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Assessment of the fermentative process from different cocoa cultivars produced in Southern Bahia, Brazil

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The "witch broom disease" caused by the fungus *Moniliophtora perniciosa* drastically reduced cocoa production in Southern Bahia (Brazil), and cloning techniques has been used with trees that showed resistance to the fungus. On the other hand, the genotype, agronomic management, soil and weather characteristics, as well as post-harvest technology directly affected the quality of beans. The present study aimed at evaluating the fermentation process of two cultivars of cocoa beans resistant to "witch broom disease" and a non-resistant cocoa cultivar susceptible to the disease, produced in south of Bahia. The fruits of all cultivars were physically evaluated and fermentations were monitored. The total phenolics and tannins were also evaluated. The results show many differences among cultivars, and the main ones include the low weight and number of seeds in SR162 cultivar and the high pulp weight in the PH16 cultivar. During fermentation, cultivars showed different behavior with regards to pH, acidity and temperature and had different reduction in tannins and total phenolics. Therefore, one may conclude that the cultivars studied showed distinct physical and chemical characteristics. Thus, it is recommended that fermentation should be conducted differently for each cultivar, in order to obtain their best features to produce high quality chocolates.

Key words: Cocoa, fermentation, phenolic, tannins.

INTRODUCTION

The "witch broom disease" caused by the fungus *Moniliophtora perniciosa* drastically reduced cocoa production in Southern Bahia (Brazil), and cloning techniques has been used with trees that showed resistance to fungus. This practice seems to be one of the most effective ways to avoid the disease (Leal et al., 2008; Almeida et al., 2009, Pires et al., 2012).

The genotype directly affects the quality of beans, contributing to flavor, aroma and taste precursors for the chocolate. Other factors such as agronomic management, soil and weather characteristics, as well as post-harvest technology, also contribute to chocolate flavor (Afoakwa, 2010).

The main reason for huge cocoa demand is related to flavor that is developed by a combined process of pre-processing and manufacturing. The unfermented cocoa beans are extremely bitter and astringent. The fermentation process is essential to prepare the cocoa beans in order to obtain the products available in the market and, it is associated with changes within the seed during this step.

During fermentation, the pulp that enfolds the seed is wiped out by successive action of microorganisms (yeasts and lactic and acetic bacteria) from the environment, raising the temperature to about 50°C (Lopez and Mcdonald, 1982).

These microorganisms act on organic acids and sugars from the pulp, which are transformed into ethanol,

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lactic acid and especially acetic acid (Schwan and Wheals, 2004). The generated organic acids penetrate the seeds, and the high temperature achieved due to aerobic fermentation, cause the death of the embryo and tissue acidification. With the embryo death, the membrane selective permeability is lost, allowing contact between enzymes and substrates, typically separated into living tissues (Lopez, 1986). These changes significantly affect the final product quality, especially the aspect involving the flavor formation (Schwan, 1996).

According to Beckett (2009), the development of cocoa flavor precursors occurs in the cotyledons during fermentation nand drying. There are two major types of cells in the cotyledons: storage cells, containing fats and proteins and, pigment cells, containing phenolic compounds and methylxanthines (caffeine and theobromine). After the seed death, cell walls and membranes are broken, allowing reactions between the various compounds and enzymes.

In studies conducted by Efraim et al. (2010), different inbehavior of different cocoa cultivars related to temperature and pH was observed during fermentation, leading to finding of differences in liquors, butter and chocolate.

Thus, the present study aimed at evaluating the fermentation process of two cultivars of cocoa beans resistant to “witch broom disease” and a sample of conventional cocoa, susceptible to the disease, produced in south of Bahia.

MATERIALS AND METHODS

Cocoa beans

Cocoa beans from cultivars resistant to “witch broom disease”, “SR162” and “PH16”, and a blend of cultivars composed by *Pará*, *Parazinho* and *Maranhão*, all belonging to *Forastero* group and non-resistant to the disease were used. These *Forastero* cultivars are grown from decades in Bahia, and considered as cultivars of high productivity and high fruit quality; however, non-resistant to the disease.

