STEVIA REBAUDIANA SUGAR CRYSTALLIZATION PROCESS

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ABSTRACT

The aim is to produce natural sugars from Stevia Rebaudiana (a perennial plant with a sweet leaf) planted in Madagascar in the Brickaville region. Studies have shown that it has been used worldwide for various times as a sweetener and medicine. In a few years, our goal is to replace table sugar containing sucrose with that produced by Stevia. And also it could possibly become a major source of very potent sweetener for natural foods. The crystallization of this sugar uses dry leaves of this plant, chromatographic columns with ion exchange, organic solvents such as ethanol and also flocculant such as calcium hydroxides. Using this new product can provide an intense sweet taste without having to worry about the side effects of sugar (sucrose). As diabetic patients have an interest in stabilizing their calorie intake, Stevia Rebaudiana could be an alternative to the sucrose in sugar.

Keywords: *Stevia Rebaudiana, sugar, stevioside, sucrose, crystallization, column chromatography.*

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1. INTRODUCTION

Nowadays, scientists are interested in products that are natural, sustainable and without adverse effects. The discovery of a sweet plant called *Stevia Rebaudiana* is truly a great success. It is a plant of the future for the sugar industry and nutritional health. It is a plant native to Latin American countries such as Paraguay and Brazil, a species belonging to the Asteraceae family [1]. Not only does it have an incredibly intense sweetening power (300 times sweeter than cane sugar) but also therapeutic virtues of great importance [2].

Madagascar is already enjoying its benefits, the industrial production of sugar from this plant is a beneficial project for our country. The question is: "how can we use it to replace cane sugar with *Stevia Rebaudiana* sugar in a short time? " . To better understand, first, what this plant is, then what is the process used to transform this plant into sugar crystals.

It is a plant native to Central America, Paraguay and Brazil, but already cultivated in Madagascar in a plantation located in Brickaville. It has been used for centuries by the Brazilians, the Guarani Indians of Paraguay as sugar and also in medicinal beverages. They called it ka'ahe'ê ("sweet grass") [3]. The leaves of this small perennial plant, which reaches 65 to 80 cm in height, can be eaten fresh, or infused into tea and food [4]. The genus Stevia includes at least 110 species but there may be up to 300 [5]. Harvesting of *Stevia Rebaudiana* leaf can be done three times a year.

Glycosides such as: steviol, dulcoside A, rubososide, steviolbioside, rebaudioside A, B, C, D, E, and F have been identified in this plant. Stevioles glycosides are molecules that give the plant its sweetening power [1]. The leaves of wild Stevia plants contain 0.3% dulcoside, 0.6% rebaudioside C, 3.8% rebaudioside A and 9.1% stevioside [4].

As we have already seen that the stevioside molecules which have a sweetening power 300 times stronger than conventional sugar are rebaudiosides (A-F), rubusoside, steviolbioside and dulcoside A [12]. The major sugar compounds are stevioside and rebaudioside A [6].

These molecules do not provide any of the calories of sugar, which is why they are classified as sweeteners. A sweetener is a product or substance with a sweet taste that does not provide calories, or provides fewer calories than table sugar (sucrose). As a sweetener, it is a solution in certain cases of diabetes or in a low-calorie diet by avoiding synthetic molecules such as aspartame [2].

Toxicological studies have shown that steviol heterosides do not have harmful effects such as cancer, no effect on reproduction. [4]
For cariogenic and mutagenic effects, a study was conducted to verify whether stevioside and rebaudioside A may be a cause of dental caries with prolonged use. Experiment carried out with a rat for 2 years at high doses almost 1kg per day. The rats were fed a diet containing 0.5% stevioside or 0.5% rebaudioside for 5 weeks. No compound has shown any potential to increase the risk of developing tooth decay [7].
Reports have indicated the lack of mutagenicity of the two compounds, steviol and stevioside [8].
Thus, it is very interesting to extract the sugar from *Stevia Rebaudiana* as an alternative to sucrose from cane sugar.

2. METHODOLOGY

Picking: picking or harvesting leaves is the most important step.
Washing: after harvesting, leaf inspection is done to remove all foreign bodies, and then rinsing with tap water to get clean material.
Drying: The cleaned leaves are now placed on aluminum trays and dried in the shade. But to completely remove the water from the leaves, they are subjected to a current of air until they lose all of its moisture. The air experienced by the leaves causes natural oxidation and changes the color of the leaves.
Grinding: Grinding is done to reduce the dry leaves to powder; this powder is shown in figure 1.

Fig- 1: Stevia dry leaf powder

Infusion: an infusion of 10% for 30 min at 70 ° C is recommended to release the molecules that give the leaves the sweetening power (example: 10 g of leaf powder in 100ml of water) [9].
Filtration: Büchner filtration is preferable: this creates a vacuum in the container, thus filtering the solution that we add from the top part.
Purification: The filtrate obtained contains numerous particles in suspension. In order to eliminate them, a treatment with calcium hydroxide Ca (OH) 2 (lime), which acts as a flocculant, is carried out. The precipitates formed will be removed by a new filtration.
Separation: Two chromatographies on an ion exchange column will be necessary [10].
-The first is done to remove pigments and other soluble materials.
-The second ion exchange column is intended to specifically absorb the steviol glycoside molecules from the previously discolored solution.
Here is Figure two which shows a model of these two chromatographic columns.

