et al. (1972); Hashim et al. (1997)	Senanayake (1997); Tomlins et al. (1993); Aremu et al. (1995); Lagunes Gálvez et al. (2007); Biehl et al. (1985)	de Muijnck (2005); Hashim et al. (1999); Reineccius et al. (1972); Nazarruddin et al. (2006)	Lagunes Gálvez et al. (2007); Doyle et al. (2001)	Guehi et al. (2010); Tomlins et al. (1993)	Wollgast and Anklam (2000)	Aremu et al. (1995); Lagunes Gálvez et al. (2007)
Microflora during fermentation	Hansen and Olmo (1998); Schwan and Wheals (2004)	Schwan and Wheals (2004)	Camu et al. (2008a); Schwan and Wheals (2004)	Schwan and Wheals (2004); Reineccius et al. (1972)	Leal et al. (2008); Samah et al. (1993)	Leal et al. (2008); Camu et al. (2008a; 2008b); Schwan and Wheals (2004); Jespersen et al. (2005); Nielsen et al. (2007); Adimpong et al. (2010)	Not relevant	Adimpong et al. (2010); Ardhana (2003); Camu et al. (2007); Jespersen et al. (2005); Nielsen et al. (2007); Schwan and Wheals (2004); Lima et al (2011b)	Schwan and Wheals (2004)	

**Table 2.** Literature related with the variables of cocoa fermentation and their effects on cocoa beans and chocolate.



### 2.3.2 *Microflora*

Schwan and Wheals (2004) give a complete explanation of the role of microbiology in cocoa bean fermentation. This publication also represents a valuable review of relevant studies concerning fermentation of cocoa beans and its role in chocolate quality published until 2004. Here we only report the findings of Schwan and Wheals (2004) relevant for the scope of this paper, and integrate them with relevant findings published after 2004. For the purpose of our paper, we will only present a relatively basic overview, for a more extensive discussion of the fermentation process the reader is referred to Schwan and Wheals (2004).

The fermentation process is characterized by a well-known systematic microbial succession (as simply illustrated in Figure 3). The initial low pH of the pulp (3.6) caused by the presence of citric acid, together with low oxygen levels favour yeasts colonization. Yeast proliferation leads to the production of ethanol and secretion of pectinolytic enzymes. Therefore, the yeast population increases considerably within the first 24 hours, after which a slow decrease is observed. The remaining conditions favour the growth of lactic acid bacteria (LAB), which reach their peak after around 36 hours from the beginning of the fermentation. The main activity of LAB is degrading glucose to lactic acid. The overall pH increases due to the metabolism of non-acid by-product. After 48 hours of fermentation the LAB population decreases giving space to the growth of acetic acid bacteria (AAB). The exothermic reactions of AAB increase the temperature up to about 50°C. The reactions consist mainly of oxidation of ethanol to acetic acid and further oxidation of the latter to carbon dioxide and water. It is believed that the conditions provoked by AAB are the cause of diffusion and hydrolysis of proteins, thus, AAB might play a key role in the formation of flavour precursors. AAB are obligatory aerobic, so increasing aeration during fermentation would increase their activity. After approximately 5-6 days of fermentation the AAB population has been observed to decrease, and the aerobic spore forming bacteria increased their population. These bacteria produce a variety of chemical compounds during the fermentation process that may contribute to the acidity and to the development of off-flavours (Lima, Kamphuis, Nout & Zwietering, 2011b; Sengun & Karabiyikli, 2011; Schwan & Wheals, 2004).

### 2.3.3 *Duration of fermentation*

*Amino acids.* Due to microbial activity, enzymes are released into the fermenting mass during the fermentation process. The enzymatic activity results in breaking down the cell wall proteins into peptides of various chain length and free amino acids (Hansen & Olmo, 1998). The formed peptides and amino acids, together with reducing sugars, represent the substrate for the Maillard reaction. The Maillard reaction takes place during the roasting process and results in the typical aromatic compounds of chocolate. The enzymes endoproteinase followed by carboxypeptidase, are during the fermentation responsible for breaking down the proteins and increasing the concentration of amino acids (Amin, Jinap & Jamilah, 1998; Kirchhoff et al., 1989a). However, these two enzymes are not the only responsible for protein degradation, also other enzymes, like aminopeptidase, invertase, polyphenol oxidase or glycosidases, have been found to play an important role in synthesizing flavour precursors (Hansen & Olmo, 1998).

As pointed out by recent publications, unfermented cocoa beans contain a very low amount of free amino acids (Rohsius et al., 2006). The increase in amino acids concentration during the fermentation is well known and demonstrated even in studies published almost 50 years ago (Rohan, 1964). In more precise terms, the total amino acids content increases about 150-200% during the fermentation (Hashim, Selamat, Kharidah & Ali, 1998; Kirchhoff, Biehel, Ziegeler-Berghausen, Hammor & Lieberei, 1989b). During this process the amino acids are however not released on a constant rate according to their statistical distribution, but their final concentration strongly depends on the pH during proteolysis (Kirchhoff et al., 1989a; 1989b). A too low pH at the beginning of the fermentation process results in reduction of flavour precursors and over-acidification of the final product (Camu, De Winter, Addo, Takrama, Bernaert & De Vuyst, 2008a). Leucine, alanine, phenylalanine and tyrosine are the amino acids with the highest production rate (Kirchhoff et al., 1989a; Rohan, 1964; Kirchhoff et al., 1989b) whereas glucine and aspergine have the highest total concentration (Adeyeye, Akinyeye, Ogunlade, Olaofe, & Boluwade, 2010). This latter finding is however discussable because other authors point out other amino acids as the most present (Rohan, 1964).

Knowing the amino acids composition is extremely important when it comes to predict the synthesis of flavour compounds later on in the production of chocolate. Findings of different studies agree when it comes to measure the increasing rate of total amino acids and amino-terminal groups, where the highest level is reached after 4 days of fermentation, after which the level remains constant (Rohan & Stewart, 1967a; de Brito et al., 2001). After fermentation it is well known that high quality cocoa beans should contain approximately 8 to 14 mg/g dry matter of total amino acids (Rohsius et al., 2006). According to these results, the protein content should decrease during fermentation because of the proteolytic activity of the previously mentioned enzymes. Controversial results are however found by Aremu, Agiang, and Ayatse (1995) where the protein content is reported to increase by 13% in the first 6 days of fermentation and then decrease with the same magnitude after 12 days fermentation. In this study, the protocol used for protein analysis is not clear, thus, it

may be, that a method that does not differentiate proteins from small peptides or amino acids was used. These data indicate, that having a thorough knowledge of the amino acids formation during ones fermentation process, can give useful information on the flavour potential of the cocoa beans in relation to the final chocolate products.

*Sugars.* The theory about fermentation of cocoa beans illustrates that the microbial activity result in hydrolyses of the starch present in the pulp to sucrose, and sucrose to glucose and fructose. Reducing sugars such as glucose and fructose, are required for allowing the Maillard reaction during the roasting process, and the development of flavours. The optimal concentration of reducing sugars in the cocoa beans is reached at about the same time as maximal flavour development, and coincides approximately with the peak in amino acid development, which is after approximately 4 days of fermentation (Rohan & Stewart, 1967a). Detailed studies dealing with the sugar profile during the fermentation process show that all sucrose is used within the first two days of fermentation, and the concentration of reducing sugars decreases 84% and 90%, respectively, during a 5-day fermentation process. After 5 days the concentration seems constant (Ardhana, 2003; Lagunes Gálvez, Loiseau, Paredes, Barel & Guiraud, 2007; Rohan & Stewart, 1967a).