The “PH16” originated from a selection performed in a commercial area. They have unknown parents and the plant was originally identified in 1996 in a population of hybridcocoa (crosses between *Amazônico* group and *Trinitario*) from “Porto Híbrido” farm in São José da Vitória County, Bahia, Brazil.

The “SR162” is a cultivar derived from genetic mutation of the common cocoa (*Alto Amazônico* group), with white seeds. The name came from the farm where they were identified; “São Roque” farm in Itagibá County, Bahia, Brazil.

Physical characterization of the fruits

Ten ripe fruits of the selected cultivars were harvested and the total mass of fruits, peel, seeds and placenta were individually determined. The number of seeds per fruit, length, width and thickness of fresh seeds were also determined.

Fermentation and drying process

The fermentation was carried out in 70 x 70 x 75 cm wooden boxes with about 20 holes (1.27 cm each) in the bottom and sides of the boxes in order to allow the liquefied pulp produced by fermentation to flow. A total mass of 400 kg in each box were processed. Turnings were performed for oxygenation and mixing of the mass 48 h from the beginning of each fermentation and after decreasing the temperature until the end of the process. Banana leaves were used to cover the mass. After fermentation, seeds were dried in the sun-roof surfaces with mobile timber for 5 to 7 days up to 8.0% of moisture.

Evaluation of the fermentation process

At the beginning of fermentation, soluble solids contentin pulp (°Brix) were determined in each material with portable refractometer Instrutherm, ModelRT-30 ATC(0-32). (°Brix) During fermentation, temperature was measured (Digital ThermometerMINIPA, MT-450 model) and samples were taken every 12 h. The pH (pH meter portable Digital PHtek) and the titratable acidity were determined in the samples according to the AOAC (1985).

The samples were frozen and then, freeze dried in a LIOTOP freeze dryer, L108 model in order to perform the total phenolics and tannins analyses.

Total phenolic content

Methanolic extracts for total phenolic determination were obtained according to Fantozzi and Montedoro (1978). 10 g of cocoa mass and chocolate were weight and defatted with petroleum ether, under shacking during 30 min for three times. From defatted samples, 5 g were weight and 80% methanol in water (v/v) solution was added and stirred for hours. After filtration, the methanolic extracts were stored in dark flask under nitrogen atmosphere at low temperature. The spectrophotometric method was used to determine the total phenolic content according to the method described by Gutfinger (1981) using Folin-Ciocalteu reagent. The phenolic content was calculated based on the catechin calibration curve.

Determination of total tannins

The tannins were determined spectrophotometrically in the same extracts by the vanillin-HCI method as described by Price et al. (1977) using catechin as standard.

Quality evaluation of fermented and dried cocoa beans by cutting test

The cut test was performed through the longitudinal section of beans (Figure 1), checking the quality according to the degree of fermentation, verifying faults as color and compartmentalization of beans, as recommended by the Ministryof Agriculture, Livestock and Supply from Brazil (Brasil, 2008) (Table 1). The fermented and dried beans of each material were randomly collected and determinations were performed in triplicate.

RESULTS AND DISCUSSION

Physical characterization of the fruits

The results of physical characterization of fruits are shown in Tables 2 and 3. According to Table 2, there was no significant difference in fruit weight between the two

Figure 1. Board for cocoa beans classification (300 beans).

Table 1. Technical regulation for cutting test of cocoa beans in Brazil (Brazil, 2008).

Product placement	Fault (%)					
	Moldy	Smoky	Damaged by insect	Slate	Germinated	Flattened
Type 1	From zero to 4.0	From zero to 1.0	From zero to 4.0	From zero to 5.0	From zero to 5.0	From zero to 5.0
Type 2	Above 4.0 to 6.0	Above 1.0 to 4.0	Above 4.0 to 6.0	Above 5.0 to 10.0	Above 5.0 to 6.0	Above 5.0 to 6.0
Type 3	Above 6.0 to 12.0	Above 4.0 to 6.0	Above 6.0 to 8.0	Above 10.0 to 15.0	Above 6.0 to 7.0	Above 6.0 to 7.0
Out oftype	Above 12.0 to 25.0	Above 6.0	Above 8.0	Above 15.0	Above 7.0	Above 7.0

Table 2. Physical characterization of cocoa fruits from different cultivars, considering the total weight and the fruits parts.

