Food Chemistry 328 (2020) 127110

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Fermentation in fine cocoa type Scavina: Change in standard quality as the effect of use of starters yeast in fermentation

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ARTICLE INFO

Keywords:
Almonds
Chocolate
Protease
Free amino acids

ABSTRACT

In the present work we aimed to demonstrate the influence of inoculum starter in support high quality fermentation. Cocoa fermentations were performed in wooden boxes and eight yeasts strains were used in separated fermentations of fine cocoa, type Scavina, as starter inoculum. Temperature, pH, titirable acidity, reducing sugar and free amino acids were evaluated during or after fermentation. The influence of starters yeasts on the decrease of acidity, sugar concentration and free amino acids was significant. The strains *Candida parapsilosis*, *Torulaspora delbrueckii* and *Pichia kluyveri* showed greater changes in the reducing sugar and free amino acids in fermented cocoa beans. These results indicate the ability of yeast used as inoculum starter to modify the end condition and further enhance the quality of fine cocoa beans.

1. Introduction

New concepts to produce special chocolates with unique characteristics, has been built. The fruit and good process quality depends on the choice of the most suitable genetic varieties, the degree of maturation, fermentation, drying and storage with defined and controlled practices. In Brazil, especially in the southern region of Bahia, producers started a movement that resulted in important awards in international quality contests for cocoa beans. This recognition led almonds to win special markets, where the quality of flavors and aromas is such to produce high value-added chocolates.

Chocolate is a food that is widely appreciated worldwide for its energy content and taste. Obtained after fermentation, drying and roasting of cocoa beans (Ouattara, Reverchon, Niamke, & Nasser, 2017; Thompson, Miller, Lopez, & Camu, 2013). Conventional chocolate has been giving way to high cocoa-based and fine chocolates. Whether obtained in conventional form or by high quality processing, is the fermentation that promotes the formation of aroma and flavor precursors as reducing sugars and free amino acids, color change and reduction of acidity, bitterness and astringency of seeds (Aprotosoaie, Luca, & Miron, 2016). It is well established that techniques applied in the fermentation as well the presence of microorganisms such as yeast, lactic acid bacteria (LAB) and acetic acid bacteria (AAB), and its metabolism greatly influences the biochemical processes inside the almonds (Ho, Zhao, & Fleet, 2014; Jinap, Siti, & Norsiati, 1994; Nielsen, Snitkjaer, & van den Berg, 2008). Although fermentation conditions that lead to good concentration of free amino acids and reducing sugars have not yet been fully established, starter cultures are studied by researchers to try to establish and standardize conditions for good fermentation (Crafack et al., 2013; Ho et al., 2014).

Cocoa coast in southern Bahia is a micro-region with extremely favorable climatic conditions for planting and obtaining cocoa of quality. Yeasts isolated from fermentation process of cocoa of Scavina type were selected by producing enzymes and killer substances. The

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https://doi.org/10.1016/j.foodchem.2020.127110
Received 7 October 2019; Received in revised form 14 May 2020; Accepted 19 May 2020
Available online 21 May 2020

Scavina variety has its origin in the ancestral group Contamana whose first representatives of this variety were obtained in the Ucayali River basin (Motamayor & Lachenaud, 2008). Scavina is known for its floral aroma notes in the fruit pulp and raw cocoa (Kadow, Bohlmann, Phillips, & Lieberei, 2013). These characteristics make this variety to be considered fine cocoa, characterized by a more aromatic and softer flavor than bulk cacao (Afoakwa, Paterson, Fowler, & Ryan, 2008). In addition, another important characteristic of the Scavina variety is recognized for The Scavina variety has its origin in the ancestral group Contamana (Motamayor & Lachenaud, 2008) whose first representatives of this variety were obtained in the Ucayali River basin. Scavina is known for its floral aroma notes in the fruit pulp and raw cocoa (Kadow et al., 2013). These characteristics make this variety to be considered fine cocoa, characterized by a more aromatic and softer flavor than bulk cacao (Afoakwa et al., 2008). In addition, another important characteristic of the Scavina variety is recognized for having genetic factors that provide greater resistance to broom-witch disease (Royaert et al., 2016) caused by the fungus *Monilioptera perniciosa*, since 1989 found in the southern region of Bahia, Brazil where the present study with Scavina hybrids was developed.having genetic factors that provide greater resistance to broom-witch disease (Royaert et al., 2016) caused by the fungus *Monilioptera perniciosa*, since 1989 found in the southern region of Bahia, Brazil where the present study with Scavina hybrids was developed. The selected yeasts were inoculated as starters in fermentation whose processing of cocoa is known as fine cocoa. Then this work aims to demonstrate the influence of inoculum starter in support high quality fermentation.

