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Optimization of Methods for Development of Fermented-Distillate of Passion Fruit Beverage

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***Abstract*—**Fermented beverages have high expression in the market for beverages in general, is increasingly valued in situations where the characteristic aroma and flavor of the material that gave rise to them are kept after processing. This study aimed to develop a distilled beverage from passion fruit, and assess, by sensory tests and chromatographic profile, the influence of different treatments (FM1-spirit with pulp addiction and FM2 – spirit with bigger ratio of pulp in must) in the setting of volatiles in the fruit drink, and performing chemical characterization taking into account the main parameters of quality established by the legislation. The chromatograms and the first sensorial tests had indicated that sample FM1 possess better characteristics of aroma, as much of how much quantitative the qualitative point of view. However, it analyzes it sensorial end (preference test) disclosed the biggest preference of the cloth provers for sample FM2-2 (note 7.93), being the attributes of decisive color and flavor in this reply, confirmed for the observed values lowest of fixed and total acidity in the samples of treatment FM2.

***Keywords*—**Fermented-distilled drink, fruit spirits, passion fruit.

I. Introduction

ONE of the most produced fermented distilled beverages in the world is the spirits [1], [2], characterized by fermentation and distillation of sugar musts of plants, particularly sugar cane. In Brazil, the production of sugar cane spirit may be considered an important economic activity, since the volume produced is 1.3 billion liters per year. Even involving an already well known simple technology and the production of spirits continues to attract the scientific interest, especially regarding the use of new raw materials that enable acquisition of different flavors, attracting new markets. For this, it is in Brazil a huge variety of fruit flavors with exotic and striking aromas, with great potential for the industry of fermented beverage [3].

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The passion fruit is one of these fruit and its main attractions include the intense yellow color of its pulp, acid and refreshing taste and an unmatched aroma, consisting mainly of esters, aldehydes, alcohols and ketones [4]. It's a well known fruit in South America, where countries such as Brazil, Colombia, Peru and Ecuador, account for 90% of exports of juice, passion fruit concentrate and pulp, with Brazil being the main producer and consumer of the fruit [5]. Therefore, the objective of this study was to take advantage of the attractions of the passion fruit and produce a fermented-distilled drink to keep the characteristics of aroma, through the use of procedures to ensure the establishment of the VOC's - Volatile Organic Compounds - present in pulp.

II. Material and Methods

A. Obtaining Spirits of Passion Fruit

Preparation of must: For the preparation of the must, were selected ripe fruits of yellow passion fruit, produced in the city of Ponto Novo (New Point) (BA). The fruits were washed and cut to remove the pulp and evaluating the yield of pulp and peel. Then the pulp was subjected to analysis of soluble solids and pH in order to be made the necessary corrections in the must.

A certain amount of fruit pulp was transferred to a fermentation vat of stainless steel, with a capacity of 10L, which were also added water and sugar, using the square of Pearson, in order to get a must of 18% soluble solids. The chaptalization is necessary since the brix of the fruit is very low (2.0 ^{o}Brix) and was made by adding crystal sugar. In this step two treatments were defined (FM1 and FM2), in which the proportions of fruit pulp/fruit varied, adopting the proportions 1:3 and 1:1 in FM1 and FM2 respectively.

Preparation of inoculum and fermentation of must: After correction of sugar was added to a commercial mixture of nutrients (FERMOPLUS) containing: ammonium salts, manganese, zinc, nitrogen, phosphorus, thiamin hydrochloride and yeast Saccharomyces cerevisiae, in dosage of 200g/hL. Then the mash was homogenized and vat closed to start fermentation, which occurred at room temperature (about 30^{o}C).

The fermentation was monitored daily by reading the content of dissolved solids in the must, and the end of fermentation determined by the stabilization of ^{o}Brix.

Distillation of fermented: The fermented must was filtered on cotton fabric for removal of solids impurities in suspension

and waste of yeast cells. The distillation occurred in copper pot still, with controlled temperature to 95°C.