Cultivar	Weight (g)			
	Fruit	Seed	Shell	Pulp
SR 162	524.90±127.99[a]*	59.50±17.76[b]	453.50±111.67[a]	7.60±3.10[b]
PH16	654.60±132.89[a]	148.40±30.38[a]	475.80±108.81[a]	27.0±8.98[a]
Non-resistant	401.10±26.33[b]	103.80±22.16[a]	286.80±15.16[b]	9.70±2.63[b]

*Results are expressed as mean ± standard deviation. Different letters in the same column show significant difference by the Tukey test at 5% confidence level.

"witch broom disease" resistant cultivars, and these cultivars were bigger than those non-resistant one, which showed the lowest weight. It was expected, since these fruits were infected by the disease (Alameida et al., 2009). However, the weight of seeds in non-resistant cultivar did not show significant difference when compared with PH16 cultivar, which was the heaviest one. The SR162 showed the lowest seed weight, about 50% of the other cultivars, indicating low productivity for industrial use. Nevertheless, studies are necessary to investigate its characteristics in order to establish the industrial potential in producing high quality chocolates.

Table 3. Physical characterization of cocoa seeds from different cultivars.

Cultivar	Seed				Soluble solid (°Brix)*
	Number perfruit	Length (mm)	Width (mm)	Thickness (mm)	
SR 162	30.6 0±7.92[b**]	24.54±1.77[a]	11.31±1.07[b]	9.14±0.54[a]	16.12±1.77[a]
PH16	42.70±9.07[a]	23.90±2.24[a]	14.28±0.77[a]	9.57±0.97[a]	17.47±0.75[a]
Non-resistant	42.20±6.81[a]	24.54±1.83[a]	13.45±1.69[a]	8.12±0.75[b]	17.00±0.92[a]

*Values fixed to 27°C; **results are expressed as mean ± standard deviation. Different letters in the same column show significant difference by the Tukey test at 5% confidence level.

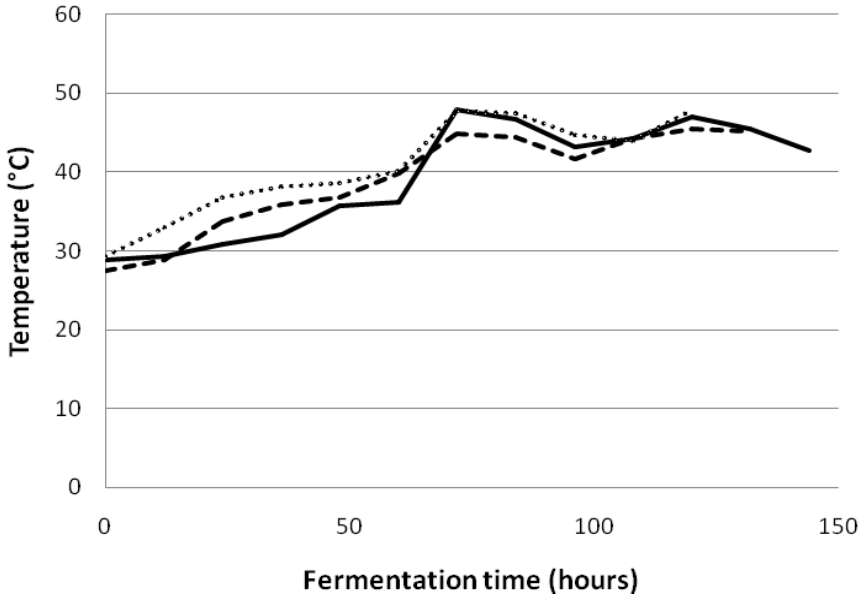

Figure 2. Temperature variationof cocoa beans massduring cultivars fermentation. The values are the mean from two fermentation replicates and three temperature measurements taken at the top, middle and bottom of theboxes. ▪▪▪▪▪, Non resistant; ▬ ▪, SR162; ▬▬, PH16.

