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Volatile Compounds and Fatty Acids Present in Sour Cassava Starch Produced with the Starter Cultures *Lactobacillus Plantarum* and *Pichia Scutulata*

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Sour cassava starch is a product obtained from cassava (*Manihot esculenta* Crantz) processing to overcome raw material toxicity and low nutritional value (lipids and proteins). It has the potential to succeed in the international market due to its innovative use in gluten-free baked products. However, the requirements of this market are high and a standardized fermentation process is necessary to obtain an innocuous product of homogeneous quality. The aim of this work was to produce sour cassava starch in a pilot-scale fermentation process with *Lactobacillus plantarum* as a single culture and in co-culture with *Pichia scutulata* and to determine the fatty acids and volatile compounds profile. The starter cultures contributed to standardization because, regardless of the culture tested, it was possible to obtain sour cassava starches safe for consumption (absence and low counts of pathogens) with satisfactory acidity (between 1and 5 %) and expansion capacity (higher than 1.00). The products obtained with single and mixed cultures exhibited a reduction of saturated fat (3.6 % and 8.1 %, respectively), an increase of the unsaturated fat (2.3 times), and an increase of the oleic, linoleic and linolenic acids proportion. These fatty acids have a positive effect on human health and wellbeing. A greater variety of desirable volatile compounds was a differential of the product obtained with the mixed culture. Therefore, *L. plantarum* with *P. scutulata* was the starter culture that exhibited better results in the pilot-scale fermentation process and has the potential for the industrial production of sour cassava starch.

1. Introduction

The cultivation and consumption of cassava (*Manihot esculenta* Crantz) are present in the culture of different nations and it is of great importance to the South American and African economies. Although widely consumed, cassava presents limitations of trade and industrial applications mainly due to fast post-harvest deterioration and intrinsic toxicity (Alamu et al., 2017). In addition, the low concentration of important nutrients, such as proteins and unsaturated fatty acids, in their roots impacts on undernourished populations that use this root and its by-products as one of the main source of calories because they are rich in carbohydrates as well as some minerals and vitamins (Burns et al., 2012). Thus, different technological processes have been applied and proposed to overcome these limitations and increase the commercial value of this crop.
The natural fermentation of the starch extracted from the cassava root followed by sun drying to obtain the sour cassava starch is a traditional technology widely used in Brazil (Demiate and Kotovicz, 2011). A varied microbiota from the raw material, water, and fermentation tanks begins a competition that culminates in the spontaneous fermentation of the starch with the participation of yeasts and the predominance of lactic acid bacteria (LAB), mainly of the genus *Lactobacillus* (Penido et al., 2018). During fermentation, the metabolism of

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these microorganisms alters the cassava starch and remove the cyanogenic glycosides, mainly responsible for its toxicity (Montagnac et al., 2009). The LAB hydrolyze the starch and acidify the medium, which leads to the improvement of sour cassava starch aroma and in the production of antimicrobial compounds, which inhibit the growth of pathogenic microorganisms (Jácome et al., 2014). On the other hand, the yeasts, when used in mixed cultures, can contribute to the improvement of the sensorial characteristics of fermented products through the synthesis of different volatile compounds (Sorrentino et al., 2013). Nevertheless, the spontaneous fermentation for the production of sour cassava starch occurs in small and medium-sized rural industries in an empirical and rudimentary way. This fermentation culminates in low homogeneity and low-quality products. The reasons are the lack of standardization and the complexity of the natural microbiota (Penido et al., 2018). The objective of this work was to produce sour cassava starch in a pilot-scale fermentation process with the use of *Lactobacillus plantarum* as a single starter culture and in co-culture with *Pichia scutulata* to evaluate its impact on physicochemical and microbiological parameters, fatty acid profile, and volatile compounds.