According to the data mentioned so far, we can state, that a fermentation process longer than 4-5 days does not improve the flavour potential of the cocoa beans, instead it might be deleterious to the quality e.g. because of moulds grow. So, this could be the maximum length of fermentation. This does however not exclude that the fermentation rate could be increased or decreased within this length, depending on the fermentation grade desired.

*Pyrazines.* The pyrazine-related compounds comprised just over 40% of the cocoa essence. However, the knowledge regarding these compounds is still very limited (Serra Bonvehí, 2005). The theory of fermentation tells us, that the pyrazines precursors (amino acids and reducing sugars) are transformed into pyrazines during the roasting process due to the Maillard non-enzymatic browning reactions. Recent studies found that this is not completely true; small amounts of several types of pyrazines in well-fermented cocoa beans have been found (Hashim, Selamat, Ali & Kharidah, 1997; 1998; Reineccius et al., 1972). A common hypothesis is, that due to the heat produced by the fermenting flora, the Maillard reactions starts during the fermentation process resulting in formation of a minor amount of pyrazines already at this process step (Reineccius et al., 1972). Alternatively, pyrazines could be formed due to the growth of *Bacillus* (Zak, Ostovar & Keeney, 1972; Ostovar & Keeney, 1973; Jinap, Siti & Norsiaty, 1994a). The truth is probably a combination of both, but further investigation is definitely needed.

When roasting fermented cocoa beans, total pyrazine concentration increase rapidly to a near maximum value which does not change during extended roasting (Reineccius et al., 1972). It is clear that the concentration of pyrazine precursors strongly depend on the fermentation process. Another import finding is that after roasting cocoa beans with different fermentation grades, similar pyrazine compounds are present, but their relative concentrations vary significantly (Sukha, Ramnath & Butler, 2004). As the exact flavour attribute of each pyrazine compound is known (and listed in Table 3). This means that by knowing the applied fermentation and roasting conditions it should be possible to predict the exact flavour profile of the final chocolate product. Before such a model exists further studies are needed but it might revolutionize the way to produce chocolate.

Pyrazine compound	Flavour attributes of pyrazine compound
2-Methylpyrazine	Chocolate, grass, green, nutty and roasted notes
2,5-dimethylpyrazine	Chocolate, roasted nuts and earthy flavours
2,3-Dimethylpyrazine	Caramel, nutty, green, sweet, malt and chocolate notes
2-Ethyl-6-methylpyrazine	Butter scotch, nutty, earthy, raw potato and roasted notes
2,3,5-Trimethylpyrazine	Baked potato, grass, musty, nutty and roasted peanuts
3-Ethyl-2,5-dimethylpyrazine	Roasted peanut flavours
2,3,5,6-Tetramethylpyrazine	Cocoa, chocolate, nutty and burnt almond notes
2,3,5-Trimethyl-6-ethylpyrazine	Roasted peanut and cocoa flavours

**Table 3.** Pyrazines formed during roasting and their flavour attribute. Source: (Hashim et al., 1997)

*pH.* In the literature many pH profiles of the fermentation process are present, and these values might seem very un-uniform and controversial. In many cases when talking about pH of cocoa beans a distinction between pulp and cotyledons is made. The initial microbial activity during the fermentation process results in death of the cocoa bean that is due to mainly penetration of ethanol and acetic acid through the husk into the cotyledons. This decreases the pH in the cotyledon from 6.5 to approximately 4.5 within the first 4 days of fermentation. After this decrease, the pH slightly increases again until reaching a value of 5 around the end of the fermentation process, which is normally 6 days. These changes are extremely important for the flavour development, because if the pH becomes too acid too fast (pH < 4.5) there will be both a final reduction in flavour precursors and an over-acid final product. This fact has to be taken into consideration when

planning the storage of cocoa beans before the fermentation process. Storage under non-optimal conditions would actually lead to spontaneous starting of the fermentation process. If this is not taken into consideration, and storage is followed by normal fermentation, it could easily result in over fermented beans, with the typical development of off-flavours (Howat, Powell & Wood, 1957; Samah, Ibrahim, Alimon, & Karim, 1993; Ardhana, 2003; Rohan, 1958; Camu et al., 2008a; Portillo, Farinas & Betancourt, 2007; Nazaruddin, Seng, Hassan & Said, 2006). When talking about cocoa bean pulp, the initial pH is very low (3.0-3.5), mainly due to the presence of citric acid. As citric acid is almost completely consumed during the fermentation the pH increases until reaching a value of 5-6 after 6 days of fermentation (Howat et al., 1957; Rohan, 1958; Lagunes Gálvez et al., 2007).

*Polyphenols.* For a thorough explanation regarding polyphenols in cocoa beans we strongly recommend Wollgast and Anklam (2000). Here, we only highlight that during fermentation the content of polyphenols decreases significantly mainly due to the activity of the enzyme polyphenol oxidase (Rohan, 1958). As this enzyme is particularly sensitive to both fermentation and drying, its activity is strongly reduced during the first days of fermentation. Thus, a low level of polyphenol oxidase activity during the rest of the process is sufficient to carry out the oxidative reactions that take place in the aerobic part of the fermentation and decrease significantly the polyphenols content. However, since polyphenol oxidase is strongly inactivated, it might also be true that non-enzymatic oxidation of the polyphenols is involved in the process (Hansen & Olmo, 1998).

The polyphenol oxidation is also responsible for the production of black, brown and red pigments of cocoa beans (Mayer, 2006). The role of polyphenols into the flavour profile of chocolate is to give astringency and bitterness, and in addition a negative correlation between polyphenols and aroma acceptability is also suggested (Delcour, Ferreira, and Roux, 1983; Camu et al., 2008a; Counet et al., 2004). With this data, we understand that shortening the fermentation process results in increasing the content of polyphenols, which in turn results in increased astringency, bitterness and antioxidant capacity.

#### *2.3.4 Aeration during fermentation*

Independently of the fermentation method, the mass size of the cocoa beans has an influence on the fermentation process. Fermenting a little mass quantity would result in low amount of free amino acids, peptides, fructose, glucose, total sugars and pyrazines. By increasing the mass size the concentration of these compounds increases until a maximum is reached (around a mass size of 55-60 kg). By increasing the mass size further the concentrations of these compounds decrease again (Hashim et al., 1998). In both studies, the fermentation was conducted in a rotary drum reactor with standard size and turning times. Under these conditions, fermenting a very high volume would lead to decreasing the aeration during the fermentation process, resulting in reduced microflora activity, thus lower temperature and proteolytic activity. On the other hand, fermenting a very small volume of cocoa beans under the same conditions leads to a high aeration, which probably causes a loss of heat, thus lowering the temperature and therefore lowering the metabolic rates of the microflora activity. If we would plot the concentration of flavour precursors versus an aeration value, the curve would look like an asymmetric bell curve. Several studies in the literature analyse the influence of aeration on the fermentation process by slightly increasing or decreasing frequency or duration of turnings. It has to be taken into consideration that probably most studies do not obtain enough data to show the whole flavour precursors versus an aeration value curve, therefore only the increasing, the decreasing or an almost constant part of the curve is shown in their results (Portillo et al., 2007; Ortiz de Bertorelli et al., 2009; Guehi et al., 2010; Senanayake, 1997; Leal, Gomes, Efraim, de Almeida Tavares & Figueira, 2008; Camu, González, De Winter, Van Schoor, De Bruyne, vandamme, Takrama, Addo & De Vuyst, 2008b; Biehl, Brunner, Passern, Quesnel and Adomako, 1985).