Considering shell, only the non-resistant cultivar was significantly different, this was also expected due to the fact that the disease is less thick, shows dark spots, wrinkle and stiff (Almeida et al., 2009).

The PH16 showed, statistically, the highest amount of pulp. This result is extremely important, since the high sugar content and low pulp pH, associated with the absence of oxygen allow yeasts proliferation at the beginning of the fermentation and the sugars turn into alcohol and CO_2 that activate pectinolitic enzymes that hydrolyze pectic polysaccharides in the pulp (Thompson et al., 2001).

The physical characterization of the cocoa seeds from different cultivars is shown in Table 3. The SR162 cultivar had lower number of seeds inside the fruit than the other cultivars. This result can justify the lower weight of seeds observed previously for that cultivar since the seeds in all cultivars had almost the same dimensions. Considering the seeds dimensions, no difference was observed in length, but SR162 showed shorter width and the non-resistant cultivars were thinner than the others. No significant differences were observed in soluble solids between the three cultivars, showing that both of them had the same potential for fermentation, making substrate and microorganisms to carry out the process, not forgetting that PH16 has about three fold more solids to be fermented than the other cultivars.

Thus, many differences could be observed between cultivars, and the main ones included the low weight and number of seeds in SR162 cultivar and the high weight of pulp in PH16 cultivar. However, all cultivars had potential for chocolate production.

Fermentation monitoring

In Figures 2, 3 and 4, the curves of temperature, acidity and pH of the mass of cocoa seeds during fermentation

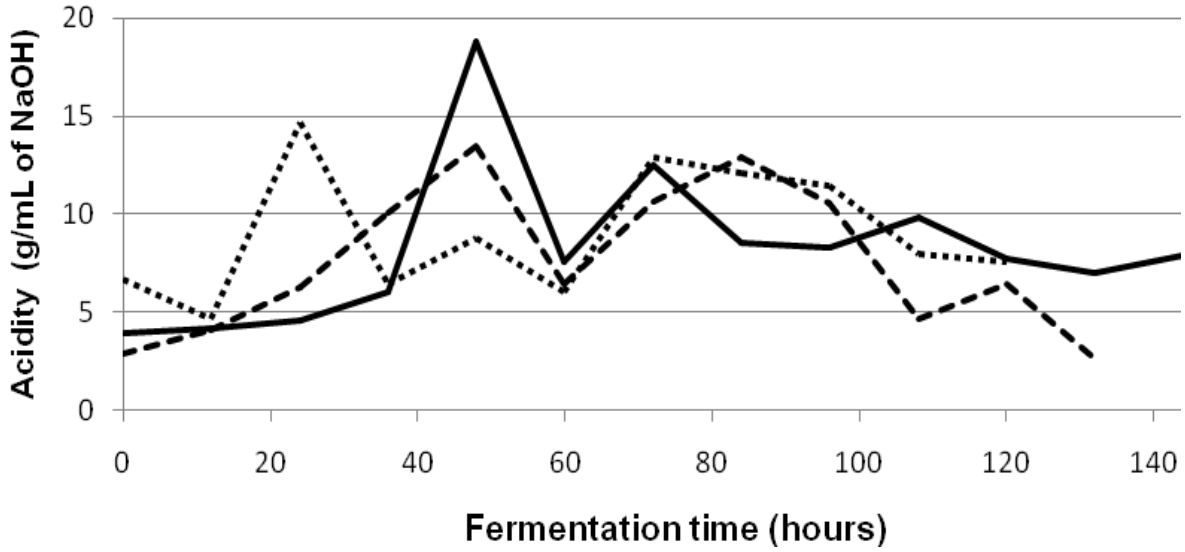

Figure 3. Changes in acidity of the cocoa beans mass during cultivars fermentation. The values shown are the average of two fermentation replicates. ▪▪▪▪▪, Non resistant; ▬ ▪, SR162; ▬▬, PH16.