2. Materials and Methods

2.1 Pilot-scale fermentation process

A single starter culture of *L. plantarum* (C1) and a mixed culture of *L. plantarum* in association with *P. scutulata* (C2) were submitted to fermentation for 21 days at room temperature to produce sour cassava starch in a pilot scale (Penido et al., 2018). The initial inoculum volume was 500 mL and the starter culture counts were 7 $\log_{10}$ CFU/mL for *L. plantarum* isolates and 5 $\log_{10}$ CFU/mL for *P. scutulata* isolates. The fermentation took place in bioreactors with 5 L of distilled water and 10 % of cassava starch (in 4 batches) and the material was sun-dried for 8 hours. The samples collected weekly were used to determine the pH and total titratable acidity (TTA) (AOAC, 2016) and to verify the viability of the starter cultures by the plate counting method (MRS agar for LAB and YM agar for yeasts). For the monitoring of the starter cultures, the *L. plantarum* isolates were submitted to DNA extraction, PCR amplification of the 16S rRNA gene and analysis of the molecular profiles obtained by Restriction Fragment Length Polymorphism (Penido et al., 2018). The molecular monitoring of *P. scutulata* isolates consisted of DNA extraction and analysis of the molecular profiles obtained by PCR fingerprinting using the microsatellite primer $(GTG)_5$ (5'-GTGGTGGTGGTGGTG-3') (Penido et al., 2018). The molecular profiles of each species were compared at different fermentation times 0 (pure starter cultures), 7, 14 and 21 days. To verify if the strain of *L. plantarum* used as starter culture remained during fermentation, the rep-PCR fingerprinting technique was used with the primer $(GTG)_5$ (Gevers et al., 2001). The profiles of the inoculated strain (*L. plantarum Lp*3) and the LAB isolated at time 0 and 21 days were compared. The final products P1 and P2 (obtained with the starter culture C1 and C2, respectively) were submitted to the determinations of TTA, expansion capacity (Maeda and Cereda, 2001) and microbiological analysis (*Bacillus cereus*, thermotolerant coliforms, and *Salmonella* spp.) (Da Silva et al., 2012). Commercial cassava starch was used as raw material for fermentation, considered as control and submitted to microbiological analysis.

2.2 Lipid extraction and fatty acid profile analysis by gas chromatography

An aliquot (200 g) of each final product (P1 and P2) and one of the commercial cassava starch (control) were subjected to lipid extraction using hexane (400 mL), under agitation (150 rpm for 1 h at 25 °C). After solvent evaporation, fatty acids were converted into methyl esters and its derivatives profile were determined (Sande et al., 2018) by gas chromatography (HP5890 gas chromatograph equipped with a flame ionization detector). The column used was Supelcowax-10 (30 m x 0.2 mm x 0.2 µm) with the following temperature gradient: 150 °C at 0 min; increasing 10 °C/min up to 240 °C. The split rate injector operated at 1/50 at 250 °C, which was also the temperature of the detector. The carrier gas was hydrogen with a flow rate of 4 mL/min and an injection volume of 1 µl. The peak identification was done by comparison with Fatty Acid Methyl Ester (FAME) C14 - C22 (Supelco-18917) standards.

2.3 Analysis of volatile compounds by Gas Chromatography / Mass Spectrometry (GC/MS)

An aliquot (25 g) of each final product (P1 and P2) and one of the commercial cassava starch (control) were subjected to volatile compounds extraction using hexane (90 mL), under agitation (150 rpm for 1 h at 25 °C). After solvent evaporation, the detection, identification, and total area (%) of the volatile compounds present in the samples were performed by a chromatograph (Agilent 7890B) equipped with a mass spectrometry detection system (Agilent 5977A-MSD), with the quadrupole type mass analyzer. The capillary column used was CP-WAX 52 CB (Polyethylene glycol, 30 m x 0.25 mm x 0.25 µm). The temperature setting of the oven started at 60 °C for 5 min and then increasing 20 °C/min up to 160 °C, remaining for another 5 min. After that, there was an increase of 5 °C per minute up to 240 °C. The analytical run totaled 37 minutes. The carrier gas

was helium with a flow rate of 1 mL/min and an injection volume of 1 µL in the splitness mode. The data acquisition occurred in the Full Scan mode, using mass ratio (m/z) from 14 to 500. The chromatograph interface with the detector was maintained at 240 °C and the electron impact ionization operated at 240 °C was used. The mass analyzer was a simple quadrupole type operated at 150 °C. The mass and fragmentation profile of the peaks found were compared to the National Institute of Standards and Technology (NIST) library.

