

## Effect of stevia extracts on total phenolics and antioxidant capacity of kale juice spheres

## Efecto de extractos de stevia en el contenido en polifenoles y capacidad antioxidante total de esferas de zumo de kale

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### **Abstract**

**Kale is a vegetable with high contents of health-promoting compounds, but its consumption as a beverage is highly limited by its bitter flavour. So, the effects of different stevia extracts (S10, S25 and S50) on the total phenolic contents (TPC) and total antioxidant capacity (TAC) by DPPH and FRAP of kale juice spheres was studied during 7 days at 5 °C. Spheres were produced with a double spherification technique which allowed to obtain hydrogel spheres with high mechanical resistance. The TPC of S25 samples showed the lowest value at day 0, with differences between other samples. The higher antioxidant capacity was observed in samples evaluated by FRAP, without differences between samples. After 3 days of storage, a decrease in TAC evaluated in both assays studied (DPPH and FRAP) was observed. These results determined that kale juice spheres with different stevia extracts and with high antioxidants levels can be produced.**

**Keywords:** *Brassica oleracea* var. *sabellica* L.; ready-to-eat; spherification, antioxidant capacity.

### **Resumen**

**Kale es una verdura con un alto contenido en compuestos beneficiosos para la salud, pero su consumo como bebida está muy limitado por su sabor amargo. Se estudió el efecto de diferentes extractos de stevia (S10, S25 y S50) sobre el contenido de compuestos fenólicos totales (TPC) y capacidad antioxidante total (TAC) mediante DPPH y FRAP de esferas de zumo de kale durante 7 días a 5 °C. Las esferas se produjeron con una técnica de doble esferificación que permitió obtener esferas de hidrogel con alta resistencia mecánica. El TPC de las muestras S25 mostró el valor más bajo en el día 0, con diferencias en relación a las otras muestras. La mayor TAC se observó en las muestras evaluadas por FRAP. Tras 3 días de almacenamiento, se observó una disminución del TAC (por DPPH y FRAP). Estos resultados determinaron que se pueden producir esferas de zumo de kale con diferentes extractos de stevia y con altos niveles de antioxidantes.**

**Palabras clave:** *Brassica oleracea* var. *sabellica* L.; listos para consumir, esferificación, capacidad antioxidante.

## 1. INTRODUCTION

Consumers are showing new interest in functional and healthy food and increasing the demand for ready-to-eat products due to their lifestyle. Kale (*Brassica oleracea* var. *sabellica* L.) is a vegetable which has attracted special attention from consumers in the last years due to its exhibits a high nutritional value since it is a rich source of vitamin C and phenolic compounds, which along with carotenoids are responsible for the high antioxidant activity of the raw material [1]. Bitterness of Brassica vegetables, due to the sulphur-containing compounds, can be masked by sweetening [2]. Stevia (*Stevia rebaudiana bertonii*) is a plant widely known to its sweetener properties with very low caloric content [3]. Furthermore, stevia contains several compounds responsible of their antimicrobial and antioxidant properties, among other health-promoting properties [4].

Kale juice consumption may be even enhanced, together with sweetening, by innovative presentations such as spheres. In that sense, a double gelification technique for vegetable juices has been recently modelled and optimized with high mechanical resistance [5]. The aim of the present study was to assess the effect of stevia addition on the TPC and TAC (evaluated by two different assays (DPPH and FRAP)) of kale juice spheres during 7 days at 5 °C.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

Kale (*Brassica oleracea* var. *sabellica* L.) was obtained from a local producer (Sacoje S.L., Lorca, Spain). Stevia (*Stevia rebaudiana bertonii*) leaves (origin from Granada, Spain) were purchased from a local store (Cartagena, Spain) and the stevia extract was obtained according to Ortiz-Viedma et al. [4].

### 2.2 Kale juice spherification and storage conditions

Spherification of kale juice was conducted according to an optimized spherification method with double gelation from Tsai et al. [5] with slight modifications. Kale juice spheres were stored in 50 mL plastic bottles (approximately 10 spheres per bottle) containing 40 mL of distilled water with 0.5 % NaCl. NaCl concentration was selected as having the same electrical conductivity (conductivity meter GLP32, Crison, Barcelona, Spain) than the obtained kale juice. Bottles containing the kale juice spheres were stored at 5 °C in darkness and analyzed at day 0, 3, 5 and 7 of storage. Three duplicates per treatment and sampling day were evaluated.

