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## Composés bioactifs

# Evaluation of antioxidant activity of steviol glucoside commercial powder in neutral and acidic pH

Évaluation de l'activité anti-oxydante de la poudre commerciale de glucoside de stéviol à pH neutre et acide

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**Abstract** ***Introduction***. Current trends on new food products aim to incorporate biopolymers to improve their physicochemical stability, such as Stevia rebaudiana. Furthermore, due to its high sweetening potential, commercial steviol glucoside (CSP) powder has been recently incorporated into food formulation for its antioxidant capacity. ***Objective***. This study focused on the evaluation of antioxidant capacity of commercial steviol glucoside powder, under both neutral and acidic pH conditions (3.5-7) at concentrations ranging from 100-500 µg/mL. ***Material and Methods***. Tests were assessed in triplicate for a sample of CSP, available in Algeria, by using 2, 2-diphenyl-1-picrylhydrazyl free radical scavenging method (DPPH). Total phenolic compounds were measured in 100µg with Folin-Ciocalteu. ***Results***. Commercial powder of steviol glucoside presented a total polyphenols content of 2.500±0.0150 mg/g of CSP dry weight, and exhibited a significant inhibitory percentage of DPPH. Values were ranged of 35.89±0.16% to 62.32±0.45% in neutral pH, and were between 40.21±0.04% to 65.00±0.26% in acidic pH. Data showed that inhibitory concentration $IC_{50}$ against DPPH was ranged of 208.204 µg/mL in neutral pH, and 269.547 µg/mL in acidic pH. ***Conclusion***. Commercial stevioside powder can be used not only as sweetener, but also as polyphenols source with antioxidant activity in the preparation of neutral and acidic low calorie functional foods.

**Keywords** : *Steviol glucoside, Polyphenols, Antioxidant capacity, Functional foods*

**Résumé** ***Introduction.*** Les tendances actuelles en matière de nouveaux produits alimentaires visent à incorporer des biopolymères pour améliorer leur stabilité physico-chimique, comme c'est le cas pour la Stevia rebaudiana. De plus, en raison de son

potentiel édulcorant élevé, la poudre commerciale de glucoside de stéviol (CSP) a récemment été incorporée dans les formulations alimentaires pour sa capacité anti-oxydante. ***Objectif.*** Cette étude porte sur l'évaluation de la capacité anti-oxydanted'un échantillon de poudre commerciale de glucosides de stéviol, sous des conditions de pH neutre et acide (3,5-7) à des concentrations allant de 100 à 500 µg/mL. ***Matériel et méthodes.*** Les tests ont été évalués en triplicata pour un échantillon de CSP disponible en Algérie, par la méthode du 2,2-diphényl-1-picrylhydrazyl (DPPH) de piégeage des radicaux libres. Les composés phénoliques totaux ont été déterminés à 100 µg avec la méthode de Folin-Ciocalteu. ***Résultats.*** La poudre commerciale de glucoside de stéviol présente une teneur totale en polyphénols de 2,500 ± 0,015 mg/g de poids sec de CSP et un pourcentage inhibiteur significatif de DPPH. Les valeurs sont comprises entre 35,89 ± 0,16% et 62,32 ± 0,45% à pH neutre et entre 40,21 ± 0,04% et 65,00 ± 0,26% à pH acide. La concentration inhibitrice $IC_{50}$ contre le DPPH est comprise entre 208,204 µg/mL à pH neutre et 269,547 µg/mL à pH acide. ***Conclusion.*** Au vu de ces résultats, la poudre commerciale de stévioside peut être utilisée non seulement comme édulcorant, mais aussi comme source de polyphénols avec une activité anti-oxydante, dans la préparation d'aliments fonctionnels, à faible teneur en calories et à pH neutre et acide.

**Mots-clés:** *Glucoside de stéviol, Polyphénols, Capacité anti-oxydante, Aliments fonctionnels*

## Introduction

Recent dietary habits influence day by day human health through the uncontrolled intake of products that generate harmful oxidative stress due to the presence of free radicals. This can have greater health implications, leading to pathological and degenerative conditions. Some plants and their derivative products, rich in antioxidant properties, have been recognized by several pharmacopoeias in various countries as substitutes for synthetic antioxidants [1]. Stevia is among those plants known as an intense sweetener up to 200-300 times sweeter than sucrose, belonging to Asteraceae family and native of Paraguay [2]. Historically, the natives of Paraguay and Brazil have been used the leaves of stevia as a sweetening to make medicines more palatable. It is also used as a sweetener in food and beverages in Japan and China. In the 1930's and 1940's, stevia was introduced in UK and Russia as a sugar substitute, and Japan has been used it extensively since early 1970 [3]. The Joint FAO/WHO Expert Committee on Food Additives reviewed the safety of steviol glycosides (steviol equivalents), and recommended an acceptable daily intake (ADI) limit of 4 mg/kg body weight/day [4].

The structure of the principal sweetening compounds was established as diterpenoid glycosides with the main genin, stevioside, being linked to four glucose molecules, and its chemical molecular formula C38H60O18 is presented in **Fig. 1**. Previous studies have shown that S. rebaudiana contains other metabolites with antioxidant potential, such as ascorbic acid, phenolic compounds including flavonoids and tannins [5].