2.4 Statistical Analysis

The physicochemical and microbiological monitoring data of the pilot-scale fermentation process at each time point, as well as the results of the final products, were compared by an unpaired, two-tailed t-test with 95 % confidence. The similarity (%) and the correlation between the TTA and pH were calculated using the Pearson correlation coefficient (r). Microbial counts over time were compared individually through a one-way ANOVA followed by Tukey's multiple comparison tests, with 95 % confidence. GraphPad Prism 5.02 was used.

3. Results and Discussion

3.1 Monitoring of pilot-scale fermentation process

The TTA values increased gradually up to the 21st day of fermentation, with no significant difference between cultures C1 and C2, over time. At time 0, the TTA values of the fermentations with different starter cultures were below 1 % and reached values above 2.0 % at the end of the fermentation with C1 ranging from 0.63 to 2.37 % and C2 varying from 0.67 to 2.30 %. This shows the fermentative efficiency of the two cultures, which was also reinforced by the presence of a strong positive correlation between the ATT values over time between C1 and C2 (r^2 = 0.91). These TTA results were confirmed by the pH analysis. During the 21 days of fermentation, the different starter cultures pH did not differ ($p < 0.05$). The pH of the fermentation with C1 and C2 ranged from 3.5 to 2.8, with the lowest pH values occurring in the last day of fermentation. The fermentative efficiency of both cultures was also confirmed by the presence of a strong positive correlation between pH values over time between C1 and C2 (r^2 = 0.93). The main role of the LAB in fermentations is to cause a fast decrease in the pH of the medium, which favors product safety by inhibiting pathogenic microorganisms and prevent undesirable changes caused by deteriorating microorganisms. In the present study, the fermentation lasted for only 21 days, less than suggested by Penido et al., 2018 (28 days) and led to the obtainment of a final product with satisfactory acidity. This reduction in time, in addition to standardizing the process, is justified by the fact that the longer the fermentation lasts, the lower the strength and stability of the sour cassava starch mass (Adegunwa et al., 2011).

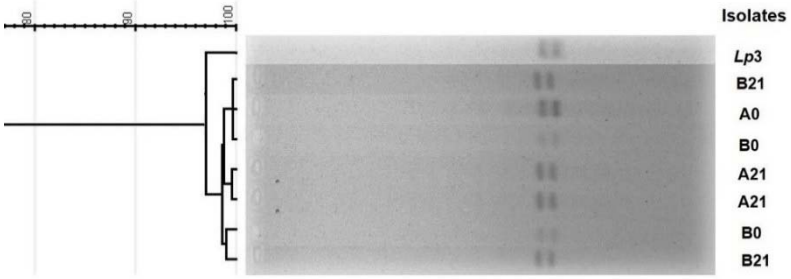

Figure 1: Dendrogram showing the genetic relatedness among L. plantarum isolated from the pilot-scale cassava starch fermentation as determined by the rep-PCR fingerprinting analysis

