Clarification of *Stevia rebaudiana* (Bert.) Bertoni extract by adsorption in modified zeolites

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ABSTRACT. Selective adsorption on zeolites X and A was studied with *Stevia rebaudiana* (Bert.) Bertoni (Asteraceae) extract clarification. Ionic exchanges were made with calcium and barium ions in zeolites NaX and NaA and the effect of contact of aqueous extract of dry *Stevia rebaudiana* Bertoni leaves with CaX, BaX, CaA and BaA zeolites was evaluated. *Stevia* extract in contact with the zeolite CaX showed highest clarification and did not present alterations of initial characteristics in concentration, glycosides and flavor. In batch tests at room temperature, the contact of *Stevia* aqueous extract and CaX zeolite provided 70-80% clarification. Since zeolite may be reused, an approximate clarification of 65-70% is possible in regenerated zeolite.

Key words: clarification, *Stevia rebaudiana* Bertoni, CaX zeolite, selective adsorption.

RESUMO. Clarificação de extrato de *Stevia rebaudiana* (Bert.) Bertoni por adsorção em zeólitas modificadas. No presente trabalho, foi estudada a adsorção seletiva do extrato de *Stevia rebaudiana* (Bert.) Bertoni (Asteraceae) nas zeólitas X e A. Foram feitas trocas com íons de cálcio e bário nas zeólitas NaX e NaA. Foi avaliado o efeito do contato do extrato aquoso de *Stevia* com as zeólitas CaX, BaX, CaA and BaA. Os resultados mostraram que o extrato que ficou em contato com a zeólita CaX foi o que obteve maior descoloração e não teve as características iniciais alteradas, em termos de concentração, glicosídeos e sabor. Com testes feitos em batelada à temperatura ambiente, o contato entre o extrato aquoso de *Stevia* e a zeólita CaX, proporcionou uma descoloração entre 70-80%. Os testes mostraram ser possível a regeneração da zeólita, sendo possível a utilização da zeólita por mais de uma vez no processo, sendo alcançada uma descoloração entre 65-70%.

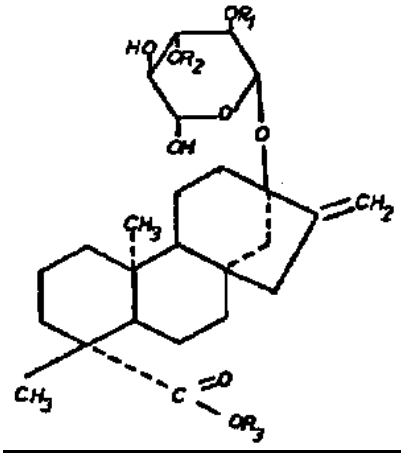
Palavras-chave: clarificação, *Stevia rebaudiana* (Bert.) Bertoni, zeólita CaX, adsorção seletiva.

The high standards of our technological society have changed the concept of health, while the introduction of new chemical products modified human alimentary habits in the interests of a more healthy life (Kinghorn *et al*., 1995). For simple aesthetic or health problems, man is substituting the well-known and popular sugar (sucrose) for products known as sweeteners. These have a flavor similar to that of sucrose, but with little or no caloric value (Angelucci, 1989).

Among the sweeteners, there are glycosides extracted from the dry leaves of a native plant called *Stevia rebaudiana* (Bert.) Bertoni (Asteraceae), or simply *Stevia*. Its leaf has several sweetener glycosides such as stevioside, rebaudioside *A, B, C, D, E* and dulcosides *A* and *B*. Those present in larger quantity are stevioside (5-10%), rebaudioside *A* (2-4%), rebaudioside *C* (1-2%); others are present in smaller concentrations. Figure 1 shows glycoside structures in which radicals R_1, R_2 and R_3 mark their difference.

Glycosides are sweeteners of natural origin, stable to a wide range of pH and heat. Since they are non-caloric, non-nutritive, non-fermenting, prevent caries and dental plaques, sweeteners are industrially interesting. Due to these advantages there are many scientific articles and patents published on *Stevia* and its sweetening glycosides. They include analytic methods, purification methods, sensorial evaluation and, chief among them, the toxicological aspects. In fact, everybody attests the safety of *Stevia* extract and its sweetening glycosides for human consumption. Stevioside is used as a nutritious sweetener in many countries of the world. In Japan it has been used since 1970 as a sweetening agent and dominates about 41% of the market. It was approved in Brazil in mid-1987 as a flavoring agent and sweetener in several classes of foods; in the United States it

started to be used in 1996 as an ingredient for dietary supplements (Cândido and Campos, 1996).