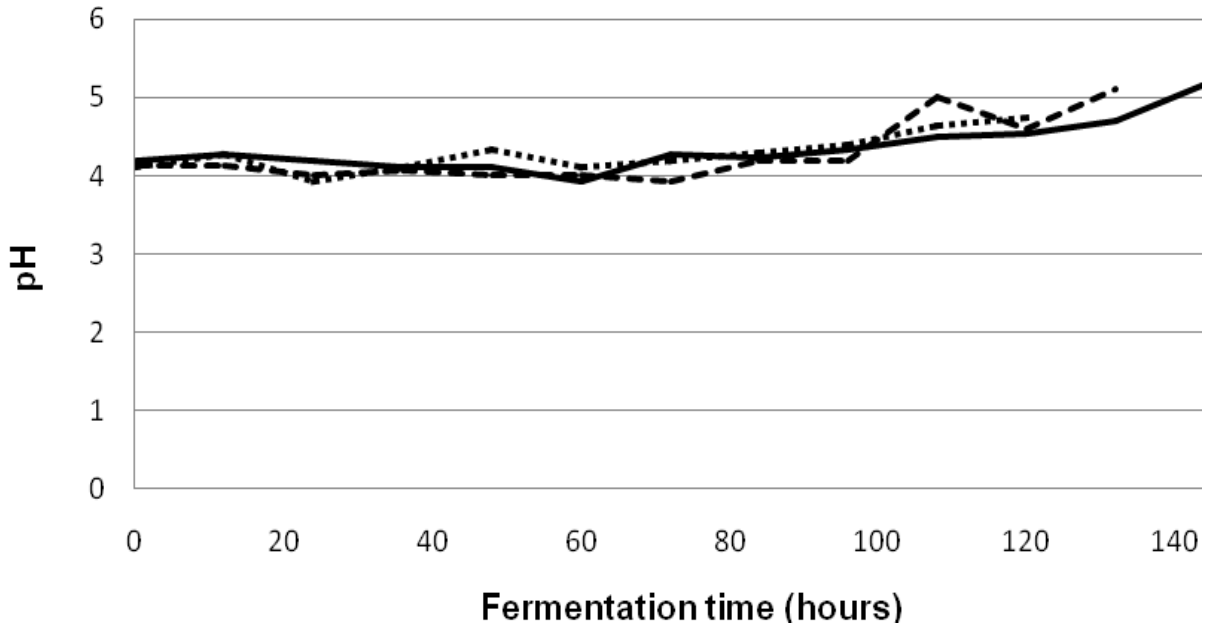

Figure 4. pH variation of the cocoa beans massduring fermentationof the cultivars. The values shownare the mean oftwofermentation replicates. ▪▪▪▪▪, Non resistant; ▬ ▪, SR162; ▬▬, PH16.

for different cultivars are shown. According to Figure 2, during the fermentation process, the temperature increased in all cultivars reaching values close to 50°C. The maximum temperatures were reached between 48 and 72 h, as noted also by Efraim et al. (2010). By comparing the three cultivars, all of them reached high temperature at the same time, though SR162 did not reach the same values achieved by non-resistant and PH16 cultivars, which may be due to the physical characteristics of the fruit with small seeds forming a compact mass, preventing greater oxygenation and decreasing the efficiency of the aerobic phase of fermentation.