2. Materials and methods

2.1. Yeast isolation and screening

Yeast were collected from cocoa fermentation during early crop in Leolinda farm (Uruçuca -BA, Brazil), where fermentation take place in wood boxes of 70 cm 2 (troughs) for 70 kg of almond. At every 12 h (0, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120) samples of cocoa beans were taken from the central portion of each trough, and placed in sterile bag and carried out to Monitoramento Ambiental Lab in Universidade Estadual de Santa Cruz. About 100 g of almonds were pulped and mixed in buffer (0.1% peptone, 0.1% Tween 80). An aliquot of the mucilaginous part was used to isolate microorganisms by plating after serial dilution on Sabouraud agar containing chloramphenicol (0.150 g/L). Plates were incubated at 25 °C for 72 h, then the colonies were isolated in pure culture. The isolated yeasts were stored in GYMP (3 g/L yeast extract, 3 g/L malt extract, 5 g/L bacteriological peptone, 10 g/L glucose and 20 g/L agar, with glycerol added at 20% v/ v) at 4 °C under a layer of sterile mineral oil. Among the isolates, 112 were randomly chosen for enzymatic tests. They were grown in Sabouraud medium with chloramphenicol for 24 h at 28 °C. The inoculum was then standardized to106 cells/mL, 10 μL were plated on solid medium containing different carbon sources and tested for various activities during incubation for 2–5 d at 28 °C. The ability to degrade starch was tested in a medium containing Yeast Nitrogen Base (YNB, DIFCO) 6.7 g/L, soluble starch 2 g/L and agar 20 g/L, with pH of 6.0 (Hankin & Anagnostakis, 1975). After growth, the degradation was revealed by the presence of yellowish halo formed around the colony by the addition of lugol. For esterase activity yeasts were inoculated in semisolid agar containing peptone 10 g/L, NaCl 5 g/L, CaCl2 x2H_2O 0.1 g/L, Tween 80 10 g/L and agar 20 g/L, pH 6.8. Esterase activity was confirmed through opaque halo formed around the colonies. The evaluation for lipase activity occurred in a medium containing peptone 5 g/L, yeast extract 3 g/L, triburtyrin10 g/L and agar 15 g/L, pH 6.0. A clear halo around the colony showed lipase activity. The protease activity was determined on casein-containing (20 g/L) YEPG (yeast extract 10 g/L, peptone 10 g/L, glucose 20 g/L and agar 15 g/L, pH 6.5) (Strauss, Jolly, Lambrechts, & van Rensburg, 2001). A clear halo around the colony revealed protease activity. Pectinase activity were determined on pectin agar medium, which consisted of YNB 6.7 g/L, pectin 10 g/L and agar 20 g/L, pH 7.0 (Strauss et al., 2001). The presence of a purple halo around the colony formed after addition of ruthenium red 0.005% (w/ v) indicated pectinase activity (McKay, 1988). Killer activity was tested on YEPG (pH 4.2 citrate–phosphate 0.05 mol /L buffered) 0.003% (w/ v) methylene blue. Saccharomyces cerevisiae NCYC 1006 and Candida glabrata NCYC Y55 yeast strains were used as sensitivity indicators of killer activity. The sensitive strains were grown on Sabouraud agar for 24 h at 28 °C and thereafter cell density was adjusted to 106 cells/mL. These suspensions were spread on YEPG-methylene blue using a sterile swab. After 30 min, 10 μL of the yeast culture, isolated from the supernatant of cocoa fermentation, previously cultivated in Sabouraud agar, were inoculated into wells made in the agar surface. The plates were incubated at 25 and 28 °C for 48 h. Killer activity was indicated by the growth inhibition halos that had formed around the wells.