The counts of *L. plantarum* did not differ over time nor among the different starter cultures ($p > 0.05$) (Figure 1) and remained close to the counts of the initial inoculum (about 7 $\log_{10}$ CFU/g). The counts of *P. scutulata*, which initially were about 5 $\log_{10}$ CFU/g, declined gradually over the 21 days ($p < 0.05$). It is important to note that *L. plantarum* counts in mixed culture were not reduced and the yeast was found until the last day of fermentation. These results were superior to those found by Penido et al. (2018) which found a reduction in the counts of *L. plantarum* and *L. brevis* when in co-culture with *P. scutulata* throughout the fermentation. All LAB isolated from the bioreactors containing the C1 and C2 starter cultures were gram-positive and catalase negative rods. All of them presented the same molecular profile, which is similar to the profile of the inoculated species *L. plantarum*. The *L. plantarum* isolates obtained during pilot-scale fermentation at the initial and final fermentation times showed the similarity of 97 % with the inoculated strain (*L. plantarum Lp*3) and similarity of approximately 98 % with each other (Figure 1). This proves that they belong to the same strain and that the starter culture used in the study can remain viable until the end of the fermentation. All yeasts isolated from the bioreactors containing the C2 starter culture had the same molecular profile, which is similar to the profile

of the species *P. scutulata*. It was observed that the molecular profiles of the single starter cultures were equivalent to those of the other isolates. Therefore, the molecular techniques used allowed the verification of the permanence of the inoculated microorganisms during the 21-day pilot-scale fermentation and demonstrated their potential use for this purpose.

3.2 Physicochemical and microbiological evaluation of the final product

In relation to the TTA, the sour cassava starches obtained with the C1 and C2 starter cultures did not differ from each other ($p > 0.05$). However, it is important to note that with both starter cultures it was possible to obtain sour cassava starch because the acidity values were higher than 1 % and, according to Brazilian legislation, the maximum acidity of 1 % characterized sweet cassava starch and the maximum acidity of 5 % characterizes sour cassava starch. The values of expansion capacities for sour cassava starches P1 and P2 did not differ among themselves ($p > 0.05$). Nonetheless, both expansion values were higher than 1.00, which indicates that the fermented cassava starch became an expandable mass, a fundamental characteristic that allows its use in bakery products. The capacity of expansion of the sour cassava starch, without the need of chemical or biological ferments and in the absence of gluten, is the main property that differentiates it from other starches (Marcon et al., 2009). Microbiological analysis of cassava starch revealed the presence of suggestive colonies of *B. cereus* (1.50×10^3 CFU/g). To verify the presence of *B. cereus* in cassava by-products is important because it is a spore-forming and toxin-producing endophytic microorganism that can cause food poisoning when present at counts above 10^5 CFU/g. For the analysis of thermotolerant coliforms, all samples presented the result < 3 MPN (Most Probable Number)/g. The other results were the absence of *Salmonella* spp. in 25 g of all samples. Therefore, both sour cassava starches produced in a pilot-scale fermentation process would be suitable for human consumption. During fermentation, the presence of the yeast, which is amylolytic, does not appear to have contributed to increased acid production by the LAB. The reason might be the presence of *B. cereus*, which is also amylolytic, in the raw material, making it possible to hydrolyze the starch when *L. plantarum* was in a single culture. However, the use of both starter cultures for the cassava starch fermentation led to the production of sour cassava starches with the satisfying values of acidity, able to expand and with good control of the pathogenic microorganisms.

3.3 Profile of fatty acids from sour cassava starches

In relation to cassava starch, the sour cassava starches P1 and P2 resulted in a reduction of saturated fat of 3.6 % and 8.1 % respectively, and in an increase of the unsaturated fat of approximately 2.3 times (Table 1).

Table 1: Fatty acids profile of cassava starch and sour cassava starch samples after gas chromatography analysis (T_R = retention time)

Fatty acids	Area (%)			
	T_R (min)	Cassava starch	P1	P2
C14:0 (Myristic acid)	3,976	4,2	1,7	1,6
C16:0 (Palmitic acid)	5,500	33,9	40,2	35,8
C16:1 (Palmitoleic acid)	5,724	3,1	4,3	4,7
C18:0 (Stearic acid)	7,090	18,6	10,7	11,0
C18:1 (Oleic Acid)	7,276	7,1	16,7	18,7
C18:2 (Linoleic acid)	7,658	0,4	3,6	3,1
C18:3 (Linolenic acid)	8,188	1,4	3,0	1,3
C20:0 (Arachidic acid)	8,835	0,6	1,2	0,9
Others		30,7	18,8	23,1