#### *2.3.5 Starter cultures*

To our concern, the use of starter cultures is still absent in cocoa bean fermentation (Adimpong, Niesen, Sørensen, Derx & Jespersen, 2010). In other food products, like cheese or wine production, starter cultures are widely used. Knowing the source of the natural microbial population could help the development of starter cultures for cocoa bean fermentation. In Schwan and Wheals (2004) only general considerations about using starter cultures are mentioned, while more recent studies give more specific data on this topic. Recently, it was found that no differences in either LAB or AAB were present when the same batch of cocoa beans was fermented at the farm or at a factory site. The results indicate that the cocoa pod surface, and not the general environment, is the main inoculum for spontaneous cocoa bean fermentation (Camu et al., 2007; 2008a; 2008b).

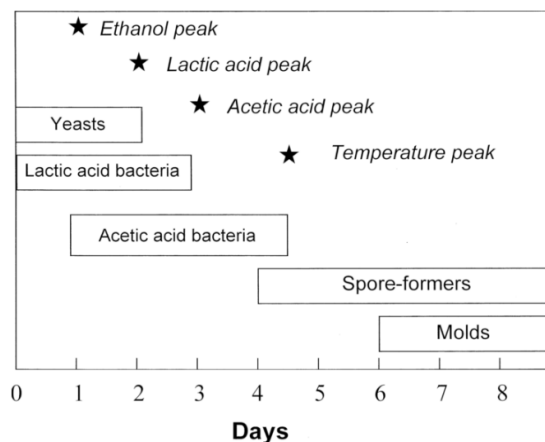


Figure 3. Schematic illustration of a microbial succession during cocoa bean fermentation. The open boxes indicate the periods during the fermentation when a particular microbial group is most abundant and/or important. The stars indicate the timing of peaks of metabolites and temperature. From Schwan & Wheals (2004).

Developing flavour precursors is probably not the only relevant activity of LAB, as recent research found that most of the strains present were also able to inhibit the growth of the pathogens: *Listeria monocytogenes*, *E.Coli* and *Salmonella* (Adimpong et al., 2010). These recent results could suggest an additional benefit to the development of starter cultures for fermentation of cocoa beans, as protection against growth of pathogens.

Different results were obtained when running the same type of experiment and analysing the yeast population. Yeasts differ in both count and specie composition when comparing the results from fermenting with heap or tray methods. However, it is not known if the differences originates from different natural inoculums or different fermentation conditions, which may favour the growth of one or other yeast species (Jespersen, Nielsen, Hønholt, & Jakobsen, 2005; Nielsen, Hønholt, Tano-Debrah, & Jespersen, 2005; Nielsen, Teniola, Ban-Koffi, Owusu, Andersson, & Holzapfel, 2007). For data on physiological characterization of yeast isolates we recommend Daniel, Vrancken, Takrama, Camu, De Vos, and De Vuyst (2009).

Recently, and also the first studies on the effect of inoculating specific strains when fermenting cocoa beans were published. Leal et al. (2008), for example, found that by inoculating a *Kluyveromyces marxianus* hybrid yeast strain, which is a strain with an increased pectinolytic activity, the flavour acceptability for chocolate consumers was improved.

In order to provide this publication with a useful tool for further research, we have collected all data regarding the microbial species from the previously mentioned studies together with data on their relevant activities when available, in a unique table (Appendix B). It is common knowledge that the microbial activity depends on the substrate in which the microbes are. Many of the mentioned microbes are already used in other food or pharmaceutical productions, but their metabolites and by-products may differ as the substrate differs. For this reason we have reported the activities only when analysed in cocoa bean fermentation.

## 2.4 Drying

The literature related with the drying process, the process parameters, and their effects on cocoa beans and chocolate, is classified in Table 4.

### 2.4.1 Duration and temperature

Drying of cocoa beans is a process of heating which reduces the moisture content of the beans to less than 7.5% (W/W). It is common practice that the duration of the drying process ends when the farmer considers, based on his own criteria, that the cocoa beans are ready (moisture content of 7.5%) (Lainé, 2001). Even if farmers are well-trained and experienced, the moisture content and the consequential overall quality of the dried cocoa beans might vary considerably between the beans (Rohsius et al., 2006; Lainé, 2001). When investigating the physical properties of category B cocoa beans from the 2000/2001 harvesting season, Bart-Plange and Baryeh (2003) found a variation in the moisture content of the dried cocoa beans between 5% and 24%. Drying is also a continuation of the oxidative stage of the fermentation process and therefore plays an important role in reducing astringency, bitterness and acidity. During this process, the characteristic brown colour of the chocolate is developed due to the enzymatic oxidation of polyphenols (Hashim, Selamat, Muhammad & Ali, 1999; Hansen & Olmo, 1998; Wollgast & Anklam, 2000).

	Effect of the variables								
	Proteins/amin o acids	Sugars	Polyphenols	Pyrazynes	pH	Flavour profile	Beans quality	Colour	Water content
Conditions of drying	Rohsius et al. (2006); Hashim et al. (1999)	Hashim et al. (1999)	Zahouli et al. (2010)	Hashim et al. (1999)	Zahouli et al. (2010); Bonaparte et al. (1998); Jinap et al. (1994b)	Sukha et al.(2008); Zahouli et al. (2010); Faborode et al. (1995); Jinap et al. (1994b); Hii et al. (2006)	Bonaparte et al. (1998); Hii et al. (2006); Zahouli et al. (2010); Faborode et al. (1995); Jinap et al. (1994b)	Bonaparte et al. (1998)	Bharath and Bowen-O'Connor (2008); Faborode et al. (1995); Lainé (2001)
Temperature of drying	Hashim et al. (1999)	Hashim et al. (1999)	Wollgast and Anklam (2000); Hashim et al. (1999); Hansen and Olmo (1998)	Hashim et al. (1999)	Hii et al. (2009); Jinap et al. (1994b)	Jinap et al. (1994b)	Hii et al. (2006); Faborode et al. (1995); Jinap et al. (1994b)	Wollgast and Anklam (2000); Hashim et al. (1999); Hansen and Olmo (1998)	Ndukwu (2009); Garcia-Alamilla et al. (2007); Hii et al. (2009)
Duration of drying	Hashim et al. (1999)	Hashim et al. (1999)	Wollgast and Anklam (2000); Hashim et al. (1999); Hansen and Olmo (1998)	Hashim et al. (1999)	Jinap et al. (1994b)	Hii et al. (2006); Jinap et al. (1994b)	Hii et al. (2006); Faborode et al. (1995); Jinap et al. (1994b)	Wollgast and Anklam (2000); Hashim et al. (1999); Hansen and Olmo (1998)	Bharath and Bowen-O'Connor (2008); Ndukwu (2009); Garcia-Alamilla et al. (2007); Hii et al. (2009); Faborode et al. (1995); Hii et al. (2006); Jinap et al. (1994b); Lainé (2001)
Drying rate			Wollgast and Anklam (2000); Hashim et al. (1999); Hansen and Olmo (1998)		Bonaparte et al. (1998); Jinap et al. (1994b)	Jinap et al. (1994b); Hashim et al. (1999); Hii et al. (2006)	Hii et al. (2006); Faborode et al. (1995); Jinap et al. (1994b)	Bonaparte et al. (1998); Wollgast and Anklam (2000); Hashim et al. (1999); Hansen and Olmo (1998)	Faborode et al. (1995); Hii et al. (2006)
Drying grade			Wollgast and Anklam (2000); Hashim et al. (1999); Hansen and Olmo (1998)		Garcia-Alamilla et al. (2007); Jinap et al. (1994b)	Sukha et al. (2008); Jinap et al. (1994b); Hii et al. (2006)	Hii et al. (2006); Faborode et al. (1995); Jinap et al. (1994b)	Wollgast and Anklam (2000); Hashim et al. (1999); Hansen and Olmo (1998)	Bharath and Bowen-O'Connor (2008)

**Table 4.** Literature related with the variables of the drying process and their effects on cocoa beans and chocolate.

It is well known that the drying rate during the drying process is of crucial importance for the cocoa beans' final quality. In case the drying rate is too fast, the beans would tend to retain an excessive amount of acids, including acetic acid, which is deleterious to the flavour. On the other hand, too slow drying rate would result in low acidity, poorer colour and high presence of moulds (Hashim et al., 1999; Bharath & Bowen-O'Connor, 2008; Jinap, Thien & Yap, 1994b; Zahouli, Guehi, Fae & Nemlin, 2010; Faborode, Favier & Ajayi, 1995; Hii, Abdul Rahman, Jinap & Che Man, 2006).