2.2. Sequencing of the D1/D2 domain of the 26 rDNA

The D1/D2 domain of the large-subunit (26S) ribosomal DNA was sequenced for identification of 8 yeasts, representing all genera found, that had shown biotechnological potential in the 109 EEA and had proven positive for killer toxin activity (Table 1). The analysis was essentially performed (Jespersen, Nielsen, Honholt, & Jakobsen, 2005) with the following modifications: primers for the amplification of the D1/D2 domain were NL-1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL-4 (5′-GGTCCGTGTTTCAAGACGG-3′). PCR was performed in an automatic thermal cycler (Mastercycler personal-Eppendorf) under the following conditions: initial denaturation at 94 °C for 5 min; 35 cycles of 95 °C for 1 min, 52 °C for 45 s, 72 °C for 1 min and final extension at 72 °C for 7 min. The amplified products were purified with EXO/SAP-protocol of PCR purification based on Exonuclease I and Shrimp Alkaline Phosphatase (Amersham Pharmacia Biotech). DNA was adjusted to 50 ng/μL using geneQuant pro (Amersham-Biosciences). PCR products were directly sequenced in the automated sequencer ABI3730XL (Applied Biosystems). The obtained sequences were compared to the sequences reported in GenBank using the BLAST algorithm.

Table 1
Yeasts identified by sequencing and enzymatic profile and phenotype killer to act as starter inoculum.

Id	Specie	Amylase	Esterase	Lipase	Protease	Killer	Pectinase
A	*Rhodotorula mucilaginosa*	+	+	+	+		
B	*Torulaspora delbrueckii*			+	+ +		
C	*Candida parapsilosis*			+			+
D	*Pichia galeiformis*			+		+	
E	*Pichia kluyveri*			+		+	
F	*Issatchenkia orientalis*	+	+	+			
G	*Saccharomyces cerevisiae*			+		+	
H	*Pichia membranifaciens*			+	+		

+ + Indicates higher production of the enzyme in solid medium.

2.3. Preparation of selected starter inoculum

Yeasts were cultivated (Leal, Gomes, Efraim, de Almeida Tavares, & Figueira, 2008) and all strains were grown previously, overnight in 250 mL of YEPD medium (1% peptone, 1% yeast extract and 2% dextrose) with stirring at 200 rpm at 28° C. This culture was then used as a pre-inoculum for 3 L of YEPD medium under the same conditions. After centrifugation, the biomass obtained was resuspended in 40 mL YEPD medium at a concentration of 3.71×10^8 cells ml^{-1} for use as inoculum in the fermentation experiments.

2.4. Fermentation

The fruits came from Leolinda farm, Uruçuca, Bahia-Brazil. The seeds were homogenized and separated in 15 lots and placed in wooden boxes of 45 kg. The boxes were inoculated with 3.26x108 yeast cells.kg^{-1} of cocoa bean. The fermentation occurred for a period of 144 h, with moving of the mass every 48 h. At every 12 h the temperature of the center of the box was checked and 200 g of almonds were removed for physicochemical analysis. At the end of the process, the almonds were dried on wooden platforms (barge system) until reaching 6–8% humidity and 200 g samples were collected for physicochemical analysis (pH, titratable acidity, quantification of free amino acids and reducing sugars). The fermentations were performed in triplicate for each yeast strain tested and a box containing the same volume of no inoculum (natural fermentation) was used as a negative control. Yeasts A, B, C, D (Table 1) were used in September / 2018 and E, F, G, H (Table 1) in December /2018.

2.5. Determination of pH of the pulp and dry beans

To determine the pH of the pulp 100 g of fresh almonds were depolluted and added with 100 mL of sterile distilled water. The final pH of the solution was then checked (using Radiometer pHmeter PHM82, Radiometer A/S, Brønshøj, China). To measure pH of the dried almonds, the samples were ground in analytical grinder (A11 basic, IKA) and five grams of the resulting powder was resuspended in 45 mL of sterile distilled water and homogenized on a magnetic stirrer (C-MAG HS4, IKA) for 10 min.