The acidity behavior was distinct for the three cultivars (Figure 3). Generally, during fermentation, there were acidity peaks just before turnings, showing the intense

action of microorganisms with the formation of acids. According to Schwan and Wheals (2004), the microorganisms acting on sugars from the pulp, transform them into ethanol, lactic acid and especially acetic acid. The PH16 showed highest values of acidity after 48 h, just before the first turning, which was expected, once this cultivar had more soluble solids in its pulp, providing better medium to microbial growth. On the other hand, non-resistant cultivar did not show the same behavior of the other cultivars, showing acidity peak after 24 h from the beginning of the fermentation. This was probably due to the microbial load in the beginning of fermentation, whereas the process occurs with the natural flora in beans surface (Lopez and Mcdonald, 1982) and beans already showed the highest level of acidity at this time. Besides, these beans came from diseased fruits, which were extremely exposed to microorganisms due to the injuries caused in fruits by the disease.

After 48 h, acidity peaks were still observed in all cultivars before turnings, but with smaller values, since the substrate for fermentation starts to exhaust. With the end of substrates permanence of turnings and high temperatures, the acidity was reduced due to acids volatilization and the fermentation process ended. As described by Beckett (2009), the embryo was dead in the end of fermentation and the membranes selectiveness was lost, allowing contact between enzymes and substrates, creating an environment propitious to the formation of cocoa flavor precursors.

The fermentation time was also different for the three cultivars. In the non-resistant one, fermentation stopped earlier than the other cultivars (120 h), due to the substrate consumption in the early stage of the process. The SR162 stopped the process in 132 h and the PH16 took the longest time (144 h), since there were much more soluble solids to be consumed in this cultivar.

The final acidity was also different between cultivars; the SR162 showed the lowest acidity with values below 3 $g.ml^{-1}$ of NaOH, while the other cultivars showed values close to 8 $g.ml^{-1}$ of NaOH. The lowest acidity showed by SR162 cultivars can be due to the seeds size, which exposed a larger surface, facilitating the acids volatilization. These findings are very important, considering that the acidity will interfere directly and in a remarkable way with chocolate flavor (Efraim, 2010).

The pH variation during fermentation was similar for all cultivars that started the process at pH of 4.1 (Figure 4). Little variation in pH was observed during fermentation for the three cultivars, but after 80 h of fermentation, the pH started to rise up and the final values were different for all cultivars. The non-resistant cultivar showed the lowest pH value (4.7), while the SR162 showed 5.0 and PH16 5.1. These differences are also very important, since it is well known that reactions that lead to the formation of flavor precursors are carried out by endogenous enzymes in cocoa seeds and the fermentation plays an important part in lowering the pH to allow those enzymes action. Furthermore, studies showed that the high potential of taste can be correlated with mild acidification (pH 5.5 to 5.0) during fermentation (Amin et al., 2002).

Tannins and total phenolics during fermentation

The initial total phenolics content differed among the three cultivars (Figure 5). The SR162 cultivar showed lower levels than PH16 and non-resistant cultivars, 33.1, 52.5 and 45.3 $mg.g^{-1}$, respectively. This occurs due to the absence of pigments (anthocyanins) in SR162.

Figure 5 shows that PH16 cultivar had decreased total phenolics content of about 36.5% during fermentation process, while SR162 and non-resistant cultivars had almost the same content found in the beginning of fermentation, though these two cultivars showed variation during the process. This behavior is related to the hydrolysis of tannins and procyanidins and the action of polyphenoloxidase; processes that generate and oxidize, respectively, smaller phenolic compounds in the medium (Brito et al., 2002; Wollgast and Anklam, 2000; Misnawi et al., 2003). According to Cros et al. (1982), total phenols in cocoa got reduced during fermentation to 30% of the initial value. Under this aspect, it can be assumed that the smaller phenolic compounds had more intense oxidation in PH16 than the other cultivars, probably due to the fermentation conditions achieved by this cultivar during the process as previously discussed.

After fermentation, there was reduction in tannin contents, and the SR162 cultivar had the greatest reduction (60.3%) for PH16; the reduction was 51.7% and non-resistant cultivar was 43%, as shown in Figure 6. This reduction is attributed to the binding of these compounds with amino acids and proteins, facilitated by the presence of compounds produced in fermentation such as acetaldehydes, alcohol and acetic acid, starting the development of flavor precursors (Ferrão, 2008; Brito et al., 2002).

