Organic acid profile of commercial sour cassava starch[1]

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SUMMARY

Organic acids are present in sour cassava starch ("polvilho azedo") and contribute with organoleptic and physical characteristics like aroma, flavor and the exclusive baking property, that differentiate this product from the native cassava starch. Samples of commercial sour cassava starch collected in South and Southeast Brazil were prepared for high performance liquid chromatography (HPLC) analysis. The HPLC equipment had a Biorad Aminex HPX-87H column for organic acid analysis and a refractometric detector. Analysis was carried out with 0.005M sulfuric acid as mobile phase, 0.6ml/min flow rate and column temperature of 60° C. The acids quantified were lactic (0.036 to 0.813 g/100g), acetic (0 to 0.068 g/100g), propionic (0 to 0.013 g/100g) and butyric (0 to 0.057 g/100g), that are produced during the natural fermentation of cassava starch. Results showed large variation among samples, even within the same region. Some samples exhibited high acid levels, mainly lactic acid, but in these neither propionic nor butyric acids were detected. Absence of butyric acid was not expected because this is an important component of the sour cassava starch aroma, and the lack of this acid may suggest that such samples were produced without the natural fermentation step.

Keywords: fermentation, cassava sour starch, quality.

RESUMO

ANÁLISE DE ÁCIDOS ORGÂNICOS EM AMOSTRAS DE POLVILHO AZEDO COMERCIAL. A presença de ácidos orgânicos no polvilho azedo, além de contribuir com aspectos como sabor e aroma, tem, conforme a literatura indica, correlação com a propriedade de expansão, que é um fator determinante no uso alimentício. Amostras de polvilho azedo foram coletadas nas Regiões Sul e Sudeste diretamente nas empresas ou no comércio. Foram preparadas para análise em cromatografia líquida de alta eficiência (CLAE), sendo que o cromatógrafo estava equipado com coluna Biorad Aminex HPX-87H para análise de ácidos orgânicos e detector refratométrico. As condições de análise envolveram o emprego da fase móvel ácido sulfúrico 0,005M, fluxo de 0,6 ml/min e temperatura da coluna de 60°C. Os ácidos quantificados foram lático (0,036 a 0,813 g/100g), acético (0 a 0,068 g/100g), propiônico (0 a 0,013 g/100g) e butírico (0 a 0,057 g/100g),

presentes na fermentação natural. Os resultados revelaram grande variação entre as amostras, com diferenças mesmo dentro das Regiões. Algumas amostras apresentaram quantidades elevadas de ácidos, especialmente do ácido lático, mas nestas amostras os ácidos propiônico e butírico não foram detectados. A ausência do ácido butírico não era esperada, uma vez que esse ácido está diretamente relacionado com o aroma característico do polvilho azedo. O fato pode sugerir que a obtenção de algumas das amostras estudadas pode ter ocorrido sem o processo fermentativo natural.

Palavras-chave: fermentação, polvilho azedo, qualidade.

1 – INTRODUCTION

Sour cassava starch (*polvilho azedo*) is traditionally produced by natural fermentation of cassava starch. The fermentative process was studied by several researchers and is predominantly lactic [7, 20, 21, 24]; starch is the medium for bacterial growth [4]. Since 1973 [8], a number of studies about the microbiology of sour starch fermentation have been published. CEREDA [8] divided the fermentation in three steps, not always very distinct. Microorganisms responsible for establishing the appropriate conditions for fermentation in the first step include *Achromobacter, Escherichia, Pseudomonas, Alcaligenes, Bacillus* and *Clostridium* genera. In the second step, acidogenic microorganisms that have higher nutritional requirements appear. Finally at the third step saprophytic microorganisms become present and are responsible for the degradation of some fermentation products. Examples of microorganisms of this step are the genus *Bacillus* and some yeasts; at this step there is generation of compounds that contribute to the typical aroma and taste of sour starch. Three types of fermentation are identified: lactic, with the presence of *Lactobacillus, Leuconostoc* and *Pediococcus* genera, propionic, where *Propionibacterium* genus is found and butyric, with *Clostridium* genus. MARTINEZ [23], who showed the importance of microaerophilic microorganisms that are present in all fermentations, isolated mainly the following gram positive bacteria: sporulated *Bacillus*, non sporulated *Lactobacillus*, *Streptococcus* and also yeasts from the *Saccharomyces* genus. According to MESTRES & ROUAU [24], the lactic bacteria population increases during the first five days of fermentation and lactic acid production continues until the 20^{th} day. At the end of fermentation, generally 30 days, total and lactic counts are relatively low, less than 10^9 CFU/g [3] and lactic acid content is around 10g/kg [24].