COMPOSITES	RADICALS			
	R_1	R_2	R_3	Sweetness
Stevioside	Gluc.	H	Gluc.	100-270
Steviolbioside	Gluc.	H	H	10-15
Rebaudioside A	Gluc.	Gluc.	Gluc.	150-320
Rebaudioside B	Gluc.	Gluc.	H	10-15
Rebaudioside C (Dulcoside B)	Rham	Gluc.	Gluc.	40-60
Rebaudioside D	Gluc.	Gluc.	$Gluc.^{2}$-$^{1}Gluc.$	200-250
Rebaudioside E	Gluc.	H	$Gluc.^{2}$-$^{1}Gluc.$	150-200
Dulcoside A	Rham	H	Gluc.	40-60

Gluc = β-D-Glucopiranosil Rham = α-L-Rhamnopiranosil

Figure 1. Glycosides structures

Several processes of extraction of the sweetening substances of *Stevia* presented in literature follow approximately the same methodology. First, extraction from the leaves of *Stevia* is made, with water or alcohols (ethanol or methanol); the obtained extract is in the form of a solution loaded with colloidal particles of dark brown color, containing all the active principles, pigments of the leaf, soluble polysaccharides and other impurities. Some processes previously remove the greases from the leaves with solvents, such as chloroform or hexane, a preliminary elimination of essential oils, chlorophyll and other apolar principles (Tanaka *et al*., 1977; Masuyama, 1980; Kinghorn *et al*., 1982).

The second stage consists of the clarification of the extract, which is usually made by metallic ions (Ishizone, 1979), ultra filtration (Fuh and Chiang, 1990) or organic solvents (Alvarez and Couto, 1984). The clarification of *Stevia* extract is that part of extraction that differentiates the methods. After clarification, all methods practically process the extract in a similar way. The solution is concentrated and dissolved again in methanol for stevioside crystallization. Nowadays, in Brazil, clarification process is done industrially by treatment with metallic ions associated with organic solvents. New processes using advanced technology are being tested. Osamu Tanaka's team, working for Mitsue Petrochemical Ind. (Tan *et al*., 1988) developed a process of extraction from *Stevia* leaves with supercritical CO_2 in the presence of methanol, extracting stevioside and rebaudioside *A*. A similar work has been done (Kienle, 1989) in Europe, extracting previously the greases and pigmentation of *Stevia* leaves by supercritical extraction and posterior conventional extracting the sweetening principles.

Extract clarification is a very important stage because it results in better visual quality of the final product. However, usual clarification processes have some disadvantages: organic solvents and metallic ions leave residues which are harmful to health and thus forbidden in some countries; ultrafiltration membranes and other advanced technologies use high cost materials and require equally operational high cost technology. Consequently, there are many published works and researches in progress on this area. On the other hand, few articles have been published in which zeolites are reported to have been used with *Stevia* (Kodaka, 1977).

The aim of our research was the evaluation of clarification efficacy of extract from *Stevia* leaves for the adsorption in zeolite. Sodium ions in zeolites NaX and NaA were exchanged with calcium and barium; then *Stevia* extract was put in contact with modified zeolites NaX, CaX, BaX, NaA, CaA and BaA, under the same conditions, with the purpose of evaluating such contact in extract clarification.

The idea of using zeolites in the process with *Stevia* hailed from its successful use in the separation of other glycosides (Ribeiro *et al.,* 1984). Silva (1998), working with zeolite in the fructose separation from dextran, was successful in the experiment.

It was believed that zeolites would behave similarly to *Stevia* glycosides, or with at least to one of them specifically. It could also occur that coloration compounds would be adsorted as the glycosides, and thus the separation of the latter from the undesired color would occur.

Present research may be considered a pioneer work because there are few publications in which zeolites are reported to have been used in the process. Our only information on the process of zeolite with *Stevia* consists of patents with limited information.

In the clarification of aqueous *Stevia* extract, the process with zeolites was advantageous because it was low cost, did not leave obnoxious residues, its operational technology and application was easily understood, and did not represent any risk to the environment.