Incorporation of sugar and fruit flavors of the drink: After distillation, it was performed the standardization of alcoholic grade of the drink to 38°GL as the minimum value established by legislation [6]. The FM1 formulation was left to stand in a glass container with 5% pulp of passion fruit, for 2 days, with the purpose of extracting pigments and aromas of the fruit. After the time of maceration, the drink has been filtered. The FM2 formulation was only distilled and bottled. The formulations FM1 and FM2 were divided into three fractions, each, and coded as follows: FM1-0, FM1-1, MF1-2, FM2-0, FM2-1 and FM2-2, and the digits 0, 1 and 2, representing the addition of sugar to the drink (0-no addition, 1-addition of 2g/L of sugar and 2 - addition of 4g/L of sugar). The determination of sucrose is within the standards allowed by Brazilian legislation, which allows a maximum addition of 6g/L, expressed in sucrose [6].

B. Physical-Chemical Analysis in the Drink

Was performed the following physical-chemical analysis in the samples: alcohol (alcoholic degree), total acidity, fixed acidity and dry extract, according to the methods described by the Adolfo Lutz (1985) [7].

C. Sensory Analysis

The sensory tests of samples were performed using a panel consisting of 10 trained tasters. For the recruitment of candidates, it was filled a questionnaire with 23 people, which enabled the assessment of the availability to participate in the work, affinity with a drink and some habits of the tasters [8]. The bodies of tasters consisted of civil servants, students and teachers of technology courses in food of plant origin in CEFET Petrolina, aged between 21 and 63 years, male. The final selection of tasters was made through the triangular test, as approach suggested by Lima *et. al* (2009) [8] and applied to two commercial samples of spirits, with a "sweetened, non-aging" and other "non-sweetened, aged 10 years".

After the selection it was made a discriminatory test for paired comparison, using as a reference sample a commercial sugar cane liquor, which was faced with the samples of fermented passion fruit of FM1-0 and FM2-0, on alternate days in order to verify if there was significant sensory difference between the beverages obtained from the passion fruit and that obtained with the traditional sugar cane. The comparison was done in triplicate.

In another section it was performed the ordering test among the six samples of fermented fruit, when the judges have the samples in increasing order of preference, according to methodology used by Dias *et al.* (2003) [3].

D. Chromatographic Analysis

To assess the profile of volatile components by gas chromatography connected the mass spectrometry, were evaluated: fruit pulp *in natura* and samples FM2-0 and FM1-0, as follows:

Sample Preparation: In a 20mL vial for headspace was weighed 3g of NaCl (Nuclear, São Paulo, Brazil) and then added 5mL of ultra-pure water and 5mL of sample. The vial was sealed with metal seal (magnetic) and silicone septum interfaced with Teflon. The sample was at rest for 10 min to ambience and then extraction was performed.

Extraction: The extraction by SPME was performed in the automatic sampler CTC CombiPal (Zwinger, Sweden) in headspace mode (HS-SPME) fiber coated with polydimethylsiloxane (100μm) purchased from Supelco (Bellefonte, PA, USA). The fiber was exposed in the headspace for 30 min at a constant temperature of 60°C and with agitation of 250 rpm. The thermal desorption was performed for 10 min at 250°C in the chromatographic injector (splitless mode).

Chromatographic conditions: The tests were performed in replicates (n = 3) in the GC-MS model SHIMADZU QP2010 Plus (Kyoto, Japan). Injector split / splitless at 250°C. Capillary column RTX-1ms (Crossbond ® 100% dimetilpolisiloxano, 30m x 0.25mm ID x 0.25 μm) purchased from Restek (Bellefonte, PA, USA) , programming of the oven: 35°C (1 min) - 4 °C.min^{-1} to 100 °C - 5°C.min^{-1} - 190°C (10 min) with a total race time of 45.25 min. It used helium gas (99.99% purity) as mobile phase with a linear speed of 27 cm.sec^{-1}. The mass spectrometer worked in SCAN mode with a mass range of 30 to 350 m/z, electron impact ionization (70eV) with temperature of the ion source heated to 230°C and interface of 250°C for data acquisition 3.5 min after the start of the race.