#### *2.4.2 Method of drying*

Even though artificial driers are increasingly popular, natural sun drying is still largely used (Hashim et al., 1999). Many studies that compare natural and artificial drying methods conclude that natural sun drying gives the best result (Bonaparte, Alikhani, Madramootoo and Raghavan, 1998; Zahouli et al., 2010). However, we do believe that artificial drying methods can improve the drying process; it just requires more research. The moisture profile during drying at different temperatures and different conditions is well known, and available in the literature (Garcia-Alamilla, Salgado-Cervantes, Barel, Berthomieu, Rodriguez-Jimenes & García-Alvarado, 2007; Bharath & Bowen-O'Connor, 2008; Ndukwu, 2009; Hii et al., 2006). Thus, by knowing the conditions of drying it would be possible to predict in real time the ideal duration of drying to reach a standardized moisture content. This prediction could easily be done with the use of prediction models, like the ones proposed by Garcia-Alamilla et al. (2007) or Hii, Law and Cloke (2009). In this way the qualitative parameters of dried cocoa beans could be standardized and the batches of cocoa beans received by chocolate manufactures will be more homogeneous.

#### *2.5 Transportation*

After drying the cocoa beans are exchanged between local buying stations, local and international traders, logistic companies, etc. On average, 7 to 10 actors are present between the cocoa farmers and the chocolate manufacturers (Anonymous, 2009; Saltini & Akkerman, 2012). No studies analysing the influence of the storage conditions during transportation on cocoa beans were found. However, as improper storage conditions might be deleterious to the beans' quality, the conditions of transportation should be taken into consideration when assessing the quality of cocoa beans.

### **3. Discussion of potential benefits**

After drying the cocoa beans are packed and ready to be transported to local buying stations, regional warehouses, trader's warehouses and shipped until reaching the chocolate production plant. During this journey, cocoa beans from many different farmers, areas, and countries are mixed together until product batches between 10 and 18 tons are assembled. To have an idea on the magnitude of this mixing; An average production capacity of a cocoa farmer in Ivory Coast is around 1000 kg per harvesting season, divided in three or four harvestings. This means that a batch of cocoa beans could contain the cocoa beans from more than 70 different farmers (Saltini & Akkerman, 2012; Lainé, 2001). After reaching the chocolate processing plant, the cocoa beans undergo a series of processes before being transformed into chocolate. The standard processing parameters and the resulting physical and chemical changes during these processes are well known and well explained in the literature (see e.g. Rousseau, 2007; de Muijnck, 2005).

Since in most cases no information regarding the farming practices used to produce the cocoa beans reaches the chocolate production plant, chocolate manufactures only have rough expectations on the flavour profile of cocoa beans based on the country of origin. Often, chocolate manufacturers blend several batches of cocoa beans in order to have a uniform and constant blend to process, and avoid country or supplier reliance. As a result, a relatively heterogeneous blend is created, as cocoa beans with different potentials are mixed. After blending, the processing parameters can then be standardized on the expected characteristics of the blend of cocoa beans (de Muijnck, 2005). In this section we discuss the potential benefits to the flavour profile, production yield and health properties of having a traceability system that allows the exchange of information between cocoa farmers and chocolate manufacturers. With such a system, the farming practices could be standardized according to the qualitative requirements of the chocolate manufacturers, or information on the farmers' activities could be included in the operational decision making process of the chocolate manufacturer.

#### *3.1 Chocolate flavour profile*

In order to illustrate the influence of the farmers' process variables on the flavour profile, we start with the example of pyrazine content. As mentioned in section 2.1.1, if the same roasting process is applied, cocoa beans of Criollo cultivar have a higher pyrazine potentiality than those of Forastero cultivar. However, Forastero roasted cocoa beans have a three times

higher amount of pyrazines than Criollo (Reineccius et al., 1972; Ortiz de Bertorelli et al., 2009). The Criollo cocoa beans used in these studies are of Sanchez variety, which is a variety known for its very short fermentation. Due to this, a small amount of proteins was likely degraded to amino acids, forming a small amount of pyrazines. This example shows how easily a great flavour potential can be lost by applying non-optimal processing parameters. Criollo beans have an extremely high potential of developing a strong flavour profile, but with a very short fermentation time, this potential is actually wasted. If a chocolate with a special high polyphenol content and soft flavour is desired, a short fermentation time for Forastero beans might suit better. Together with the increased availability of techniques to identify the genetic structure of cocoa beans (Sounigo et al., 2005), improved traceability information provides the basis for utilizing cultivar information.

During the roasting process flavour precursors are transformed into flavour compounds. The direct correlation between amino acids, reducing sugar degradation and roasting temperature is well known. In the literature, it is reported that the reduction of amino acids during roasting could vary between 24.1 to 71.8%, and the decrease in sugar content (fructose and sucrose) could vary between 47 and 60%. The variations depend on cultivar, fermentation method, temperature and duration of roasting (de Brito et al., 2001; Serra Bonvehí & Ventura Coll, 2002). The decrease in amino acids is not constant throughout the roasting process. It occurs mainly during the first 30 minutes of the process, and is directly dependent on the temperature. On the other hand, the decrease rate of sugar depends on the temperature and the sugar content is constantly decreasing during roasting until all sugar is degraded (Rohan & Stewart, 1967b). The formation yield of pyrazines is known, and reaches the highest yield at high temperatures (typically 150/170 °C) (Reineccius et al., 1972; Keeney, 1972; Serra Bonvehí & Ventura Coll, 2002; Jinap, Rosli, Russly & Nordin, 1998; Krysiak, 2006). By knowing the roasting temperature, the amino acids and sugar contents, the synthesis of pyrazines could easily be predicted using predictive models like the one proposed by Noor-Soffalina, Jinap, Nazamid, and Nazimah (2009).

The exact flavour attribute for each pyrazine compound (see Table 3) is known, meaning that the flavour profile of the finished chocolate can be predicted. When it comes to analysing the flavour, also polyphenols must be taken into consideration. Alteration in the content and composition of polyphenols mainly occurs during roasting and to a smaller extent also during grinding, refining and conching. Generally, higher processing temperatures and/or longer processing times reduce the amount of polyphenols available in cocoa beans. If an alkalisating step is present in the process, this also leads to a remarkable decrease in polyphenol content (Jinap et al., 1998; Wollgast & Anklam, 2000). However, as the polyphenol potential for different cultivars is known, and the polyphenol reduction during processing is also known, the effect of polyphenols can be taken into consideration when predicting the flavour profile.