Other researchers attempting to identify organic acids present in sour starch found lactic, acetic, propionic and butyric acids [9, 11, 14]. Formic, succinic [14] and valeric [9] acids were detected in commercial sour starch samples, as well as in laboratory produced samples, but the methodology employed for analyzing such samples was different from that used in this paper. CÁRDENAS & De BUCKLE [6] found lactic acid representing 66 to 82% of the sour

starch total acidity, whereas CARVALHO *et al*. [7] 60%, and ASTÉ [1] 95%; acetic, propionic and butyric acids were also detected in amounts of 2.5, 1.5 and 2%, respectively. MESTRES & ROUAU [24], when analyzing samples produced in Colombia and employing the same methodology of this work found *ca.* 0.6 g of lactic acid per 100g of starch. CARVALHO *et al*. [7] reported values of 0.40 to 0.53g/100g for total acidity, expressed as lactic acid. BANGOU [2] studied cassava starch fermentation at two different temperatures, 20 and 35° C, for a period of 15 days and determined lactic and butyric acid contents. The results showed that at 35° C, butyric acid was the major component of the total acidity (1.38mg/g), followed by lactic acid (0.93mg/g). At 20° C, on the other hand, the result was different, 0.13mg/g of butyric acid and 2.47 mg/g of lactic acid were produced. CEREDA & CATÂNEO [13] had already concluded in an earlier study that at lower fermentation temperatures, and consequently lower fermentation rates, there was higher generation of lactic acid, whereas higher temperatures or higher fermentation rates, resulted in higher production of butyric acid.

In a bibliography review, FIGUEROA [20] showed that lactic, acetic, butyric, propionic and valeric acids were found on cassava sour starch samples and that total acidity was highly correlated with the lactic acid content. Heterogeneous samples from Colombia also exhibited lactic, acetic, propionic and butyric acids [16]. ESCOBAR & MOLINARI [19] detected lactic, acetic and butyric acids and found a positive correlation between acid content and the expansion and water binding capacity.

Quality differences observed among samples commercialized in the geographic area of this study [17, 18] called our attention to the importance of determining their profile of organic acids. Sour starch baking ability is its most interesting characteristic [12, 25] that makes sour starch technologically different from natural cassava starch. The denomination "sour" is due to the natural fermentation that occurs as explained above, with the formation of organic acids. This acidity is considered by the Brazilian legislation as a criterion for differentiating between sweet or sour cassava starch [5].

CEREDA [10] evaluated 12 commercial samples from three Brazilian States (São Paulo, Paraná and Minas Gerais) and concluded that it was not possible to correlate any physico-chemical property with the place of production. The author found great variation on ash content (CV = 65.7%) and titrable acidity (CV = 68.2%).

Nowadays there is a growing market for sour starch, mainly due to the increase in the number of small industries that manufacture cheese bread dough for both home and fast food store consumption. The fermentation step for the production of cassava sour starch is a time consuming process and the substitution of this naturally produced starch for chemically modified ones may appear attractive.

The aim of this study was to provide information about the organic acid profile of sour cassava starch samples collected in South and Southeast Brazil, compare results with published data and try to group samples according to this characteristic. It must be stressed that sample origin is difficult to be ascertain because sometimes a

sour starch is produced in one region and packaged and sold in another, without identification of the production state.

2 – MATERIALS AND METHODS

2.1 – Materials

Sour cassava starch samples (n=29) were collected on South and Southeast Brazil, directly on factories or bought on market; one sample of natural cassava starch was a gift from a factory from São Paulo state and was analyzed for comparison. All reagents employed were compatible with chromatographic analysis and were prepared for sample and standard (lactic, acetic, propionic and butyric acid, all from Merck) injections.

The HPLC equipment had a Biorad Aminex HPX-87H column (300x7,8mm), oven for column temperature control and a refractometric detector. Chromatograms were generated and integrated by using a personal computer and the software Chroma [15].

2.2 – Methods

2.2.1 - Sample preparation

Samples weighing 15 g (DM) were suspended in 30 ml of a 0.005M sulfuric acid solution for a period of contact of 24 hours at room temperature and with occasional agitation. After this period of contact, the suspensions were centrifuged twice. Firstly at 3000 rpm for 10 minutes and then at 10000 rpm for 15 minutes.

2.2.2 - HPLC analysis

The 0.005M sulfuric acid mobile phase was filtered through a 0.22m m membrane. The flow rate was set at 0.6 ml/min and the column temperature kept at 60° C. At injection, the samples were also filtered to 0.22m m and the volume injected was of 20m l (BRABET [3]; BRABET & DUFOUR [4]).