### 2.3 Total phenolic contents and Total Antioxidant Capacity

Total phenolic contents (TPC) and the antioxidant capacity (TAC) by DPPH and FRAP assays were evaluated according [6, 7, 8]. The TAC was expressed as Trolox equivalent antioxidant capacity (TEAC) per kg<sup>-1</sup> fw and the TPC was expressed as gallic acid equivalent per kg<sup>-1</sup> fw.

### 2.4 Statistical analysis

An analysis of variance (ANOVA) was performed to compare different treatments and storage times at a significant level of  $P \leq 0.05$  using PASW Statistics 22 for Windows (SPSS Inc., Chicago, IL, USA). In some cases, when significant differences were observed, the Tukey's HSD (Honestly Significant Difference) test was applied.

## 3. RESULTS AND DISCUSSION

The initial total phenolic content (TPC) of kale spheres were 791.4±40.8, 807.1±9.3, 582.7±4.8, and 900.2±35.4 to CTRL, S10, S25 and S50, respectively (Fig. 1). S25 samples showed

the lowest value, with significant difference between other samples. Yu et al. [9] observed TPC values of kale extracts between 1.59 to 2.33 mg of gallic acid per g fresh kale. This difference might be explained by the different kale cultivars and extraction methods. After 3 days of storage, the TPC of all samples decrease around 55-65% (Fig 1.)

On the other hand, the antioxidant capacity (TAC) of kale spheres evaluated by DPPH and FRAP assays is presented in the Figure 2. TAC evaluated by DPPH assay at day 0, in kale spheres samples, showed values around 1194.9±99.4, 1304.7±11.7, 1028.9±7.9 and 1327.9±56.4 mg TAEC kg<sup>-1</sup> fw to CTRL, S10, S25, S50 treatments, respectively. The S10 and S50 samples showed significant differences in relation to S25 samples (Fig. 2). In relation to TAC evaluated by FRAP assay, a higher antioxidant capacity was observed regarding DPPH values, with values of 2145.9±211.3, 2203.4±212.6, 1994.9±136.6 and 2453.5±107.4 to CTRL, S10, S25 and S50, respectively, at day 0, and was not observed significant differences between treatments (Fig. 2).

After 3 days of storage, a decrease in the TAC by DPPH and FRAP assays was observed in all samples, which probably was due to active enzymes involved in the degradation of antioxidant compounds or because of softening or disruption of plant cell walls and the destruction of complex phenolics [10], since the values of TPC also decreased after 3 days of storage. Furthermore, kale leaves have high levels of vitamin C, which is a vitamin with high antioxidant potential, then, the previous finding may be owed to a vitamin C degradation, since vitamin C is degraded during food storage. Accordingly, higher vitamin C degradation rates have been reported in untreated green vegetables smoothies compared to heat treated samples [11].

#### 4. CONCLUSIONS

It has been found that kale spheres with high levels of antioxidants can be produced by an innovative double spherification technique.

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#### 6. REFERENCES

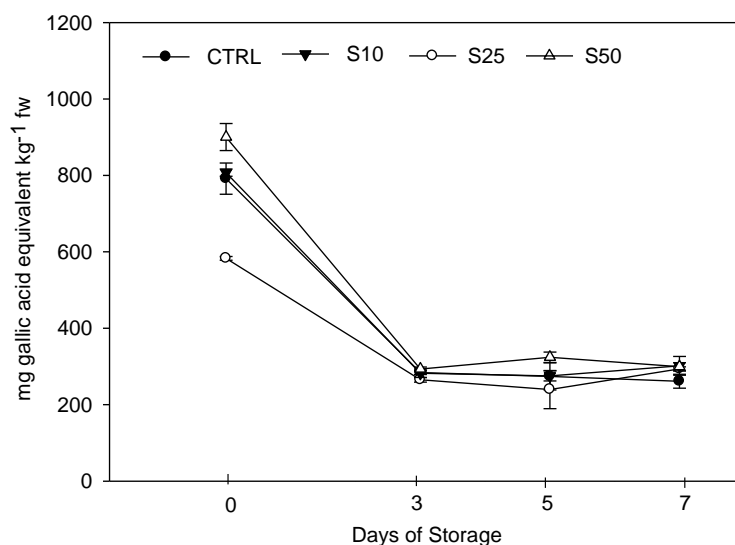
- [1] Sun B., Yan H., Zhang F., Wang Q. 2012. Effects of plant hormones on main health-promoting compounds and antioxidant capacity of Chinese kale. *Food Res. Int.* 48: 359–366.
- [2] Sindhu S., Maya P., Indira T.N. 2012. A method for preparation of mustard (*Brassica juncea*) powder with retained pungency and reduced bitterness. *LWT - Food Sci Tech.* 49: 42–47.
- [3] Megeji N.W., Kumar J.K., Singh V., Kaul V.K., Ahuja P.S. 2005. Introducing Stevia rebaudiana, a natural zero-calorie sweetener. *Current Science*, 88(5): 801-804.
- [4] Ortiz-Viedma J., Romero N., Puente L., Burgos K., Toro M., Ramírez L., Rodríguez A., Barros-Velázquez J., Aubourg S.P. 2017. Antioxidant and antimicrobial effects of stevia (*Stevia rebaudiana Bert.*) extracts during preservation of refrigerated salmon paste. *Eur. J. Lipid Sci. Technol.* 119: 1-9.
- [5] Tsai F.H., Kitamura Y., Kokawa M. 2017. Liquid-core alginate hydrogel beads loaded with functional compounds of radish by-products by reverse spherification: Optimization by response surface methodology. *Int. J. Bio. Macromol.* 96: 600-610.
- [6] Singleton, V. L., Rossi, J. A. (1965). Colourimetry of total phenolics with phosphomolibdic-phosphotungstic acid reagents. *Am. J. Enol. Vit.* 16: 144–158.
- [7] Brand-Williams W., Cuvelier M.E., Berset C. 1995. Use of free radical method to evaluate antioxidant activity. *LWT - Food Sci Tech.* 28: 25–30.