The conventional processes of clarification have some disadvantages: they leave obnoxious residues with health hazards; they have a high implantation and maintenance cost; and the operational technology consumes reagents and specialized labor, which elevates cost too. Consequently, a simple technology that does not result in toxic residues in the final product is interesting and necessary.

Material and methods

Stevia rebaudiana extract preparation

Steviafarma S.A (localized in Maringá, Paraná, Brazil) supplied the leaves of *Stevia* for the experiments. The leaves of *Stevia* were mixed with hot water (1:9, w/w) at 328K for 30 minutes. After cooling the resulting extract was filtered.

Characterization of the zeolites

The zeolites consisted of powdered NaA and powdered and grain NaX; the size of the grain particles were 1.0-4.0 mm. Both zeolites were of the Baylith-type, Bayer Brasil S.A. The chemical composition of zeolites was determined by Atomic Absorption Spectrometry. For the analyses, the Varian Spectra AA-10 Plus equipment was used. Table 1 gives the results.

Table 1. Composition of zeolites NaX and NaA

Zeolite	% Al_2O_3	%SiO_2	%Na_2O	Rate Si/Al
NaA	35.9	43.3	20.8	1.02
NaX	30.3	52.6	17.1	1.47

Ion exchange of sodium for calcium and for barium

The initial zeolites were powdered NaA and powdered and grain NaX. Sodium ions in initial zeolites were exchanged for calcium and barium, always with chloride as anion, which facilitates its elimination. The relationship in the calculation of the mass of salt was:

Ca^{+2}/Na^{+} and Ba^{+2}/Na^{+} = 2 equivalent / equivalent.

Both ion exchanges followed methodology by Silva and Machado (1994).

- ($CaCl_2.2H_2O$) - an aqueous suspension at 15% (w/w) of zeolite was made and shaken for 24 hours when in powder form and for 1 hour when in granulated form. The adjustment of pH was done with HCl 6% solution (w/w), while pH was adjusted between 5 and 6. The reactor was placed in a heating plate shaken at 348K; solution of 16% of calcium chloride was then added slowly, and the mixture was shaken during 24 hours.
- ($BaCl_2.2H_2O$)- an aqueous suspension at 10% (w/w) of zeolite was made and shaken for 24 hours when in powder form and for 1 hour when in granulated form. Adjustment of pH was made, with HCl 6% solution (w/w); pH was adjusted between 5 and 6. The reactor was placed in a heating plate and shaken at 348K; the solution of 20% of barium chloride was then added slowly, and the mixture was shaken during 24 hours.

In all ion exchanges the suspensions were filtered in Büchner Funnel in vacuum. Cake was washed three times, the first washing with a solution of salt equal to the one added to the suspension; the second and third washings with the same amount of water used in the solution of the salt. Washings were done at the same temperature of ion exchange. After being washed, cake was dried at 393K for 24 hours.

Analytic methods used

Phenol sulfuric method for total carbohydrates. The sweetening principles of the *Stevia* extract were determined by the Phenol Sulfuric Method. The latter determines total carbohydrates and the correlation that exists between total carbohydrates and glycosides of the *Stevia* leaf (Alvarez *et al*., 1986).

Procedure: 10 µL of the *Stevia* extract are diluted in 10mL of water: diluted *Stevia* extract; a standard and a blank solutions are prepared for comparison.

Sample = 500 µL Phenol 5%+ 500 µL *Stevia* extract diluted + 2.5mL Concentrated Sulfuric Acid.
Standard = 500 µL Phenol 5%+ 500 µL Glucose 4.5%(w/w)+ 2.5 mL Concentrated Sulfuric Acid
Blank = 500 µL Phenol 5% + 500 µL water + 2.5 mL Concentrated Sulfuric Acid.

The mixture was left to rest from 10 to 15 minutes; developed color was measured in a Spectrophotometer Shimadzu (UV-1203), at 490 nm, against Blank. The content of total

carbohydrates in the *Stevia* leaf was calculated by Equation (1):

$$TCH(g\%) = \frac{L_A}{L_S} \times 4.5045 \quad (1)$$

TCH - level of total carbohydrates (g%), L_A - sample absorbance, L_S - standard absorbance

Total soluble solids method. The value of soluble solids in a solution, called Brix, was determined by a Refractometer Atago-N1 (Brix 0-32 % ± 0.10).