When looking directly at the flavour acceptability, several studies investigate the best combination of time and temperature of roasting. However, these best combinations differ quite a lot from study to study. This heterogeneity is likely caused by the fact, that cocoa beans produced with even slight differences in the farmers' practices have different best combination of time and temperature for roasting. For this reason we believe that it is extremely important to know how the cocoa beans have been handled by the farmer in order to be aware of the flavour potential, and thus set the optimal processing parameters. It is for instance well known that cocoa beans subjected to an improper roasting procedure generates undesirable flavour compounds; If the cocoa beans are not roasted enough, the resulting chocolate would be very bitter and alternatively, if the beans are over roasted, burned and off-flavours will be developed (Serra Bonvehí & Ventura Coll, 2002; Jinap et al., 1998). In general, the literature agrees that the higher the roasting grade is, the better the flavour profile will be, until an over roasting point is reached. However, the specific process conditions listed in the literature vary. Indications on the over roasting point range from 120 °C for more than 60 minutes through 150 °C for any duration of roasting to 150 °C for more than 45 minutes. Other studies even suggest the latter combination as the best one, and more discrepancy can be found in the literature (Rohan & Stewart, 1967b; Jinap et al., 1998; Ramli, 2006; Krysiak, 2006; Plumas, 1996). Based on this, it is clear that the optimal roasting parameters strongly depend on the raw material processed.

Regarding the mouthfeel, it is known that chocolates with a low cocoa content are characterized as melting and creamy, whereas the chocolates with higher cocoa content are characterized as dry, mealy and sticky (Thamke et al., 2009). For this reason, it is not possible to produce a chocolate with the flavour profile of dark chocolate, and the mouthfeel of milk chocolate. However, we have just that the flavour potential of the beans can be increased by optimal roasting parameters. Because of this stronger flavour potential, it might be possible to produce a chocolate with less cocoa percentage without changing the flavour profile. In such a chocolate the cocoa butter fraction could be increased, with the consequent improvement of the rheological mouthfeel (Afoakwa, Paterson & Fowler, 2007).

The above discussion emphasizes that if a batch of raw material is very heterogeneous, it is inevitable that flavour potential is to some extent wasted. We believe that this problem can be solved in two ways:

- Standardization of the farmers' activities: If a higher standardization is reached, the batch will be more homogeneous, and the flavour potential of all cocoa beans will be leveraged. This solution would take big efforts in standardizing the practices of many family size farmers.
- Implementation of a traceability system: The chocolate manufacturer receives data on how the cocoa beans have been processed. This is possible because the cocoa beans normally reach the chocolate plant packed in the farmers' bag, with a size of approximately 15 kg (Saltini & Akkerman, 2012). Thus, the cocoa beans can be sorted on similarities, and optimal processing parameters can be chosen for a more homogeneous blend of cocoa beans.

By leveraging the flavour potential of all cocoa beans, fewer raw materials would be needed for achieving a certain degree of flavour, and the production yield could be significantly higher.

### 3.2 Process optimization

Cocoa beans are often alkalinized with potassium carbonate or sodium hydroxide in order to improve the microbiological conditions (de Muijnck, 2005). Many studies report that increasing the pH from 5.7 to 7.5 is deleterious to the flavour acceptability (Serra Bonvehí & Ventura Coll, 2002; Noor-Soffalina et al., 2009; Andres-Lacueva, Monagas, Khan, Izquierdo-Pulido, Urpi-sarda, Permanyer & Lamuela-Raventos, 2008). As mentioned in section 2.2.4 some Lactic Acid Bacteria (LAB) involved in the fermentation process are also able to inhibit the growth of pathogens (Adimpong et al., 2010). The use of LAB starter cultures could lead to a more controlled fermentation process, and thereby the possibility of controlling the flavour, as also argued in Schwan (1998). In some cases, this might make the alkalization step redundant, avoiding its potential negative aspects (see e.g. Miller, Hurst, Payne, Stuart, Apgar, Sweigart & Ou, 2008).

Recently, it was found that increasing roasting time and temperature also significantly increases the butter extraction yield (Asep, Jinap, Tan, Russly, Harcharan & Nazimah, 2008). This finding is very relevant when planning the chocolate production. For example, if a chocolate manufacturer desires to produce a chocolate with medium polyphenol content and very soft aromatic profile, a supply chain with the improved traceability system passing on information from farming during storage and transportation to chocolate manufacture, it will be possible to select cocoa beans with short fermentation time. Short fermentation time leads to high content of polyphenols and little flavour potentiality, due to the few flavour precursors formed during fermentation. Then, these short time fermented cocoa beans could be treated with high temperature and long time, so that the polyphenol content will decrease to the desired level, and the cocoa butter extraction process will have a high yield. A similar approach could be used in relation to the reduction of particle size during grinding, as this would also improve the butter extraction yield (Asep et al., 2008).

Another step where the chocolate production system could benefit by having data containing information on the farmers activities is in relation to the storing process. As cocoa harvesting is not constant during the year, there is a need of storing the cocoa beans for ensuring a constant production through the whole year. If the cocoa beans are not properly stored, moulds could grow and spoil the product. For this reason chocolate manufacturers often prefer to store the cocoa beans themselves, as their storing conditions are considered to be better than the storing conditions of local buyers in West African countries. Very often the drying process ends when the farmer considers the beans to be ready. Most of the time, no moisture analysis is done. If information regarding the drying conditions, method of drying, duration and temperature variations, would reach the chocolate production plant, the moisture content could be calculated. As is well known, low and high moisture cocoa beans have quite different shelf life (Doyle et al., 2001). By having these data, low moisture beans could be selected for longer storage times while high moisture beans could be used straight for production; ensuring a better utilization of the raw material.

Finally, the price paid for cocoa beans depends on the quality of the beans. In some cases the colour is used to assess the quality, and thus the price (Ilangantileke, Wahyudi & Bailon, 1991). In many cases cocoa beans are categorized by origin. For example, Accra quality does not refer to any actual quality parameter, but to cocoa beans purchased from a specific harbour in Ghana. In many cases this categorization based on origin indeed reflects a specific set of quality parameters, but it also obstructs the development of new producing countries or areas. A system that allows an additional exchange of data regarding well-defined parameters about the cocoa beans quality would probably allow more accurate categorization of the beans, and more accurate prices can be set.

### 3.3 Health promotion

The antioxidant and health promoting proprieties of chocolate have been thoroughly investigated over the past years. It has been found that many polyphenol compounds are directly correlated with antioxidant properties, free-radical scavenging



properties, treatment of dementia and maintenance of overall cardiovascular health (Schmid, 2007; Porter, Ma & Chan, 1991; Mao, Van de Water, Keen, Schmitz & Gershwin, 2003). A lot of work has been done on the health potential of cocoa and most of it is discussed in a recent review by Cooper, Donovan, Waterhouse and Williamson (2008). In general, recent research shows that heat treatment and alkalisation are mainly responsible for decreasing the polyphenol content and thereby reducing the health benefits of chocolate (Wollgast & Anklam, 2000; Arlorio et al., 2008; Camu et al., 2008; Andres-Lacueva et al., 2008). Even though more research is definitely needed, it is however possible to start planning the production parameters that allow the production of chocolate with increased health effects, for example as recently published by Tomas-Barberán, Cienfuegos-Jovellanos, Marín, Muguerza, Gil-Izquierdo, Cerdá, Zafrilla, Morillas, Mulero, Ibarra, Pasamar, Ramón and Espín (2007).

#### 4. Further research

This paper shows that much technical material about the relationship between farming practices and cocoa bean quality and especially flavour potential is available. However, there is a lack in coordination as many studies are repetitive in both goals and results. We do believe that further research should focus on the use of the available data more than on finding new data. Regarding the flavour profile for example, we know how aromatic compounds are formed, what their precursors are, and how such precursors are formed. Also, data on the sensorial properties and human acceptability of such aromatic compounds are available (Frauendorfer & Schieberle, 2006; Stark, Bareuther & Hofmann, 2006; Serra Bonvehí, 2005).