3 – RESULTS AND DISCUSSION

The total organic acid content is highly correlated with lactic acid in cassava sour starch [20]. This fact was evident when a linear regression was made with these two values; the r^2 obtained for the 30 samples of cassava starch was 0.9779, as shown on *Figure 1*. It is possible to conclude that lactic acid is the most important component of sour starch acidity. The presence of lactic acid on cassava natural starch (sample 11) may be explained, once it is known that the production technology allows an incipient fermentation process [7].

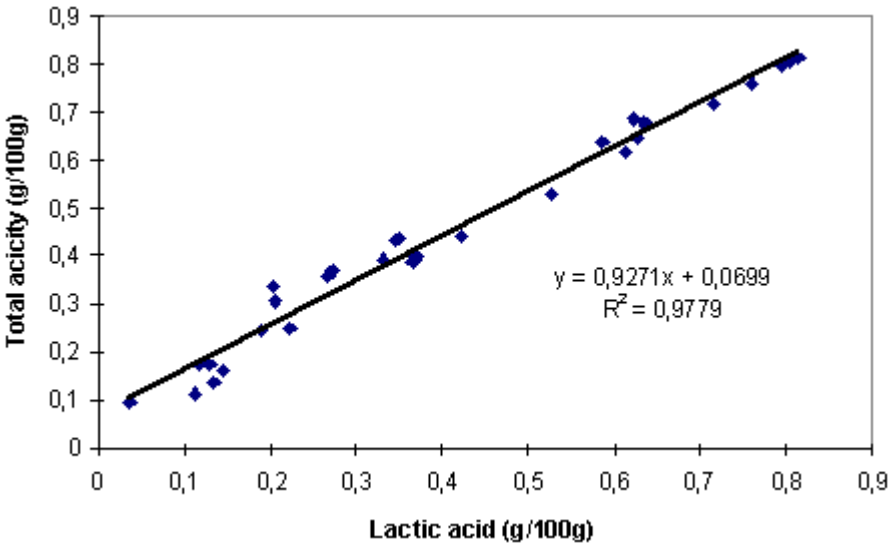

FIGURE 1. Linear regression between lactic acid content and total acidity of the cassava starch samples.

When considering lactic acid as a percentage of total acidity, it may be observed that these values varied from 38.2 to 100%. From the 30 samples, including sample 11 (natural starch), 10 samples had lactic acid as the only component of the total acidity and in 7 other samples this acid was responsible for more than 90% of the total acidity. In the other samples, lactic acid contents were from 60.5 to 89.0% and in one sample (number 22] this acid represented only 38.2% of the total acids, whereas acetic accounted for 45.2 and butyric acid for 16.6%. This sample did not contain propionic acid.

Table 1 illustrates differences among sour cassava starch samples according to their organic acid profile. When comparing with previously published data it was possible to conclude that not all samples presented the typical organic acid profile. Propionic and butyric acids were detected in only a few samples. It must be observed with attention the absence of butyric acid in some of the samples. This acid is produced during the cassava starch natural fermentation and was expected to be present [9,11,14]. Employing a different methodology, CEREDA [9], analyzed sour cassava starch samples from Paraná and Santa Catarina states and found butyric acid as the main organic acid, followed by lactic and acetic acids.

TABLE 1. Amounts of organic acids in sour cassava starch samples (dry matter).

Sample	Acid (g/100g; % of total acids)								
	Lactic		Acetic		Propionic		Butyric		total
1**	0.371	93.1	0.028	6.9	0	0	0	0	0.399
2**	0.366	94.2	0.022	5.8	0	0	0	0	0.388
3**	0.135	100.0	0	0	0	0	0	0	0.135
4	0.204	60.5	0.063	18.6	0.013	3.9	0.057	19.0	0.337
5	0.271	72.7	0.050	13.4	0.008	2.1	0.043	11.8	0.373
6**	0.222	88.9	0.028	11.1	0	0	0	0	0.250
7**	0.145	89.0	0.018	11.0	0	0	0	0	0.163
8	0.267	74.5	0.048	13.4	0.005	1.5	0.038	10.6	0.359
9**	0.422	95.1	0.021	4.9	0	0	0	0	0.443
10**	0.528	100.0	0	0	0	0	0	0	0.528
11*	0.013	100.0	0	0	0	0	0	0	0.013
12**	0.112	100.0	0	0	0	0	0	0	0.112
13	0.350	80.3	0.046	10.7	0	0	0.039	8.9	0.436
14	0.623	91.1	0.025	3.7	0.004	0.5	0.032	4.7	0.684
15	0.331	84.2	0.043	11.0	0	0	0.019	4.8	0.393
16	0.585	91.6	0.035	5.5	0	0	0.019	2.9	0.639
17**	0.717	100.0	0	0	0	0	0	0	0.717
18**	0.761	100.0	0	0	0	0	0	0	0.761
19	0.190	77.4	0.023	9.2	0	0	0.033	13.4	0.246
20**	0.813	100.0	0	0	0	0	0	0	0.813
21**	0.804	100.0	0	0	0	0	0	0	0.804
22	0.036	38.2	0.043	45.2	0	0	0.016	16.6	0.095
23**	0.795	100.0	0	0	0	0	0	0	0.795
24	0.118	67.9	0.032	18.7	0.005	2.7	0.018	10.7	0.173
25	0.131	74.7	0.014	8.0	0	0	0.030	17.3	0.175
26	0.206	67.2	0.068	22.3	0	0	0.032	10.5	0.306
27	0.346	79.9	0.050	11.6	0	0	0.037	8.5	0.434
28	0.634	93.7	0.026	3.9	0	0	0.017	2.4	0.677
29**	0.627	96.6	0.022	3.4	0	0	0	0	0.648
30**	0.614	100.0	0	0	0	0	0	0	0.614