Percentage of clarification of the aqueous extract *Stevia*. Absorbance analysis was made and a correlation between absorbance and clarification was used (Fuh and Chiang, 1990). Pigments in the solution were analyzed by measuring absorbances at 420nm (A420) and 670 nm (A670), the wavelengths of pigments maximum absorbances, by spectrophotometer Shimadzu (UV-1203). Clarification percentage (% clarification) was calculated by Equation (2):

$$\%Clarification = \left(1 - \frac{(A_{420}(orA_{670}))after}{(A_{420}(orA_{670}))before}\right) \cdot 100 \quad (2)$$

where

A420 or A670 - absorbance of sample in the respective wavelengths of 420 and 670 nm, before and after the purification.

Results and discussion

First test with exchanged zeolites in contact with the aqueous extract of *Stevia*

In a preliminary experiment the exchanged zeolites were put in contact with *Stevia* extract and tests in a batch reactor were made at room temperature, with a contact time of 24 hours without any shaking. The suspension was then filtered. Results of contact zeolite/aqueous extract of *Stevia rebaudiana Bertoni* are in Table 2.

Table 2. Result of contact zeolite/aqueous extract of *Stevia rebaudiana Bertoni*

Sample	% Brix	TCH (g%)	Taste
Pattern	4.2	1.602	
CaX	3.1	1.519	Non-altered characteristics of extract.
BaX	3.8	1.474	Saline taste.

CaA and BaA zeolites did not change any characteristics of *Stevia* extract; but CaX and BaX clarified the extract by adsorption and became loaded with pigments. Table 2 shows non-significant variations in TCH. This is a very important fact because it shows that glycosides initially extracted from the leaf of *Stevia* did not suffer alterations in their contact with the zeolite. Brix variations, almost 25%, were related to pigment extraction. Since BaX introduced a saline taste, CaA, BaA and BaX zeolites were discarded and only the CaX zeolite remained.

Analysis of the efficiency of the ion exchange

As in the above test, it was difficult to separate clarified extract from zeolite in powder form. Ion exchange with NaX zeolite in grain form was made. Three ions series exchanges on zeolite in grain form were necessary to obtain the same percentage of ion exchange in powder form.

Efficiency of ionic exchange (Silva, 1998) is calculated by percentage of sodium cathion exchanged (PSE), given by Equation (3). Results are in Table 3. It is interesting to note that ion exchange presents practically the same efficiency in the powder as well as in the grain form.

$$PSE = \frac{(inicial\ sodium\ concentration - final\ sodium\ concentration\ X\ 100}{inicial\ sodium\ concentration} \quad (3)$$

Table 3. Percentage of ion exchange in NaX zeolite

Zeolite	PSE
CaX (powdered)	83.0 %
CaX(grain)	80.0 %

Tests with variation of the zeolite mass

Tests in batch reactor were made with samples of CaX zeolites in grain form, zeolite mass varying from 10 to 60% (w/w). Samples were placed in a thermostatic bath with magnetic shake at 303K/1 hr.

Figure 2 shows that the mass of 40% (w/w) of CaX zeolite in solution is ideal for the process of clarification. Starting from that value the increment in the clarification percentage did not compensate an increase in the relative quantity of zeolite.

Figure 2. Varying of zeolite mass by clarification

Clarification test with granulated and powdered CaX zeolite

40% (w/w) CaX zeolite was in contact during 24 hr with the *Stevia* extract by shaking. Table 4 shows that clarification obtained with CaX granulated zeolite was similar to that obtained with powdered CaX zeolite.

Table 4. Test with granulated and powdered CaX zeolite

Sample	pH	Brix (%)	TCH (g/100 mL)	%Clar. 420nm	%Clar. 670nm
Standard Extract	5.6	4.0	1.580		
Extract + powdered CaX	5.8	3.0	1.497	81.3	70.2
Extract + grain CaX	5.8	3.2	1.502	80.8	69.8

Where % Clar. = percentage of clarification

Tests with temperature variation with granulated CaX zeolite

Tests were made with varying temperature ranging from 303 to 343K, by shaking, for 1 hr contact time for each temperature. Table 5 shows that the temperature increase did not improve the process of clarification. Therefore, room temperature is best.