A higher content of mono and polyunsaturated fats aggregates higher nutritional value to the food (Sande et al., 2018). Oleic acid (omega-9), linoleic acid (omega-6) and linolenic acid (omega-3) are essential fatty acids that play key roles in the proper functioning of human metabolism, as they can not be synthesized by the human body (Tan and Muhamad, 2017). Fermentation of cassava starch using *L. plantarum* and *L. plantarum* with *P. scutulata* was able to increase considerably the proportion of these three acids in the final product (Table 1). This increase in nutritional quality justifies the use of starter cultures for the production of sour cassava starch. Cassava is cultivated for its starch-rich tuberous roots, but its seeds are known to be rich in oils and fatty acids (Alves et al., 2014). These fatty acids were found, in lower concentrations, in the cassava starch. In addition to the fatty acids from the raw material, microorganisms can also contribute to their production because lipids, especially polyunsaturated fatty acids, are components of the cell membrane. Palmitic, stearic, oleic, linoleic and linolenic acids were identified as cell fatty acids of strains of *L. plantarum*

(Coulibaly et al., 2009) and as metabolites produced by isolates of this species (Vanaja et al., 2011). In the fermentation of *yakupa*, beverage obtained from cassava fermentation, palmitic, stearic, linoleic and oleic acids were detected (Freire et al., 2015). The yeasts also showed an important role in the production of fatty acids in cassava fermentation. The increase of lipids positively alters the physicochemical properties of starchy foods, such as sour cassava starch. This happens because the formation of complexes between the amylose fraction of the starch and the lipids affects the formation of the structure and the texture of these foods (De Pilli et al., 2011). Therefore, the use of starter cultures benefits the quality of the final product, increasing the content of unsaturated fatty acids.

3.4 Volatile compounds and fatty acids

A total of 19 compounds were identified by GC-MS in the samples (Table 2). Palmitic acid, a very common fatty acid, was the only compound that was present in all samples. Only one phenol, 2,4-Di-*tert*-butyl phenol, was detected. This volatile organic compound presented antifungal activity, antioxidant activity and demonstrated the potential for the development of a food additive (Varsha et al., 2015). Sclareol is perfumed bicyclic diterpene alcohol, which has already been found in essential oils (Tongnuanchan and Benjakul, 2014). The P2 sample showed the highest diversity of compounds. The 4-hexyl-2,5-dihydro-2,5-dioxo-3-furanacetic acid is a compound naturally found in small amounts in a wide variety of foods, including wines (García-Carpinteiro et al., 2011) and is characterized by woody odor. Geranyl isovalerate, on the other hand, is associated with a fruity aroma. The compounds detected in the samples of cassava starch and sour cassava starches are volatile components that present a desirable aroma, antioxidant and antimicrobial activity.

Table 2: Identification of volatile compounds and fatty acids in cassava starch and sour cassava starch samples after analysis by GC-MS