Fig- 2: Column chromatographies

Crystallization: The steviol glycoside powder obtained is then purified by crystallization in ethanol to obtain a solid and pure product [11].
All of these processes do not involve any solvent or chemical compound, only calcium hydroxide and alcohol, natural products, are used apart from water. This makes it possible to obtain a finished product free from any residual solvent or any exogenous chemical product. All of these processes are summarized in figure 3.

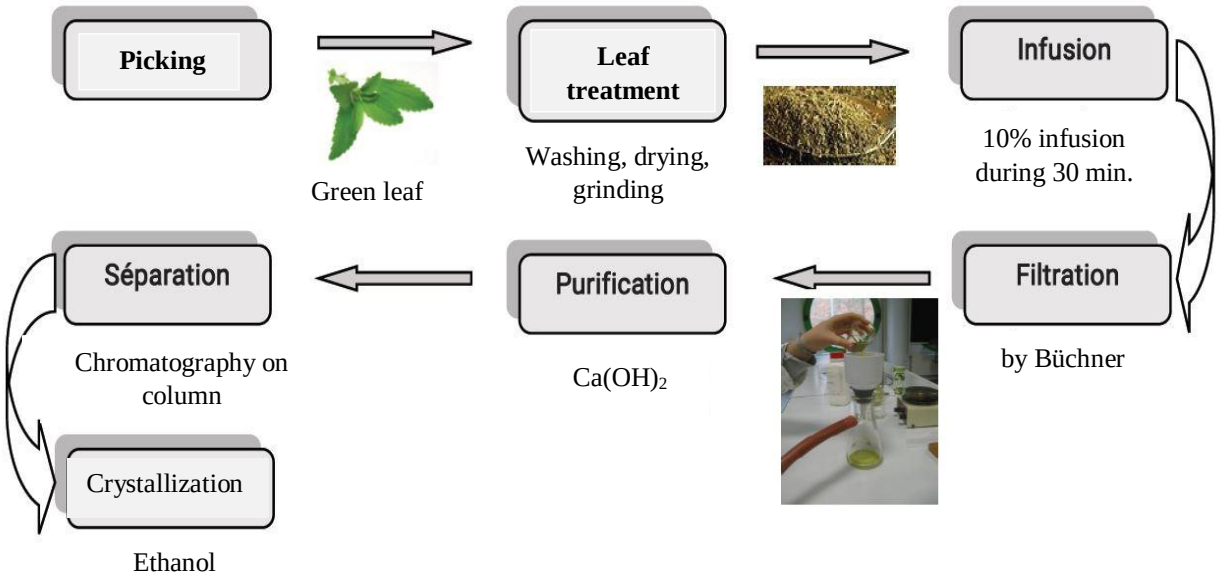

Fig- 3: Summary of Stevia-based sugar crystallization processes from Madagascar

3. RESULTS and INTERPRETATIONS

The result is a pure crystalline product with a reddish color and above all without adverse effects since it is natural.

Fig- 4: Sugar from the leaf of Stevia Rebaudiana

The quality control method adopted for the physico-chemical control of Stevia sugar is according to the ICUMSA standard (International Commission for Uniform Methods for Sugar Analysis) in order to assess the quality of the sugar of the Stevia leaf. The table 1 summarizes the sweetening powers of molecules present in Stevia sugar.

Table- 1: Sweetening power of the molecules that make up the sugar in Stevia

Molecules	Sweetening power			
	1	2	3	Average
Stevioside	291	277	280	**283**
Rebaudioside A	433	421	425	**426**
Rebaudioside B	305	304	305	**305**
Rebaudioside C	60	57	59	**59**
Rebaudioside D	38	42	39	**40**
Rebaudioside E	115	113	119	**116**
Rubusoside	95	94	109	**99**
Dulcoside A	72	70	75	**72**
Steviolbioside	96	95	95	**96**

Here are the nutritional values summaries of the sugar in the Stevia leaf.

Table- 2: Nutritional value in percent of the sugar of a stevia leaf

	%		%
Calcium	0.5440	**Manganese**	0.0147
Magnesium	0.3490	**Sodium**	0.0892
Phosphorus	0.3180	**Lipid**	0.0000
b-Carotene	0.0075	**Protein**	11.200
Potassium	1.7800	**Vitamin**	0.0110

Performance study

On 1 hectare of plant, 1 000 to 1 200 kg of dried leaves are harvested which contain 60 to 70 kg of steviosides. If we compare these figures with those for sugar cane or sugar beet, we see that the yield is much lower. However, the 70 kg of steviosides which are 300 times sweeter than the sucrose in cane sugar, equivalent to 21 000 kg of sucrose.

4. CONCLUSION

Stevia Rebaudiana is a perennial herb with oblong, crenellated leaves and a sweet flavor, introduced to Madagascar. The leaves of steviol such as: stevioside, rebaudiosides, dulcoside. Stevioside can make up to 10% of the leaf mass. It can be extracted in water first, then purified by Buchner filtration adding $Ca(OH)_2$, separated by column chromatography, and finally, crystallizing it from purifying ethanol.

After crystallization and physicochemical analyzes, we obtained red-colored stevia sugars, with a high sweetening power coming mainly from molecules of stevioside of the order of 283, rebaudioside A of the order of 426 and rebaudioside B of the order of 305, in addition to mineral nutrient supply such as calcium, magnesium, phosphorus, b-carotene,…; and protein and vitamin intake.

Stevioside has a sweetening power, about 300 times greater than that of sucrose and in addition, it is devoid of toxicity. And since rebaudioside A has been approved as a sweetener in France and Europe, this increases the interest in doing more research on the *Stevia Rebaudiana* plant.

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