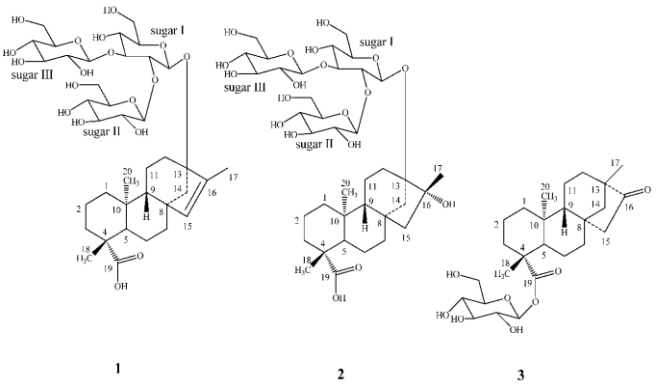


**Fig. 1. Chemical molecular formula of Stevia rebaudiana** [6]

This functional property has widely attracted much attention and interest, so that in published data, complete and extensive research works on the formulation of high-potency sweet taste products, without nutritional value as Stevia rebaudiana fresh leaf and commercial powder steviol [7]. The fruit juice and beverage industry has extensively embraced the stevia incorporation in the development of new formulations [8]. Moreover, recent investigations have focused on studying the stability of the

stevia functional properties under various treatment conditions applied in food technology, such as temperature variation, applied technological processses, and preservation.

For this reason, this study aimed to assess the antioxidant capacity of steviol glucosides commercial powder in both neutral and acidic pH in range of most used concentrations.

## Material and methods

### Characterization of commercial stevioside powder (CSP)

Commercial Steviol glycosides powder (95% Rebaudioside A) from STEVIA NATURA SAS-France was used in this study. A basic characterization of commercial stevioside powder was carried out. Total Moisture content, ash and density were calculated as per the described method of AOAC [9]. Water solubility index (WSI) was determined according to Yang *et al.,* (2023) [10]. All tests were triplicated.

Organoleptic test (color, taste) was conducted by 25 non-smoking volunteers, aged 21±1 years from the Agri-food Department of the University of Blida 1. Test was carried out using discrimination, descriptive and panelist of 10 hedonic points.

### Total polyphenols content

Total polyphenols content (TPC) was measured by the Folin-Ciocalteu (Biochem, Chemopharma, France) method with optimization according to Carbonell-Capella *et al.,* (2015) [11]. Calibration curve established using gallic acid 1-hydrate (GA) (Panreacquimica, Barcelona, Spain) solutions was used for the measures. The correlation coefficient $R^2$ was 0.998, and the results were calculated from the equation:

$$y = 0.081 (\times) - 0.1789$$

and expressed as mg GA/100 mL of juice [12].

### DPPH radical scavenging activity

Antioxidant activity (AA) using DPPH (Sigma-Aldrich, Germany) free radical method, varying quantities (100, 200, 300, 400 and 500 µg) of Commercial Steviol glycosides powder (CSP) were taken in different 10mL volumetric flask, and the volume was made up to 1 mL with methanol.

The percentage of antioxidant activity was determined using DPPH free radical as a reagent with minor modification. Briefly, a DPPH solution was prepared by dissolving 40mg/100 mL of methanol. Then, 3 mL of DPPH was added to 1mL of each sample in a test tube. After shaking, test tubes were incubated for 30min in dark at 25°C, and the scavenging capacity was measured using spectrophotometer (JENWAY 7305), at 517 nm against the bland. Test was triplicated for each sample and the percent-tage of antioxidant activity (AA%) was calculated using the following equation:

$$AA\% = (A° - A1)\ A°\times 100$$

A°: Absorbance of DPPH solution. A1: Absorbance of the mix DPPH and sample [13, 14].

The same steps were followed for the DPPH radical scavenging activity in the acid pH which was maintained at 3.5 by the addition of citric acid concentrated at 1.355 $10^{-4}$ mol/L acetic acid.

### Statistical analysis

All tests were triplicated for each sample and standard errors were performed using IBM SPSS Statistics version 20.0 for Windows. All results were shown in the significant cases ($P< 0.05$).

## Results

### Characterization of commercial Steviol glycosides powder

According to **Table1**, preliminary characterization tests showed an accepted quality of the studied CSP in term of solubility. Results showed a moisture content ranging from 5.55%, and ash levels close to 0.66%.

Based on **Table 2,** data showed that all the volunteers had felt the color of CSP to be very pleasant with a score of 9.84. Participants found that CSP flavor was highly acceptable with a score of 9.5/10. All the volunteers had mostly perceived a fruity after taste scoring it at 9.78/10.

### Total polyphenols content

The total phenolic content of Commercial Steviol glycosides powder was 2.5 mg of total polyphenols per gram of CSP dry weight.