[8] Benzie I.F., Strain J.J. 1999. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Method Enzymol.* 299:15–27.

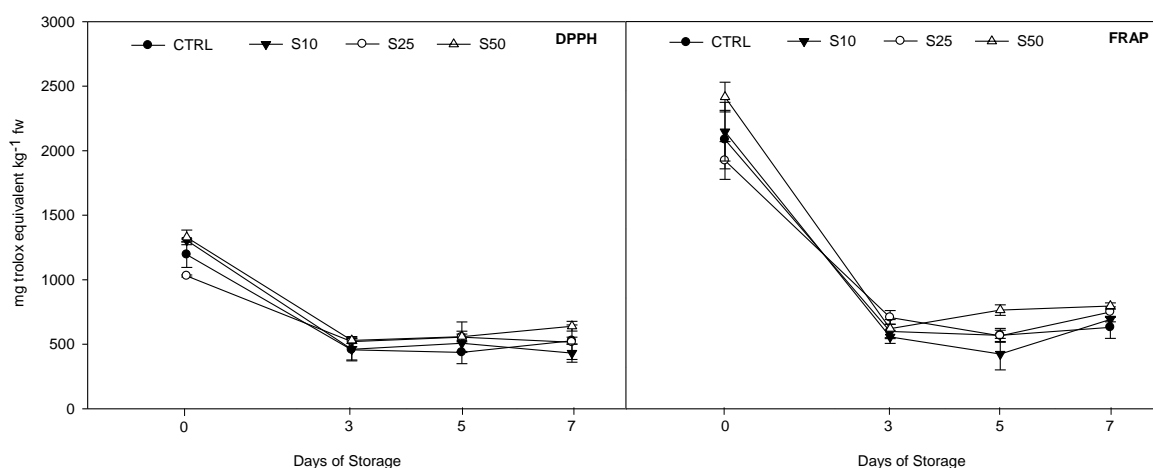
[9] Yu L., Gao B., Wang T.T.Y., Luo Y., Wang J., Yu L.L. 2018. Home food preparation techniques impacted the availability of natural antioxidants and bioactivities in kale and broccoli. *Food Funct.* 24:585-593.

[10] Bernhardt S., Schlich E. 2005. Impact of different cooking methods on food quality: retention of lipophilic vitamins in fresh and frozen vegetables. *J. Food Eng.* 17: 327–333.

[11] Castillejo N., Martínez-Hernández G.B., Mónaco K., Gómez P.A., Aguayo E., Artés F., Artés-Hernández F. 2017. Preservation of bioactive compounds of a green vegetable smoothie using short time-high temperature mild thermal treatment. *Food Sci. Technol. Int.* 23: 46-60.



**Figure 1:** Total phenolic compounds of kale juice spheres with stevia during 7 days at 5 °C.



**Figure 2:** Total antioxidant capacity by DPPH and FRAP assay of kale juice spheres with stevia during 7 days at 5 °C.