III. Results and Discussion

A. Physical-Chemical Analysis

The study of the yield of the yellow passion fruit pulp showed that this plant has potential for industrial use, as the pulp fraction is 58.51% (± 6.25), on average, of the ripe fruit. Adds to this the fact that the pulp is very acid and concentrated and can be used in diluted form, with no loss of aroma and flavor.

During the fermentation of the must of passion fruit, the behavior of the soluble solids content (sugar) was monitored daily until they had settled, setting the end of the fermentation process. The graph shown in Fig. 1 shows the curve resulting from this monitoring and shows that the must °Brix has remained at 5% from the fourth day of fermentation.

The fermentated must (wine) was submitted to the traditional destillation in copper still, when the fractions of ″head″, ″heart″ and ″tail″ had been separate.

The fractions ″heart″ of the spirits had been standardized (38°GL) through the adjustment of mass and after that the physical-chemical parameters had been evaluated: total acidity, fixed acidity and dry residue, studied in the six samples of spirits passion fruit (FM1-0; FM1-1; FM1-2; FM2-0; FM2-1 and FM2-2) and are shown in Table I.

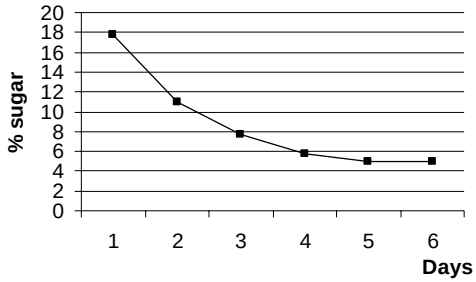

Fig. 1 Sugar content in the must during fermentation

TABLE I
VALUES OF THE ALCOHOLIC GRADE, TOTAL ACIDITY, FIXED ACIDITY AND DRIED RESIDUE, IN SAMPLES OF SPIRITS PASSION FRUIT

Spirits samples	Physical-chemical parameters			
	Total acidity % (v/v)	Fixed acidity % (v/v)	Dry residue % (m/m)	Alcoholic degree % (v/v) 20°C
FM1-0	1.00	0.68	0.1720	38.0030
FM1-1	1.70	1.20	0.5120	38.0030
FM1-2	3.20	1.80	1.0430	38.0030
FM2-0	0.70	0.28	0.0420	38.0030
FM2-1	0.70	0.12	0.1930	38.0030
FM2-2	0.70	0.28	0.3450	38.0030

Samples FM1 had presented greaters levels of total acidity, fixed acidity and dry residue, in relation to samples FM2. This behavior this related with the technique proposal for setting of volatile in spirits FM1, that involves the maceration of the drink distilled with 5% of added pulp. Taking itself in account that the fixed acidity also represents acid ones contained in the fruit and those produced during the fermentation [9], it was of if waiting that to these two fractions were also added acid ones dissolved of the portion of the fruit added during the maceration. Evaluating the results shown in Table I, is possible to perceive that, for the two treatments (FM1 and FM2), the levels of dry residue increase between the samples. The increase of these levels is consequence of the addition of increasing amounts of sucrose, made with the intention to sweeten the spirits and to improve the flavor.

B. Chromatographic Analysis

Tables II, III and IV presents the identified volatile compounds in the passion fruit pulp and passion fruit spirits FM1 and FM2, caught by the headspace-SPME technique, with their respective relative areas and retention time.

TABLE II
MAIN IDENTIFIED VOLATILE COMPONENTS IN THE PASSION FRUIT PULP, TIME OF RETENTION (T_R) AND AREA OF PEAKS

Number	T_R (min.)	VOCs*	Area
1	4.98	Alanine	3323396
2	6.49	Ethyl butanoate	5894098
3	13.35	Ethyl hexanoate	10963888
4	20.40	Hexyl butanoate	3894803
5	21.33	5-hydroxymethyl-2-furfuraldehyde	2453990
6	23.74	Ethyl decanoate	483331506
7	26.22	Octanoate 3-methylbuthyl	48672434
8	34.88	Oleic acid	19723005
9	41.10	Pentadecanoic acid	59507753