Table 5. Clarification in various temperatures

T (K)	303	313	323	333	343
% Clar. λ= 420 nm	83.6	81.3	79.5	78.9	78.3
% Clar.λ= 670 nm	71.5	70.7	69.6	68.5	68.1

Influence of contact time

Room temperature and zeolite content (40%) were kept constant by varying the contact time from 20 to 160 min. Grain CaX by shaking was employed. It could be observed that only 90 minutes were necessary to saturate CaX. Figure 3 illustrates this.

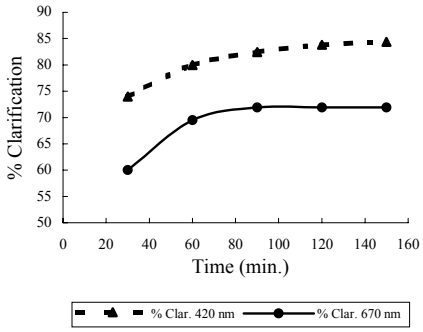


Figure 3. Clarification Time

Process with reused zeolites

The zeolite used in this experiment was CaX, previously in contact with *Stevia* extract for 90 minutes, at room temperature, by shaking. This sample was washed with hot water (353K) that removed pigments retained in clarification process. This same sample was submitted to the clarification test in the same conditions as the previous one. Results obtained, shown in Table 6, proved the regeneration capacity of CaX. It was verified that the zeolite could be regenerated and thus allowing its use more than once in the process. A clarification between 65-70% was possible by this method.

Table 6. Clarification with reused zeolite

% Clar. λ= 420 nm	% Clar. λ= 670 nm
70.0	65.0

Continuous process of clarification

After obtaining the above results with batch tests, a module was set up (Figure 4) with a fixed bed of CaX zeolite. *Stevia* extract percolated the bed in ascending flow. The flow rate was controlled so that the time of contact would be sufficient to reproduce clarification obtained on batch tests. The zeolite mass used amounted to 40% of *Stevia* extract. Results were obtained for two different flows, represented in Figures 5 and 6.

The continuous process of clarification provides results similar to those obtained with the batch tests. This shows its feasibility.

Figure 5 indicates that one may obtain in the first 2 hours a high clarification percentage, followed by a subsequent gradual decline. With a flow rate of 1.25 mL/min, clarification becomes approximately constant during 90% of the time and a clarification between 55-60% in the whole process.

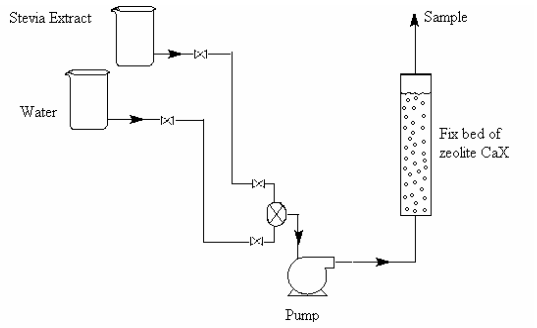


Figure 4. Experimental Module

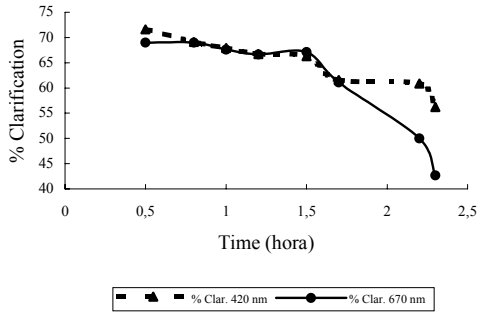


Figure 5. Continuous process of clarification, flow rate=1mL/min, final time = 2hr30min

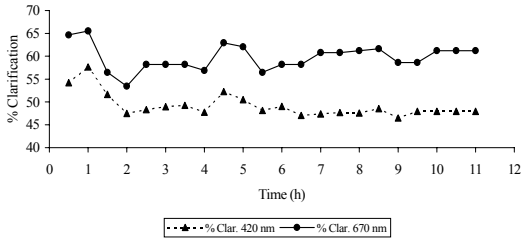


Figure 6. Continuous process of clarification, flow rate=1.25 mL/min, final time = 11hr

Conclusion

The process of *Stevia* extract clarification with zeolites proved to be advantageous because of its low cost (zeolite is cheap), because it leaves no obnoxious residues and uses easy operational technology. In addition, it does not present any risk to the environment.

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