2.6. Determination of titratable acidity of cocoa almonds

The dried and crushed cocoa beans were degreased using Soxhlet, with petroleum ether as organic solvent for 24 h (Noor-Soffalina, Jinap, Nazamid, & Nazimah, 2009). The resulting defatted cocoa powder was stored at −4 for further analysis. Then, the collected petroleum ether was evaporated at 40 °C, the residual fat was mixed with a 1:1 (v/v) solution of 0.1 N KOH: ETOH plus two drops of phenolphthalein. The acidity of the final solution was determined by titration with 0.1 N NaOH. Anova one-way and tukey test were made in Graph Prism 5.0.

2.7. Cut test

The cut test was performed on almonds (Wood, 1985), considering 100 almonds, for the lot of 45 kg of cocoa.

2.8. Analysis of reducing sugars

The defatted cocoa powder (1 g) was resuspended in 10 mL of ultrapure water and homogenized for 30 min. Then filtered with Whatman paper no. 1 and in 0.45 μm membrane under vacuum. Reducing sugars present in the filtrate were determined using the dinitrosalicyclic acid reagent (Miller, 1959). Reactions were adjusted to 300 μL volumes on a microplate and the absorbance of the solution was determined on an Elisa (Shimatzu) reader at a wavelength of 575 nm.

2.9. Free amino acid analysis

The extraction of free amino acids was performed (Kirchhoff, Biehl, & Crone, 1989). The dried and defatted cocoa powder (0.7 g) was incubated with 1.4 g of polyvinylpyrrolidone (PVPP) and 15 mL of distilled water. It was homogenized for 1 h at 4 °C. The pH was adjusted to 2.5 with glacial acetic acid, α-amino butyric acid was used as an internal standard, and the volume of the final blend was adjusted to 25 mL. The proteins were separated by the addition of acetone. After 30 min the precipitate was separated by centrifugation at 3 °C and discarded. The supernatant was used for the free amino acid analyzes by HPLC. Prior to quantification the amino acids were derivatized with phenylisothiocyanate (PITC) (Sigma Chemical Co.) (Heinrikson & Meredith, 1984). The derivatized amino acids were separated by reverse phase HPLC with UV detector at 254 nm and the column was a 3.9 × 300 mm Waters Peak-Tag free amino acid (Waters Associates, Milford, Mass., U.S.A.). Eluent A (50 mM sodium acetate buffer at pH 5.7) and eluent B (acetonitrile: water = 60:40) were used as the mobile phase. A gradient of (1) 0.5 min, 100% (A): 0% (B), (2) 5 min, 75% (A): 25% (B), (3) (B), (4) 0.5 min, 0% (A): 100% (B), (5) 14 min, constant 100% (A): 0% (B) was applied. Amino acids were grouped into other (glycine, arginine, threonine, proline, methionine, cystine and lysine) acids (aspartic, gutamic, serine and histidine), hydrophobic (alanine, tyrosine, valine, isoleucine, leucine, phenylalanine) acids to facilitate discussion.

2.10. Statistical analysis

All experiments were performed in triplicate and a variance analysis (ANOVA) and a Turkey's multiple comparison test were applied to verify significant differences between fermentations with and without inoculum. GraphPad Prism 5.0 software (GraphPad Software, Inc.) was used for analysis.

3. Results

3.1. Selection of yeasts to use as inoculum starter

Eight yeasts strains were selected and used as starter inoculants in fine cocoa fermentation to verify if the inoculum influences the obtaining higher values of flavor precursors (Table 1). The temperature of the fermentations that received inoculum did not present significant difference in comparison with controls without inoculum in any of the fermentation periods (September and December). The average temperatures of the first fermentation (September) that started at 24° C and reached the maximum 45.2° C after 48 h, remained around 43° C until 120 h and declined to 37.2 °C in 132 h, at the end of the process. In the second fermentation (December), the initial temperature was 27.2 °C and reached 45 °C at 48 h, kept the temperature high up to 120 h and declined to 34.6 °C at the end of the fermentation process. The pH of the pulp of the first and second periods of fermentation increased progressively from 3.59 and 4.1 at the beginning of the process, to 5.60 and 4.9 at the end, respectively. No significant differences were observed in pH between fermentations with controls without inoculum.