*VOCs – Volatiles Organics Compounds

TABLE III
MAIN IDENTIFIED VOLATILE COMPONENTS IN THE PASSION FRUIT SPIRIT FM1, TIME OF RETENTION (T_R) AND AREA OF PEAKS

Number	T_R (min.)	VOCs*	Area
10	5.23	1-butanol,3-methyl	179838169
11	8.93	3-methylbutyl acetate	59254111
3	13.40	Ethyl hexanoate	52135031
12	20.76	Ethyl octanoate	247586158
13	21.07	Octanoic acid	26311943
14	22.10	2-phenylethyl acetate	24646576
15	26.25	Hexyl hexanoate	39088778
6	26.66	Ethyl decanoate	483331506
7	27.92	Octanoate 3-methylbuthyl	48672434
16	31.67	Ethyl dodecanoate	370170677
17	32.74	Pentadecanoate 3-methylbuthyl	63239090
18	33.47	1-dodecanol	22714393
19	36.02	Ethyl tetradecanoate	90511909
20	41.10	Ethyl 9-hexadecenoate	43802728
21	42.13	Ethyl hexadecanoate	119979925

*VOCs – Volatiles Organics Compounds

TABLE IV
MAIN IDENTIFIED VOLATILE COMPONENTS IN THE PASSION FRUIT SPIRIT FM2, TIME OF RETENTION (T_R) AND AREA OF PEAKS

Number	T_R (min.)	VOCs*	Area
22	4.90	Ethane, 1,1-diethoxy	11331027
10	5.25	1-butanol,3-methyl	122366601
2	6.51	Ethyl butanoate	2039271
23	8.90	Heptyl acetate	8619619
3	13.36	Ethyl hexanoate	8037993
12	20.69	Ethyl octanoate	17917200
24	26.07	Decanoic acid	1407227
15	26.21	Hexyl hexanoate	10159535
6	26.51	Ethyl decanoate	52069211
7	27.83	Octanoate 3-methylbuthyl	1825807
16	31.51	Ethyl dodecanoate	48872031
17	32.66	Pentadecanoate 3-methylbuthyl	4599284
19	35.95	Ethyl tetradecanoate	6975270
9	40.92	Pentadecanoic acid	7241834
21	42.98	Ethyl hexadecanoate	15186054

*VOCs – Volatiles Organics Compounds

Taking into account the main objective of this study, which focuses on verifying the presence of VOC's from the pulp of passion fruit beverages, is observed that three esters found in the pulp were also detected in two samples (FM1 and FM2), these are ethyl hexanoate, ethyl decanoate and octanoate of 3-methylbutyl. It seems that both processes used to obtain the fermented beverage can retain important VOC's that belongs to the fruit original aroma. However, from the chromatograms and peaks areas of these compounds, the concentration are more significative for sample FM1, in which the peaks areas are 6, 9 and 26 times higher that for sample FM2 for the same esters.

The presence of esters ethyl hexanoate, ethyl butanoate, hexyl hexanoate and hexyl butanoate identified as major components in the passion fruit pulp, was also reported by other authors [4], [10], [11].

Comparing Tables III and IV, is possible to observe new VOC's in the beverages, these from the fermentation process, they are the alcohol 1-butanol 3-methyl and the esters: butyl acetate 3-methyl, ethyl octanoate, hexyl hexanoate, ethyl dodecanoate, butyl pentadecanoate 3-methyl, ethyl

tetradecanoate, ethyl hexadecanoate. The production of these compounds is independent of the fruit, as they were cited by Nóbrega (2003) in their work with the volatile spirit of sugar cane [12].

C. Sensory Analysis

The sensorial panel used to evaluate the passion fruit spirits quality was made by ten panelists, selected according to their interest in participate in this study and none of them were taking medicines or presented any disease that could influence the analysis. The main criterions to select the group were: afinity to distilled beverages (80%), non smoking person (70%) and the percentage of individual hit in the triangle teste equal or greater than 66.66% in the sections.