* natural cassava starch

** samples that presented acid profile different from that of the literature [9, 11, 14]

The predominantly lactic fermentation [22] seems to be initially heterofermentative, followed by a homofermentative process [21]. MESTRES & ROUAU [24], analyzing samples of sour cassava starch

produced in a pilot plant in Colombia and employing the same methodology of the present work, were able to detect only lactic acid and traces of acetic acid. There was one difference between those authors' organic acid extraction procedure and the one employed here. While MESTRES & ROUAU [24] made a suspension of 6.25% (DM) sour starch, in this work a more concentrated sour starch suspension was made (33.3% DM), which made it possible to detect acids present in lower levels.

Some of the samples studied exhibited several peculiarities such as the absence of typical aroma, very low granulometry and whiter color than usual, resembling the native cassava starch. Coincidentally these samples contained only lactic acid or, in some cases, lactic plus a small amount of acetic acid.

Analysis of the organic acid profiles allowed us to make further considerations:

- In nine samples lactic acid was the only component of the total acidity, in amounts from 0.112 to 0.813g/100g.
- Six samples presented lactic and acetic acids as components of the total acidity. In this group lactic acid contents varied from 0.145 to 0.627 g/100g and acetic acid from 0.018 to 0.028g/100g. On a percentage basis, lactic acid was responsible for 88.9 to 96.6% and acetic acid for 3.4 to 11.1% of the total.
- Nine samples did not have propionic acid as a component of their acidity and their total acid contents varied from 0.095 to 0.677 g/100g (38.2 to 93.7% for lactic, 3.9 to 45.2% for acetic and 2.4 to 16.6% for butyric acid).
- Five samples presented all the acids generally found on sour starch produced by natural fermentation and the total acid contents varied from 0.173 to 0.684g/100g. On percentage basis, these values corresponded to 60.5 to 91.1% for lactic acid, 3.7 to 18.7% for acetic acid, 0.5 to 3.9% for propionic acid and 4.7 to 19.0% for butyric acid.

Table 2 summarizes sample separation and shows the region (state) where they were produced.

TABLE 2. Total acid content on sour cassava starch samples.

Acids present	Total acidity g/100g	State (number of samples)
Lactic	0.112 to 0.813	SC (3), PR (1), SP (1), MG (4)
Lactic and acetic	0.163 to 0.648	SC (2), SP (3), MG (1)
Lactic, acetic and butyric	0.095 to 0.677	SC (5), PR (3), MG (1)
Lactic, acetic, propionic and butyric	0.173 to 0.684	SC (1), PR (4)

Observing *Table 2* it was possible to conclude that (a) from the eight samples from Paraná State, only one did not present butyric acid in its composition, (b) from the 11 samples from Santa Catarina State, 6 presented the typical sour cassava starch acids, including butyric acid, whereas 5 had lactic and acetic or only lactic acid, (c) out of the 6 samples from Minas Gerais State, 5 presented lactic and acetic or only lactic acid and only one sample had butyric acid in its composition. This sample, on the other hand, had the lowest total acid content among all 29 sour starch samples studied. Total acid content of this sample was 0.095%, which represented 8.5 times less than the sample with the highest acid content, coincidentally also from Minas Gerais State and (d) all 4 samples from São Paulo State presented both lactic and acetic or only lactic acid in their compositions.

4 – CONCLUSIONS

- The organic acids detected and quantified were: lactic (0.013 to 0.813 g/100g), present in all 30 samples studied, acetic (0 to 0.068 g/100g) in 20 of the samples, propionic (0 to 0.013 g/100g), in five samples and butyric (0 to 0.057 g/100g), in 14 samples.
- Analysis of organic acids by the methodology employed in this work may prove to be an important tool for cassava sour starch quality evaluation.
- The samples of sour cassava starch commercialized in the regions considered in this study exhibited a heterogeneous organic acid profile, which suggests the necessity of improving the production process with the purpose of quality standardization.

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