Evaluation of fermented and dried cocoa beans

In Table 4, the results of the cut test of fermented and dried cocoa beans according to the Brazilian law (Brasil, 2008) are shown. A great number of slate beans were found, suggesting unfermented or poorly fermented beans. The PH16 cultivar had 14.60% of slate beans, SR162 had 37.67% and non-resistant cultivars had 19.30%, all of them were out of type according to the Brazilian law, since the values are above 12% (Table 1). The good quality of cocoa beans is heavily dependent on the degree and time course of the cotyledons acidification during the fermentation process. The slate beans are indicative of faults in the fermentation process, such as

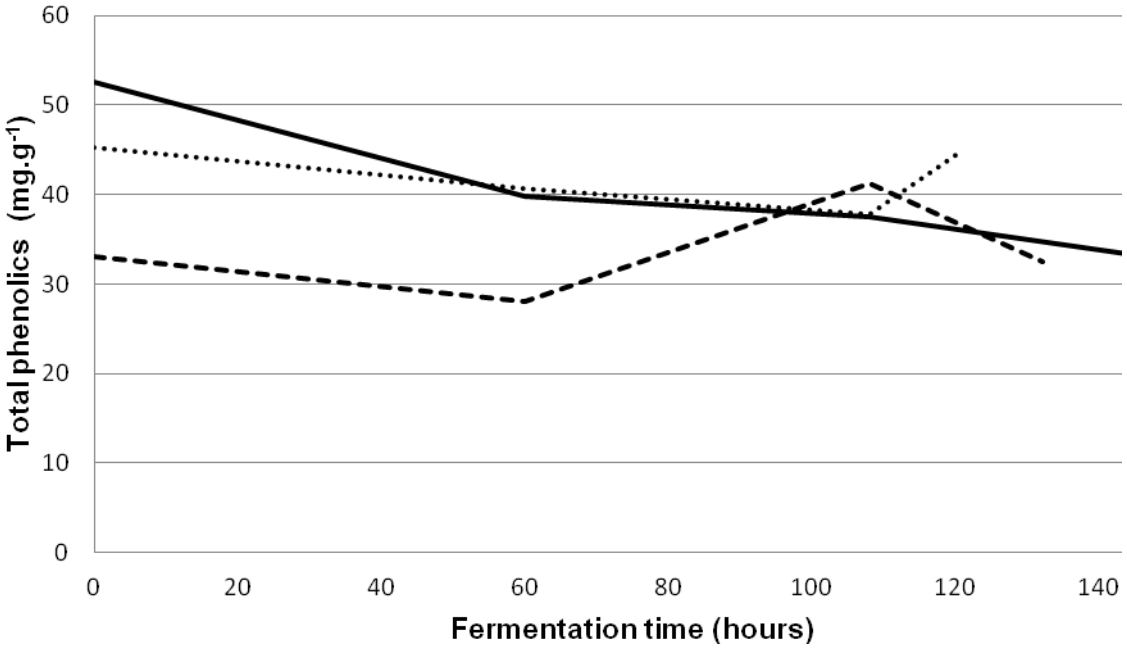

Figure 5. Total phenolics content in cocoa mass during fermentation. The values are the mean between two replicates of fermentation. ▪▪▪▪▪, Non resistant; ▬ ▬, SR162; ▬▬, PH16.