After selecting the panelists, each one were submited to paired comparision test in two sections, where a comercial sample were used in both sections to compare it with samples FM1-0 (section I) and FM2-0 (section II). The test was done in triplicate. Its objective was to verify if the passion fruit spirits had sensorial characteristics that would make them different from the comercial or if it was possible to notice different ethanol VOCs, that would make the sensorial profile of passion fruit spirit, being different from sugar cane spirit. The difference between them was quite obvious for the percentage of hit of the provers, as shown in Fig. 2.

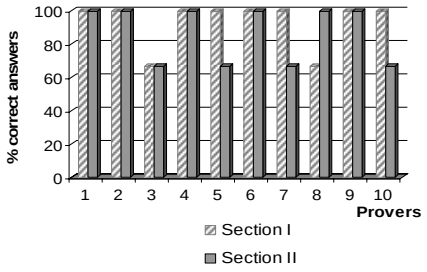

Fig. 2 Percentage of hit of the provers in two sections of the paired evaluating

As shown in Fig. 2, even with the provers realizing difference in both samples (percentage of hit above 66.66 %), the perception was better observed in the section I, when the evaluated sample was FM1 and eight of the provers obtained 100% of hit. In section II, only six provers got this performance with the sample FM2.

Again, the best answers of the experiments adresses the conclusions for the technique of preparation of passion fruit spirit by maceration of the pulp (FM1).

The last stage of sensory evaluation was the test of preference, which took into account the parameters of color, aroma and taste of the spirits, punctuated through the ordering of the samples FM1-0, FM1-1, FM1-2, FM2-0, FM2-1 and FM2-2, in the growing order of the scale of preference, which contained six positions. To each position a respective note was attributed: 1.66, 3.32, 4.98, 6.64, 8.30, 9.96.

In Fig. 3 it is represented the sequence of final preference of the provers, obtained through the average of the notes attributed by them.

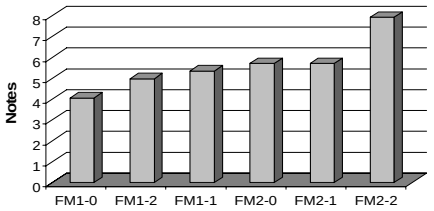

Fig. 3 Growing order of preference of passion fruit spirits

In this test, it was verified that the samples preferred by the provers panel are those obtained with higher proportion of pulp in the must subjected to fermentation and that does not involves maceration after distillation. In other words, the most accepted spirits were FM2-0, FM2-1 and FM2-2, which notes were, respectively, 5.72, 5.72 and 7.93.

This result seems contradictory in relation to the what was presented previously, which showed a certain superiority of the spirits obtained by maceration of the pulp (FM1). This can be explained because in the initial tests, the main evaluated parameter was related to the spirit aroma, which is more complex in ample FM1, as can be confirmed by Tables III and IV and their chromatograms. However, the attributes of color and taste evaluated in the test of preference were decisive in the general evaluation of the drinks. The lightly yellowed color obtained in samples FM1 had not a very positive effect in this provers panel, which opted for the transparency, clearness and total absence of pigments of the samples FM2.

Beyond the visual aspect, another attribute that influenced in the choice of the provers was the taste, therefore, in accordance with what it was observed during them you analyze physical-chemical, levels of fixed and total acidity had been bigger in samples FM1, causing reduction of the gustative quality of the samples.

Among the samples FM2-0, 2 FM-2-1 and FM2-2, the best acceptance of the last one is related to its ″sweetness″, as its processing involved the addition of 4g of sucrose per liter of spirit.

IV. Conclusion

The results of the present study showed which spirits prepared with yellow passion fruit (*Passiflora edulis f. flavicarpa*) can be a good alternative for the market of drinks fermented and distilled, as the principals COVs characteristics from the fruit remain in the drink, making it more attractive from the sensory point of view. The prepared drinks (FM1 and FM2) obtained a good acceptance, being the best acceptance observed for the samples FM2, in which the processing involves the use of higher proportion of passion fruit pulp (1:1), maintaining the transparence and the ausence of color in the final product. The addition of sugar (4g/L) also contributed with the high acceptance of this sample.

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