Therefore, we suggest that further research should focus on finding how to use the available data. An example could be the development of flavour synthesis simulation models, similar to the simulation models presented in e.g. Saltini and Akkerman (2012), Akkerman and Van Donk (2008) or Ruiz-Garcia, Steinberger and Rothmund (2010). The inputs of such a simulation model could be the cocoa cultivar and all the parameters for all processes, from storing prior to splitting, fermenting, drying, roasting, conching and so on. By using the available data the model could predict the sensorial characteristics of the finished chocolate. This idea is illustrated in Figure 4. Obviously, also the opposite might be possible, where the inputs to the model would be the desired characteristics of the finished product, and the output would be the processing parameters that would produce chocolate with that specified flavour profile. A model like this could help chocolate producers in setting the optimal parameters for processing the cocoa beans in order to control their flavour potential, or produce chocolate with a higher production yield. However, nowadays data on the farming practices are not available for the chocolate manufacturers, but once the benefits of having them are clear, investments for implementing traceability systems will be appealing.

Additionally, efforts for further research could also focus on standardizing the farming practices, for example by developing starter cultures for the fermentation process. The use of starter cultures is already very common for other food products, like cheese or wine. Even if studies for the development of such products have recently started (Adimpong et al., 2010; Leal et al., 2008), much work is still to be done. We hope to contribute to research in this direction by collecting all the known yeasts, LAB and AAB strains in a unique table (see Appendix B). This overview could help the selection of strains for inoculum trials. In the studies mentioned in Appendix B the strains were selected with standard techniques. This standard methodology with plating on specific substrates has always been well considered, but recent findings show that in the case of very complex populations, only a little part of the population is actually identified by this method. Due to recent advances in relation to metagenomics, data on the composition of the entire population can be obtained (Riesenfeld, Schloss & Handelsman, 2004). Based on such data, the optimal inoculum for cocoa fermentation could be obtained and commercialized. In order to further improve the microflora of fermentation, metabolic engineering could be used to construct strains with superior yield and productivity (Lima et al., 2011a; Stephanopoulos, 1994). This approach would fit perfectly into fermentation of cocoa beans so that, once a starter culture is defined, it could be strengthened to increase the cells' production of a certain substrate.

#### 5. Conclusions

In this paper we review the literature on the influence of different farming practices on cocoa bean and chocolate quality parameters. The aim of this paper was to identify the potential benefits of including information on the farming practices used to produce the cocoa beans into the operational decision making process of a chocolate manufacturer. This information is increasingly becoming available through the implementation of traceability systems, which allow such data exchange. The magnitude of the benefits should cover the expenses for setting up well-designed traceability systems – as they are possibly beyond the legal traceability requirements.

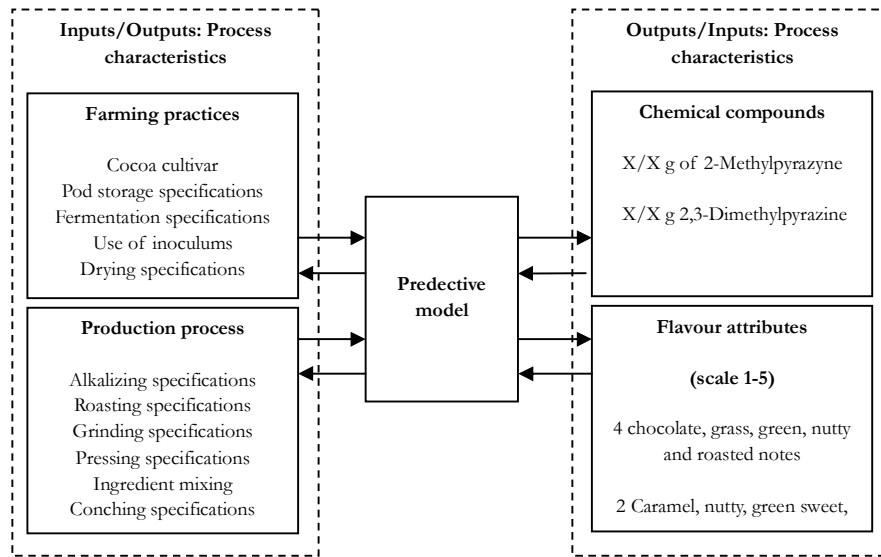


Figure 4. Inputs and outputs of the flavour development simulation model.

After an extensive analysis of the literature, we created a matrix relating each variable in the farming practises to its influence on the eventual consumer product. For each of these relations, we discuss the relevant literature. Based on this, we have identified several potential benefits, which can be classified in the following three categories.

- Benefits to the production process: skipping/reducing unnecessary processes; increasing the cocoa butter extraction yield; increasing the overall production yield; optimizing the warehousing activities; reducing spoilage during storage.
- Benefits to the flavour profile of chocolate: optimizing the blend of cocoa beans based on similarities; improve the flavour profile by controlling the flavour potential of the cocoa beans; reducing cocoa powder fraction without changing the flavour profile; increasing the flavour potential by optimizing the fermentation process by introducing starter cultures; increasing the flavour acceptability by reducing alkalisation.
- Benefits to health promotion: selecting beans with high antioxidant capacity; setting up processing parameters that minimally ruin the antioxidant capacity.

In this paper, we propose and discuss several potential benefits, but further research needs to be done in order to be able to quantify the benefits in specific situations. With this publication, we aim to contribute to the literature by organizing and classifying the state of the art, by highlighting possible directions for further research, and by broadening the discussion on the purpose of traceability systems to a more proactive use in the optimization of production systems.

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## Appendices

- A. Matrix relating the variables in the farming practices and their effects on cocoa beans and chocolate
- B. Microbes involved in cocoa fermentation



*Appendix A. Part 1/2. Matrix relating the variables in the farming practices and their effects on cocoa beans and chocolate.*

		Effect of the variables					
		Proteins/ Amino acids concentration in beans	Concentration of sugars	Concentration of polyphenols	Concentration of pyrazynes	pH	Flavour profile of chocolate
Farming	Cocoa cultivar			Counet et al. (2004)	Counet et al. (2004); Reineccius et al. (1972)	Tomlins et al. (1993)	Sukha et al. (2008); Keeney (1972); Reineccius et al. (1972); Bailey et al. (1962); Clapperton et al. (1994)
	Area of origin /crop year	Kirchhoff et al. (1989a); Rohsius et al. (2006); Sukha et al. (2004); Serra Bonvehí & Ventura Coll (2002); Caligiani et al. (2007)		Counet et al. (2004); Arlorio et al. (2008); Caligiani et al. (2007)	Counet et al. (2004)		Sukha et al. (2004); Jinap (1995)
	Storing	Ortiz de Bertorelli et al. (2009)				Ortiz de Bertorelli et al. (2009); Meyer et al. (1989); Faborode et al. (1995)	Nazaruddin et al. (2006); Howat et al. (1957); Samah et al. (1993); Ardhana (2003); Lima et al. (2011a)
Fermentation	Conditions of fermentation	Rohsius et al. (2006); Hashim et al. (1998)	Portillo et al. (2007); Hashim et al. (1998)	Portillo et al. (2007)		Guechi et al. (2010); Howat et al. (1957); Biehl et al. (1985)	Sukha et al. (2008); Tomlins et al. (1993); Doyle et al. (2001); Lainé (2001); Biehl et al. (1985)
	Duration of intervals between mixing during fermentation	Hashim et al. (1998); Senanayake (1997)	Portillo et al. (2007); Hashim et al. (1998); Senanayake (1997)	Portillo et al. (2007)	Hashim et al. (1998)	Portillo et al. (2007); Senanayake (1997); Guechi et al. (2010)	Leal et al. (2008) -
	Fermentation grade	Kirchhoff et al. (1989a; 1989b); Adeyeye et al. (2010); Sukha et al. (2004); de Brito et al. (2001); de Muijnck (2005); Hashim et al. (1998); Rohan & Stewart (1967a; 1967b); Reineccius et al. (1972); Rohan (1964); Aremu et al. (1995); Amin et al. (1998)	Reineccius et al. (1972); Rohan & Stewart (1967a); Hashim et al. (1998)	Arlorio et al. (2008); Portillo et al. (2007); Camu et al. (2008b); Caligiani et al. (2007); Biehl et al. (1985)	Sukha et al. (2004); Reineccius et al. (1972); Hashim et al. (1997)	Senanayake (1997); Tomlins et al. (1993); Aremu et al. (1995); Lagunes Gálvez et al. (2007); Biehl et al. (1985)	de Muijnck (2005); Hashim et al. (1999); Reineccius et al. (1972); Nazaruddin et al. (2006)
	Microflora during fermentation	Hansen & Olmo (1998)		Camu et al. (2008a)		Leal et al. (2008), Samah et al. (1993)	Leal et al. (2008); Camu et al. (2008a; 2008b); Schwan & Wheals (2004); Jespersen et al. (2005); Nielsen et al. (2007)
Drying	Conditions of drying	Rohsius et al. (2006)				Zahouli et al. (2010)	Sukha et al. (2008); Zahouli et al. (2010); Faborode et al. (1995)
	Temperature of drying					Hii et al. (2009)	Hii et al. (2006)
	Duration of drying	Hashim et al. (1999)	Hashim et al. (1999)				Jinap et al. (1994b); Hashim et al. (1999)
	Drying rate					Bonaparte et al. (1998)	Sukha et al. (2008)
	Drying grade					Garcia-Alamilla et al. (2007)	