3.2. pH, acidity and cut test of almonds

In the first fermentation period, the inoculums A (*R. mucilaginosa*) and B (*T. delbrueckii*) generated dry almonds with lower pH (5.6 and 5.7 respectively) and significantly different ($P < 0.05$) from the fermentation of control 1 (5.9). In the second fermentation period only the inoculum G (*Saccharomyces cerevisiae*) obtained a higher pH (6.0) and statistically different ($P < 0.05$) of control 2. The titratable acidity of the seeds fermentated by inoculum starter were statistically lower ($p < 0.05$) than those without inoculum (controls 1 and 2) (Fig. 1). The fermentation with the inoculum C (*Candida parapsilosis*), presented

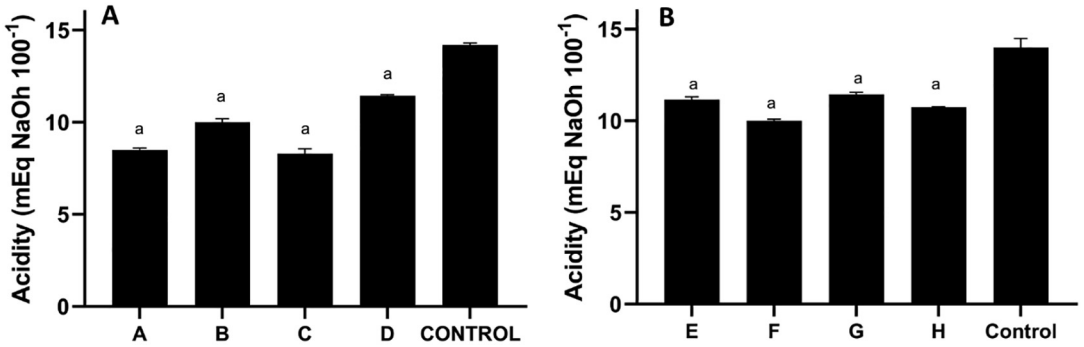

Fig. 1. Acidity in mEq NaOH 100 g −1 of fermented almonds with and without inoculum in September (A) and December (B). A-H correspond to different strains that are listed in Table 1. The error bar corresponds to the standard deviation. The letter "a" indicate significative difference among sample and control in ANOVA one-way and tukey test ($p < 0,005$).

Table 2
Results of the test of fermented almonds (n = 100) with and without inoculum in two different fermentative periods.

	A	B	C	D	C1	E	F	G	H	*C2*
Brown[1]	100	100	100	95	90	97	100	100	100	89
B/V[2]	0	0	0	5	2	3	0	0	0	5
Violet[3]	0	0	0	0	8	0	0	0	0	6

[1] Equivalent to well-fermented seeds; [2] Partially fermented seeds; [3] Unfermented seeds.

seeds with the lowest acidity. The cutting test showed uniformity of well fermented seeds, with brown color content for more than 95% in all processes (Table 2).

3.3. Analysis of reducing sugars

The concentration of reducing sugars from the fermentations conducted with inoculums B (*T. delbrueckii*) and C (*C. parapsilosis*) (14.3 and 15.3 g/kg, respectively) were significantly higher (p greater than 0.05) than those from control 1 (8.9 g/kg). While the fermentations conducted with F (*I. orientalis),* G (*S. cerevisiae*) and H (*P. membranifaciens*) (11.2, 11.0 and 10.3 g/kg, respectively) were significantly lower ($p < 0.05$) than control 2 (16.8 g/kg). The other fermentations were not statistically different from the controls of the respective periods.

3.4. Analysis of free peptides and amino acids

The amount of free amino acids of all fermentations conducted with inoculum presented a significant difference ($p < 0.05$) in relation to the controls 1 and 2 (Table 3). In the first fermentation period all fermentations with inoculum were higher than the control, while in the second period, only with the inoculum E (*P. kluyveri*) was higher than the control 2. Among the fermentations with and without inoculum of the different evaluated periods, the percentages of hydrophobic amino acids represented between 48 and 55%, acids amino acids between 12 and 18% and others between 26 and 35%. The percentage of acidic amino acids were higher in the fermentations of the second period. The percentage of hydrophobic amino acids were higher in the first period. Leucine and phenylalanine (both hydrophobic) counting the highest concentrations in all fermentations (Table 3).