	Compounds	Molecular formula	T_R (min)	Cassava starch	P1	P2
FATTY ACIDS:	Linoleic acid	$C_{18}H_{32}O_2$	26.140			x
	Palmitic acid	$C_{16}H_{32}O_2$	31.612	x	x	x
	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	32.203		x	x
	Stearic acid	$C_{18}H_{36}O_2$	35.669			x
	4-Hexyl-2,5-dihydro-2,5-dioxo-3-furanacetic acid	$C_{12}H_{16}O_5$	17.692			x
ALCOHOLS:	2-Octyl-1-dodecanol	$C_{20}H_{42}O$	12.972	x		
	2-Hexyl-1-decanol	$C_{16}H_{34}O$	16.705			x
	trans-9-Hexadecen-1-ol	$C_{16}H_{32}O$	23.069	x		
	2-Methyl-1-hexadecanol	$C_{17}H_{36}O$	26.664	x		x
	Sclareol	$C_{20}H_{36}O_2$	32.621		x	
KETONES:	2-Isopropyl-5,5-dimethylcyclohex-2-enone	$C_{11}H_{18}O$	19.174			x
	4,4a,5,6,7,8-hexahydro-4a-methyl-2(3H)-Naphthalenone	$C_{11}H_{16}O$	22.384		x	
	13β-methyl-13-vinyl-Podocarp-7-en-3-one	$C_{20}H_{30}O$	32.515		x	
ESTERS:	6-Pentyl-2H-pyran-2-one	$C_{10}H_{14}O_2$	19.163		x	
	Phthalic acid, butyl tetradecyl ester	$C_{26}H_{42}O_4$	25.828			x
	Phthalic acid, butyl octyl ester	$C_{20}H_{30}O_4$	28.335	x		
	Geranyl isovalerate	$C_{15}H_{26}O_2$	30.258			x
ETHER:	14-Oxatricyclo [9..2.1.0(1,10)]tetradecane, 2,6,6,10,11-pentamethyl-	$C_{18}H_{30}O$	31.038		x	
PHENOL:	2,4-Di-*tert*-butyl phenol	$C_{14}H_{22}O$	21.810	x	x	

4. Conclusions

Both starter cultures exhibited good performance for the production of sour cassava starch in a pilot-scale fermentation process. *L. plantarum* contributed to the acidification of the medium and inhibition of the pathogens. *P. scutulata* contributed to the production of desirable aromatic compounds. Therefore, *L. plantarum* in association with *P. scutulata* was the starter culture that exhibited better results in a pilot-scale fermentation process and led to the obtainment of a cassava starch safe for human consumption, with satisfactory acidity, expansion capacity, higher unsaturated fat content, and with the presence of desirable volatile compounds.

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References

Adegunwa M.O., Sanni L.O., Maziya-Dixon B., 2011, Effects of fermentation length and varieties on the pasting properties of sour cassava starch, Afr. J. Biotechnol., 10, 8428-8433.

Alamu E.O., Maziya-Dixon B., Dixon A.G., 2017, Evaluation of proximate composition and pasting properties of high quality cassava flour (HQCF) from cassava genotypes (Manihot esculenta Crantz) of β-carotene-enriched roots, LWT – Food Sci. Technol., 86, 501-506.

Alves A.A.C., Manthey L., Isbell T., Ellis D., Jenderek M.M., 2014, Diversity in oil content and fatty acid profile in seeds of wild cassava germplasm, Ind. Crop. Prod., 60, 310-315.

AOAC (Association of Official Analytical Chemists), 2016, Official methods of analysis, 20th ed., AOAC International, Gaithersburg, USA <eoma.aoac.org> accessed 09 11 2016.

Burns A.E., Gleadow R.M., Zacarias A.M., Cuambe C.E., Miller R.E., Cavagnaro T.R., 2012, Variations in the chemical composition of cassava (Manihot esculenta Crantz) leaves and roots as affected by genotypic and environmental variation, J. Agric. Food Chem., 60, 4946-4956.

Coulibaly I., Amenan A.Y., Lognay G., Fauconnier M.L., Thonart P., 2009, Survival of freeze-dried Leuconostoc mesenteroides and Lactobacillus plantarum related to their cellular fatty acids composition during storage, Appl. Biochem. Biotechnol., 150, 70-84.

Da Silva N., Taniwaki M.H., Junqueira V.C.A., Silveira N.F.A., Nascimento M.S., Gomes R.A.R., 2012, Microbiological examination methods of food and water: a laboratory manual, CRC Press, Boca Raton, USA.

De Pilli T., Derossi A., Talja R.A., Jouppila K., Severini C., 2011, Study of starch-lipid complexes in model system and real food produced using extrusion-cooking technology, Innov. Food Sci. Emerg. Technol., 12, 610-616.