<b>Processing</b>	<b>Roasting</b>	Adeyeye et al. (2010); Granvogel & Schieberle (2006); de Brito et al. (2001); Reineccius et al. (1972); de Brito et al. (2001)	Reineccius et al. (1972); Rohan (1966)	Arlorio et al. (2008); Oliviero et al. (2009); de Brito et al. (2001)	Ramli (2006); Serra Bonvehí & Ventura Coll (2002)		Plumas (1996); Ramli (2006); Jinap et al. (1998); Keeney (1972); Reineccius et al. (1972); Krysiak (2006); Bailey et al. (1962); Ramli (2006)
	<b>Finished product</b>					Not relevant	Noor-Soffalina et al. (2009)
	<b>pH</b>						Delcour et al. (1983)
	<b>Oxidation of Polyphenols</b>	Not relevant	Not relevant				de Muijnck (2005) Keeney (1972)
	<b>Concentration of flavor precursors</b>	Not relevant	Not relevant	Not relevant			
	<b>Other chemical compounds</b>	Not relevant	Not relevant	Not relevant	Not relevant	Not relevant	Frauendorfer & Schieberle (2006); Sukha et al. (2004)

*Appendix A. Part 2/2. Matrix relating the variables in the farming practices and their effects on cocoa beans and chocolate.*

		Effect of the variables					
		Microflora during fermentation	Beans quality (moulds, purple beans...)	Colour	Water content (moisture)	Production yield	Health benefits
Farming	Cocoa cultivar	Not relevant			Not relevant	Motamayor et al. (2003; 2008)	
	Area of origin / crop year		Chaiseri & Dimick (1989); Caligiani et al. (2007)	Arlorio et al. (2008); Counet et al. (2004)	Not relevant		Arlorio et al. (2008); Counet et al. (2004)
	Storing		Tomlins et al. (1993)		Ortiz de Bertorelli et al. (2009)		
Fermentation	Conditions of fermentation		Guehi et al. (2010); Carr et al. (1979)				
	Duration of intervals between mixing during fermentation	Camu et al. (2008b)	Guehi et al. (2010)				
	Fermentation grade	Lagunes Gálvez et al. (2007); Doyle et al. (2001)	Guehi et al. (2010)		Aremu (1995); Lagunes Gálvez et al. (2007)	Asep et al. (2008)	
	Microflora during fermentation		Adimpong et al. (2010); Ardhana (2003); Camu et al. (2007); Jespersen et al. (2005); Nielsen et al. (2007)				
Drying	Conditions of drying		Bonaparte et al. (1998)		Bharath & Bowen-O'Connor (2008); Faborode et al. (1995); Lainé (2001)		
	Temperature of drying				Ndukwu (2009); Garcia-Alamilla et al. (2007); Hii et al. (2009)		
	Duration of drying				Bharath & Bowen-O'Connor (2008); Ndukwu (2009); Garcia-Alamilla et al. (2007); Hii et al. (2009); Faborode et al. (1995); Hii et al. (2006); Jinap et al. (1994b); Lainé (2001)		
	Drying rate			Bonaparte et al. (1998)	Faborode et al. (1995); Hii et al. (2006)		
	Drying grade				Bharath & Bowen-O'Connor (2008)		
Processing	Roasting			Krysiak (2006)	Arlorio et al. (2008); Keeney (1972)	Asep et al. (2008)	Arlorio et al. (2008); Oliviero et al. (2009); de Brito et al. (2001); Oliviero et al. (2009)
Finished product	pH						
	Oxidation of Polyphenols			Oliviero et al. (2009)	Mayer (2006)		Mao et al. (2003)
	Concentration of flavor precursors						

*Appendix B. Microbes involved in cocoa fermentation*

	Microbe	Notes	Main activity	Specifications	Reference
Yeasts	Candida spp.	Increased in number after 24h.	Ethanol and lactic acid assimilation. Assimilation of glucose and sucrose. Able to assimilate citrate.	Brazil, Ghana, Malaysia, Belize, Dominican republic	Schwan & Wheals (2004); Lagunes Gálvez et al. (2007); Daniel et al. (2009)
	Candida krusei	Dominant species. Detected in different fermentation methods.	Ethanol fermentation, citric and lactic acid assimilation. Acetic acid production.	Ghana, Dominican republic	Jespersen et al. (2005); Nielsen et al. (2005); Lagunes Gálvez et al. (2007)
	Candida tropicalis	Important presence. Pathogen.	Assimilation of glucose and sucrose.	Indonesia	Ardhana (2003); Wingard et al. (1979); Daniel et al. (2009)
	Hansenula spp.		Largely used in difference food and pharmaceuticals productions. Assimilation of glucose and sucrose.	Ghana, Malaysia	Schwan & Wheals (2004); Daniel et al. (2009)
	Hanseniasspora guilliermondii	Detected in several fermentation methods. Dominating yeast during early stages.	Ethanol fermentation, acetoin and 2,3-butanediol production. Already used in wine production.	Ghana, Dominican republic	Jespersen et al. (2005); Nielsen et al. (2005); Nielsen et al. (2007); Lagunes Gálvez et al. (2007); Zironi et al. (1993)
	Kloeckera spp.	Disappeared after 24h.	Already used in wine production.	Brazil, Ghana, Malaysia, Belize	Schwan & Wheals (2004); Zironi et al. (1993)
	Kluyveromyces marxianus	Grow slowly and disappeared. Artificially inoculating a hybrid of this strain improves flavour acceptability.	Pectinolytic activity.	Brazil.	Schwan & Wheals (2004); Leal et al. (2008)
	Kluyveromyces thermotolerans	Found when temperature higher than 50 °C.		Brazil.	Schwan & Wheals (2004)
	Kodamaea ohmeri		Assimilation of glucose and sucrose.		Daniel et al. (2009)
	Lodderomyces elongisporus	Disappeared after few hours.		Brazil.	Schwan & Wheals (2004)
	Meyerozyma		Assimilation of glucose and sucrose.		Daniel et al. (2009)
	Pichia spp. - Pichia membranifaciens	Disappeared after few hours. Detected in different fermentation methods. Dominating yeast in late stages. Detected in early stages.	Ethanol fermentation, lactic and citric acids assimilation. Assimilation of glucose and sucrose.	Brazil, Ghana, Beliza, Dominican republic.	Schwan & Wheals (2004); Jespersen et al. (2005); Nielsen et al. (2007); Lagunes Gálvez et al. (2007); Daniel et al. (2009)
	Rhodotorula spp.			Malaysia.	Schwan & Wheals (2004); Jespersen et al. (2005)
	Saccharomyces spp.	Dominant strain during the whole process.	Well known and used in wine production. Assimilation of glucose and sucrose.	Brazil, Ghana, Malaysia, Belize, Trinidad, Indonesia.	Schwan & Wheals (2004); Jespersen et al. (2005); Nielsen et al. (2005); Ardhana (2003); Zironi et al. (1993); Daniel et al. (2009)
	Saccharomycopsis spp.			Ghana, Beliza.	Schwan & Wheals (2004)
	Schizosaccharomyces spp.			Ghana, Beliza.	Schwan & Wheals (2004)
	Torulopsis pretoriensis	Found when temperature higher than 50 °C.		Brazil.	Schwan & Wheals (2004)
Torulopsis spp.			Ghana.	Schwan & Wheals (2004)	
Trichosporon asahii			Ghana.	Jespersen et al. (2005)	