4. Discussion

During experiments with inoculum starter the kinetics of temperature and pH averages, as a function of the fermentation time, are in accordance with the fermentations with and without inoculum described in the literature (John et al., 2019; Lagunes Gálvez, Loiseau, Paredes, Barel, & Guiraud, 2007; Leal et al., 2008; Nielsen et al., 2008; Ooi, Ting, & Siow, 2020; Schwan, 1998). It is suggested that these variables are not influenced by the presence of inoculums starter. The generation of dried beans and more acidity in the fermentation during the first period (September) was observed when it was used inoculums A (*R. mucilaginosa*) and B (*T. delbrueckii*). In the second fermentation period (Dec), it was observed that only in the use of inoculum G, the pH was higher, suggesting lower production of organic acids. Endogenous acids such as malic, tartaric, oxalic, citric and phosphoric are less important to the final pH of almonds than lactic and acetic acid which are produced by degradation of sugar by microbial action in the pulp (Haile & Kang, 2019; Hernández, Núñez, Gómez, & Tovar, 2019). The final pH of the almonds is much more influenced by the sugar concentration of the pulp, the presence of fermenting microorganisms and the diffusion of its acidic metabolites from the pulp to the cotyledon. This acidified environment activates cotyledon enzymes (Amin, Jinap, & Jamilah, 1998).

The titratable acidity allows better access and more sensitive detection of the acidity composition of the almonds in the end of the process. Thus, the ability of the inoculums A, B, C, D, E, F, G and H to reduce the acidity indexes, increasing the quality of the almonds, validates data previously found in the literature (Leal et al., 2008; Schwan & Wheals, 2004). The fermentation with the inoculum C (*C. parapsilosis*), presented almonds with the lowest acidity. This may have occurred due to its pectinolytic activity, which leads to a higher aeration of the fermentative mass during fermentation and promotes the reduction of lactic acid and lactic acid bacteria, with an increase in the oxidation of the acetate to CO_2 and H_2O, and consequently a decrease in the acidity of the almonds (Haile & Kang, 2019; Nielsen et al., 2008; Schwan & Wheals, 2004; Thompson et al., 2013).

The almonds used in this work were homogenized and then distributed in lots, reducing the interference of difference of initial sugar concentration as a function of the genetic origin of the almonds. These findings agree with the premise that the observed differences are related to the microbial processing with inoculum starter, despite the intrinsic characteristics of the fruit. The increase in the concentration of free amino acids in fermentations led by inoculum suggests the ability of these inoculants to contribute to the change in concentration of flavor precursors that lead to the formation of flavor in the Maillard reactions (Santander Muñoz, Rodríguez Cortina, Vaillant, & Escobar Parra, 2019). The proteolytic activity of inoculum B (*T. delbrueckii*) may have contributed to proteolysis within the cotyledon, as this inoculum generated the highest concentration of free amino acids in the first period. This result strongly suggests that there is diffusion of proteolytic extracellular enzymes from yeast to cotyledons. Inoculum A (*R. mucilaginosa*), however, showed less proteolytic activity. Generating free amino acids concentration less than the inoculum inoculum B (*T. delbrueckii*) and those that did not have this activity.

Table 3
Free amino acids in fermented cocoa beans with and without inoculum start in two different periods. The results are expressed as g kg −1. The values followed by different letters in the amino acid sum line represent a significant difference ($P < 0.05$) with the respective control of the period.