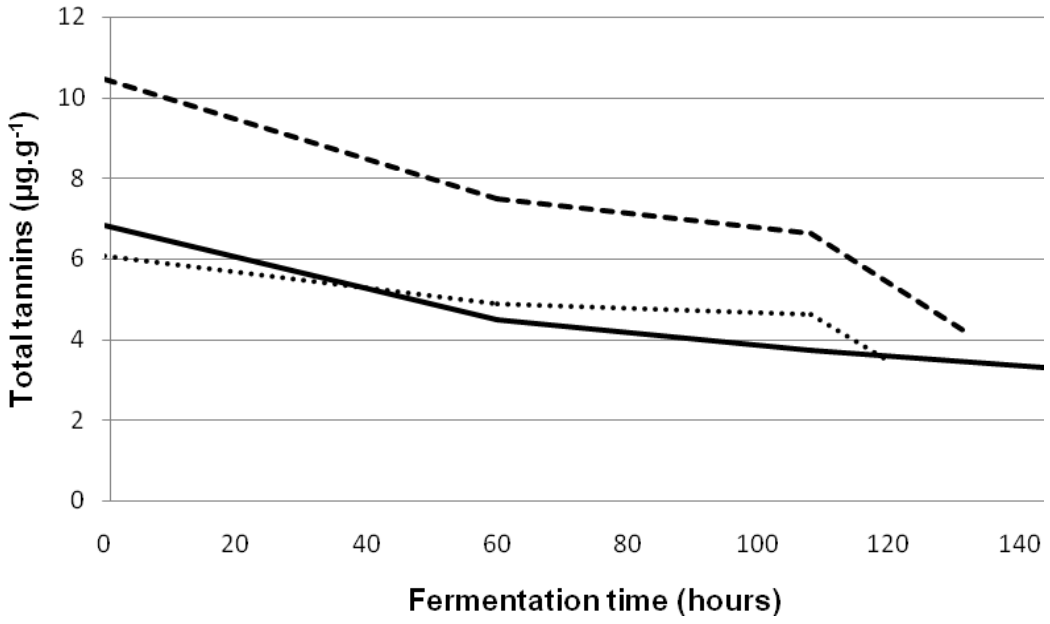

Figure 6. Total tannins content in cocoa mass during fermentation. The values were mean between two replicates of fermentation. ▪▪▪▪▪, Non resistant; ▬ ▬, SR162; ▬▬, PH16.

lack of aeration and dryness, caused by inappropriate turnings of the mass, in which the beans in surface suffer dehydration, and those ones inside the mass did not receive the necessary oxygen for acetic fermentation. This fact can explain the highest level of slate beans in SR162 cultivar that by having smaller seeds are exposed to these facts. More turnings would be necessary for all cultivars to improve aeration inside the mass and reduce the dryness effects. In other words, the fermentation conduction should be specific for each cultivar in order to obtain better products.

The amount of damage by insects and flattening is

Table 4. Cutting test results of fermented and dried cocoa beans.

Cultivar	Moldy	Smoky	Damaged by insects (%)	Slate (%)	Germinated	Flattened (%)
SR162	-	-	-	37.67	-	1
PH16	-	-	0.33	14.60	-	-
Non-resistant	-	-	0.33	19.30	-	-

acceptable for all cultivars, putting them inside type 1 beans.

Conclusions

Although the cultivar SR162 has seeds with lower weight and smaller size, soluble solids showed no significant difference between the cultivars studied, providing them the same potential for fermentation. However, the physical characteristics displayed by the seeds of SR162 cultivar had influence the fermentation process, since the mass did not reach temperatures around 50°C as observed with other cultivars, probably due to the number of seeds that declined to efficiency of aerobic phase of fermentation by compressing the mass to be fermented.

The PH16 cultivar decreased total phenolics content during fermentation to about 36.5%, while SR162 and non-resistant cultivars had almost the same content found in the beginning of fermentation, though these two cultivars showed variation during the process. Concerning tannin contents, the SR162 cultivar had the greatest reduction (60.3%), for PH16, the reduction was 51.7% and non-resistant cultivar was 43%. These variations in phenolic compounds and tannins between cultivars will certainly influence the chocolate flavor, since these compounds give the chocolate characteristics like bitterness and astringency.

Therefore, one may conclude that the cultivars studied showed distinct physical and chemical characteristics, which will directly influence the fermentation process and the fermented product used for chocolate production. Thus, it is recommended that fermentation should be conducted differently for each cultivar in order to obtain its best features to produce high quality chocolates.

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