	Yamadazyma		Assimilation of glucose and sucrose.		Daniel et al. (2009)
Lactic acid bacteria (LAB)	Lactobacillus. Acidophilus		Degrading glucose to lactic acid. Most of them already used in various industrial fermentations, like cheese, beer or wine production.	Brazil, Africa.	Schwan & Wheals (2004); Adimpong et al. (2010)
	Lb. brevis	Present between 48 and 96h.		Brazil, Belize, Dominican republic.	Schwan & Wheals (2004); Lagunes Gálvez et al. (2007)
	Lb. buchneri			Belize.	Schwan & Wheals (2004)
	Lb. casei			Brazil, Belize.	Schwan & Wheals (2004)
	Lb. cellobiosus	Principal spices		Belize, Indonesia.	Schwan & Wheals (2004); Ardhana (2003)
	Lb. Delbrueckii			Brazil, Belize.	Schwan & Wheals (2004)
	Lb. fermentum	Most abundant in first 24h – dominating LAB strain		Brazil, Ghana, Belize.	Camu et al. (2007; 2008a; 2008b); Schwan & Wheals (2004); Nielsen et al. (2007)
	Lb. fructivorans			Belize.	Schwan & Wheals (2004)
	Lb. gasseri			Belize.	Schwan & Wheals (2004)
	Lb. kandleri			Belize.	Schwan & Wheals (2004)
	Lb. Plantarum	Principal spices		Brazil, Ghana, Malaysia, Belize, Indonesia, Africa, Indonesia, Dominican republic.	Camu et al. (2007; 2008a; 2008b); Schwan & Wheals (2004); Adimpong et al. (2010); Ardhana (2003); Lagunes Gálvez et al. (2007)
	Lb. paracasei	Present after 48h.		Dominican republic.	Lagunes Gálvez et al. (2007)
	Lb. pentosus	Present after 48h		Dominican republic.	Lagunes Gálvez et al. (2007)
	Lb. collinoides			Ghana, Malaysia.	Schwan & Wheals (2004)
	Lb. Lactis			Brazil.	Schwan & Wheals (2004)
	Lb. mali			Ghana.	Schwan & Wheals (2004)
	Lactococcus lactis	Most abundant in first 24h		Brazil, Africa.	Schwan & Wheals (2004); Adimpong et al. (2010)
	Leuconostoc mesenteroides	Most abundant in first 24h		Brazil, Belize, Africa.	Schwan & Wheals (2004); Adimpong et al. (2010)
	Leuconostoc oenos			Belize.	Schwan & Wheals (2004)
	Leunostoc paramesenteroides			Belize, Ghana.	Schwan & Wheals (2004); Camu et al. (2007)
	Leuconostoc pseudoficulneum	Might be important		Ghana.	Nielsen et al. (2007)
	Leuconostoc pseudomesenteroides			Ghana.	Camu et al. (2007)
	Pediococcus acidilactici			Brazil, Africa.	Schwan & Wheals (2004); Adimpong et al. (2010)
P. dextrinicus		Brazil.	Schwan & Wheals (2004)		
Weissella		Africa, Ghana.	Adimpong et al. (2010); Camu et al. (2007)		

Acetic acid bacteria (AAB)	Acetobacter spp.	Most common species. Its metabolism is improved by the presence of oxygen.	Oxidation of ethanol to acetic acid and further oxidation of the latter to carbon dioxide and water. They are obligatory aerobic.	Belize.	Schwan & Wheals (2004)
	A. aceti			Brazil, Indonesia.	Schwan & Wheals (2004)
	A. ascendens			Ghana.	Schwan & Wheals (2004)
	A. ghanensis			Ghana.	Camu et al. (2007; 2008b)
	A. lovaniensis	Present between 72 and 96 h.		Malaysia, Dominican republic.	Schwan & Wheals (2004); Lagunes Gálvez et al. (2007)
	A. pasteurianus	Predominant AAB		Brazil, Indonesia, Ghana, Indonesia.	Camu et al. (2007; 2008a; 2008b); Schwan & Wheals (2004); Nielsen et al. (2007); Ardhana (2003)
	A. peroxydans			Brazil.	Schwan & Wheals (2004)
	A. rancens			Ghana, Malaysia.	Schwan & Wheals (2004)
	A. senegalensis			Ghana.	Camu et al. (2007; 2008b)
	A. syzygii	Predominant AAB		Ghana.	Nielsen et al. (2007); Camu et al. (2007)
	A. tropicalis	Predominant AAB		Ghana.	Nielsen et al. (2007); Camu et al. (2007)
	A. xylinum			Ghana, Malaysia.	Schwan & Wheals (2004)
	A. xylinum			Malaysia.	Schwan & Wheals (2004)
	Gluconobacter oxydans			Brazil, Malaysia, Belize, Ghana.	Schwan & Wheals (2004)

<b>Spore forming bacteria</b>	Bacillus spp.	Dominant in late stages.	Under fermentative conditions they might produce 2,3 butanediol, pyrazines, acetic and lactic acid, off flavours.	Brazil, Trinidad, Ghana, Malaysia, Indonesia.	Schwan & Wheals (2004); Nielsen et al. (2007); Ardhana (2003)
	B. brevis			Brazil.	Schwan & Wheals (2004)
	B. cereus	Development of off-flavours.		Brazil, Trinidad.	Schwan & Wheals (2004)
	B. circulans			Brazil.	Schwan & Wheals (2004)
	B. coagulans			Brazil, Trinidad.	Schwan & Wheals (2004)
	B. firmus			Brazil.	Schwan & Wheals (2004)
	B. laterosporus			Brazil.	Schwan & Wheals (2004)
	B. licheniformis			Brazil, Trinidad, Ghana, Malaysia, Indonesia.	Schwan & Wheals (2004); Nielsen et al. (2007); Ardhana (2003)
	B. macerans			Brazil.	Schwan & Wheals (2004)
	B. megaterium	Development of off-flavours.		Brazil.	Schwan & Wheals (2004)
	B. pasteurii			Brazil.	Schwan & Wheals (2004)
	B. polymyxa			Brazil.	Schwan & Wheals (2004)
	B. pumilus			Brazil, Trinidad, Indonesia.	Schwan & Wheals (2004); Ardhana (2003)
	B. stearothermophilus			Brazil, Trinidad.	Schwan & Wheals (2004)
B. subtilis	Development of off-flavours.	Brazil, Trinidad, Ghana, Malaysia.	Schwan & Wheals (2004)		