Demiate I.M., Kotovicz V., 2011, Cassava starch in the Brazilian food industry, Food Sci. Technol., 31, 388-397.

Freire A.L., Ramos C.L., Schwan R.F., 2015, Microbiological and chemical parameters during cassava based-substrate fermentation using potential starter cultures of lactic acid bacteria and yeast, Food Res. Int., 76, 787-795.

García-Carpinteiro E.G., Sánchez-Palomo E., González-Viñas M.A., 2011, Aroma characterization of red wines from cv. Bobal grape variety grown in La Mancha region, Food Res. Int., 44, 61-70.

Gevers D., Huys G., Swings J., 2001, Applicability of rep-PCR fingerprinting for identification of Lactobacillus species, FEMS Microbiol. Lett., 205, 31-36.

Jácome S.L., Fonseca S., Pinheiro R., Todorov S.D., Noronha L., Silva J., Gomes A., Pintado M., Morais A.M.M.B., Teixeira P., Vaz-Velho M., 2014, Effect of Lactic Acid Bacteria on Quality and Safety of Ready-to-eat Sliced Cured/Smoked Meat Products, Chem. Eng. Trans., 38, 403-408.

Maeda K.C., Cereda M.P., 2001, Avaliação de duas metodologias de expansão ao forno do polvilho azedo, Food Sci. Technol., 21, 139-143.

Marcon M.J.A, Kurtz D.J., Raguzzoni J.C., Delgadillo I., Maraschin M., Soldi V., Reginatto V., Amante E.R., 2009, Expansion Properties of Sour Cassava Starch (Polvilho Azedo): Variables Related to its Practical Application in Bakery, Starch, 61, 716-726.

Montagnac J.A., Davis C.R., Tanumlhardjo S.A., 2009, Processing techniques to reduce toxicity and antinutrients of cassava for use as a staple food, Compr. Rev. Food Sci. Food Saf., 8, 17-27.

Penido F.C.L., Piló F.B., Sandes S.H.C., Nunes A.C., Colen G., Oliveira E.S., Rosa C.A., Lacerda I.C.A., 2018, Selection of starter cultures for the production of sour cassava starch in a pilot-scale fermentation process, Braz. J. Microbiol., 49, 823-831.

Sande D., Colen G., Dos Santos G.F., Ferraz V.P., Takahashi J.A., 2018, Production of omega 3, 6, and 9 fatty acids from hydrolysis of vegetable oils and animal fat with Colletotrichum gloeosporioides lipase, Food Sci. Biotechnol., 27, 537-545.

Sorrentino A., Boscaino F., Cozzolino R., Volpe M.G., Ionata E., Guerriero S., Picariello T., La Cara F., 2013, Characterization of Free Volatile Compounds in Fiano Wine Produced by Different Selected Autochthonous Yeasts, Chem. Eng. Trans. 32, 1837-1842.

Tan N.A.H., Fonseca S., Muhamad I.I., 2017, Optimisation of Omega 3 Rich Oil Extraction from Elateriospermum Tapos Seed by Microwave Assisted Aqueous Enzymatic Extraction, Chem. Eng. Trans. 56, 1783-1788.

Tongnuanchan P., Benjakul S., 2014, Essential oils: extraction, bioactivities, and their uses for food preservation, J. Food Sci., 79, 1231-1249.

Vanaja G., Gotcheva V., Angelov A., Agrawal R., 2011, Formation of volatiles and fattyacids of therapeutic importance in the probiotic Lactobacillus plantarum LPcfr adapted to resist GIT conditions, J. Food Sci. Technol., 48, 110-113.

Varsha K.K., Devendra L., Shilpa G., Priya S., Pandey A., Nampoothiri, K.M., 2015, 2,4-Di-tert-butyl phenol as the antifungal, antioxidant bioactive purified from a newly isolated Lactococcus sp., Int. J. Food Microbiol., 211, 44-50.