**Table 1. Characterization of Commercial Steviol glycosides powder at 1%**

| Color | Water solubility | Density % | Ash % | Moisture % | pH | Taste % |
|---|---|---|---|---|---|---|
| White | Easy in water | 0.24±0.05 | 0.66±0.02 | 5.55±0.06 | 4,84±0.07 | Consistent sweet |

**% :** *Average of tests results in three samples of CSP at 1% expressed in g/100mL.*

**Table 2. Organoleptic test scoring of volunteers**

| | Color | | Flavor | | After taste | | Acceptability | |
|---|---|---|---|---|---|---|---|---|
| | **Light** | **Dark** | **Chemical** | **Fruity** | **Sweet** | **Licorice** | **Sweet** | **Better** |
| **Volunteers** | 25 | 0 | 22 | 3 | 18 | 7 | 5 | 20 |
| **Average Score/10** | 9.84±0.66 | | 9.5±0.38 | | 9.78±0.57 | | 9.86±0.62 | |

*N = 25 volunteers.*

**Antioxidant activity of Commercial Steviol glycosides powder**

The CSP at concentration of 500µg showed the higher radical scavenging inhibition percentage, and the results represented 65.00% and 62.32% in acidic pH. and neutral pH, respectively **(Fig. 2)**. All values were ranged from 35.89±0.16 % to 62.32±0.45 % in neutral pH, and were between 40.21±0.04 % to 65.00±0.26 % in acidic pH. The commercial Steviol glycosides powder studied had an inhibitory concentration $IC_{50}$ against DPPH of 208.204 µg/mL in neutral pH, and 269.547 µg/mL in acidic pH.

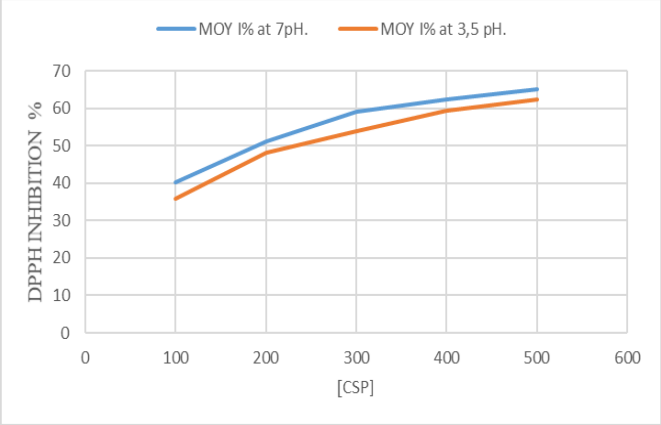


**Fig. 2. Antioxidant activity of Commercial Steviol glucoside powder**

*Average values of inhibition % at different concentrations of three samples of CSP expressed in µg/mL. CSP: Commercial Steviol glucoside powder.*

## Discussion

This study was focused on the evaluation of the antioxidant activity of commercial steviol glycolsides powder in both neutral and acidic pH. Data on pH, and ash of commercial stevioside powder (CSP) showed acceptable quality compared to the international standards, as well as to the product of M/s Stanpack Pharma Pvt. Ltd., Mumbai, India studied by other authors [10, 15].

Moisture content and density represented 5.55% and 0.24%, respectively. These results were close to those obtained by Zorzenon *et al.,* (2020) [16], and slightly higher than the values indicated earlier by Mishra *et al*., (2010) [17].

In terms of overall acceptance, commercial Steviol glycosides powder received a high score of around 9.86, confirming its excellent organoleptic quality.

The total polyphenols content in commercial stevia powder was found to be equal to the values found by Rao (2014) [3]. According to Tadhani et *al.,* (2007), Kim *et al.,* (2011), Zeng *et al.,* (2013), and Ibrahem *et al.,* (2020), polyphenols are present in both Stevia leaf powder and commercial stevioside, but the higher contents were found in leaf powder [18-21].

The higher antioxidant capacity of stevia solutions was primarily attributed to the elevated polyphenols content as reporting by Balaswamy *et al.,* (2014) [15].

The slight increase in inhibition percentage of antioxidant potential in acidic pH was related to the variation in the structure of steviol glucoside with the decrease of pH. Specifically, changes in the polyphenols charges might affect inhibition percentage. These results are also consistent with those obtained by Ibrahem *et al.,* (2020) [21] in water and methanolic extracts prepared with stevia rebaudiana leaf powder. Data were also consistent with those obtained by Rao (2014) who found an inhibition percentage of 47.64 % for 100 µg/mL with CSP [3,19]. Values were also similar to those of Shruti *et al*., (2012) who found 64.26 % and 72.37 %, respectively for 100 µg and 200 µg of stevia aqueous extract.

All the findings have clearly confirmed that the studied commercial steviol glucoside powder exhibitted an effective antioxidant potential against DPPH [20].

## Conclusion

According to the current study, the incorporation of steviol glycoside powder with its stable antioxidant capacity, both in neutral and acidic environments, presents a significant advantage for the light products industry, notably in the case of fruit juices. This stabi-

lity enhances the potential for developing healthier and more appealing product formulations while meeting the growing demand for light and low-calorie options in the market.

## Conflict of interests

The authors have declared no conflict of interests for this article.

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