AMINO ACIDS	A	B	C	D	Control1	E	F	G	H	Control2
Acids (%)	12,48	14,62	14,86	18,05	15,23	15,71	15,98	16,82	17,32	15,96
(abs.)[1]	1,09	1,43	1,42	1,63	1,32	1,86	1,69	1,62	1,65	1,86
Asp	0,32	0,33	0,34	0,36	0,31	0,41	0,34	0,32	0,33	0,42
Glu	0,36	0,70	0,68	0,85	0,65	0,96	0,91	0,89	0,93	0,98
Ser	0,26	0,25	0,24	0,30	0,23	0,34	0,30	0,29	0,28	0,32
His	0,14	0,15	0,15	0,12	0,12	0,15	0,13	0,11	0,12	0,14
Hydrophobic (%)	52,07	50,82	49,53	55,70	48,93	50,79	50,58	50,02	49,52	49,94
(abs.) [1]	4,53	4,97	4,73	5,04	4,23	6,00	5,33	4,81	4,72	5,82
Ala	0,68	0,70	0,70	0,91	0,64	1,04	0,92	0,92	0,94	1,10
Tyr	0,56	0,64	0,60	0,63	0,51	0,72	0,66	0,57	0,55	0,69
Val	0,46	0,51	0,48	0,56	0,42	0,66	0,59	0,54	0,53	0,60
Ile	0,33	0,36	0,33	0,36	0,29	0,42	0,38	0,33	0,34	0,38
Leu	1,44	1,60	1,51	1,55	1,39	1,84	1,57	1,46	1,40	1,80
Phe	1,05	1,16	1,11	1,04	0,98	1,32	1,21	0,99	0,96	1,24
Others (%)	35,46	34,56	35,61	26,25	35,83	33,51	33,44	33,15	33,16	34,11
(abs.) [1]	3,08	3,38	3,40	2,38	3,10	3,96	3,53	3,19	3,16	3,97
Gly	0,15	0,14	0,13	0,14	0,12	0,16	0,12	0,12	0,12	0,15
Arg	0,72	0,80	0,83	0,76	0,82	0,91	0,77	0,69	0,66	0,87
Thre	0,23	0,25	0,23	0,29	0,22	0,32	0,28	0,26	0,26	0,30
Pro	0,81	0,92	0,85	0,00	0,82	1,15	1,20	0,97	1,03	1,18
Met	0,27	0,29	0,28	0,25	0,23	0,29	0,23	0,22	0,22	0,29
Cys	0,37	0,38	0,49	0,31	0,38	0,50	0,40	0,40	0,37	0,54
Lys	0,53	0,61	0,60	0,62	0,51	0,64	0,52	0,52	0,51	0,64
Total	8,70a	9,79b	9,55c	9,05d	8,65e	11,82f	10,55 g	9,62 h	9,53i	11,65j

[1] Absolut values.

5. Conclusions

It is expected that a low titratable acidity, a quality parameter for cocoa, and high reducing sugar and free amino acids result in a best flavor, thus, the results of reducing sugars, free amino acids and acidity ratify the ability of the inoculum with yeast starters to modify the final condition and the quality of the almonds. Among yeast selection characteristics, only the proteolytic and pectinolytic enzymes seem to have contributed to the greater physicochemical alterations in the fermented almonds. The strains that accumulated the most physicochemical changes, observed in free amino acids, titirable acidity and reducing sugar analysis, that are relevant to the flavor (as flavor precursors) in this experiment were *Candida parapsilosis* (C), followed by *Torulaspora delbrueckii* (B) and *Pichia kluyveri* (E).

CRediT authorship contribution statement

Denise Sande Santos: Conceptualization, Methodology, Writing - original draft, Data curation, Investigation, Formal analysis, Data curation. **Rachel Passos Rezende:** Conceptualization, Methodology, Writing - original draft, Funding acquisition, Supervision. **Thalis Ferreira dos Santos:** Conceptualization, Formal analysis, Data curation, Writing - review & editing, Investigation. **Eric de Lima Silva Marques:** Conceptualization, Formal analysis, Writing - original draft. **Adriana Cristina Reis Ferreira:** Conceptualization, Data curation, Investigation. **Adriana Barros de Cerqueira e Silva:** Conceptualization, Validation, Writing - review & editing. **Carla Cristina Romano:** Conceptualization, Validation, Writing - review & editing. **Damiao Wellington da Cruz Santos:** Conceptualization, Validation, Writing - review & editing. **João Carlos Teixeira Dias:** Funding acquisition, Project administration, Supervision, Conceptualization, Methodology. **João Dias Tavares Bisneto:** Resources, Methodology, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was funded by National Council for Scientific and Technological Development (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) financial code 001 and Fundação de Amparo a Pesquisa do Estado da Bahia (FAPESB).

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