Whey protein obtained by membrane separation processes fortified with microencapsulated antioxidant fraction from *Stevia rebaudiana*

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Abstract

The aim of this study was to develop a whey protein concentrate obtained by membrane separation processes and fortify it with a microencapsulated fraction of *Stevia rebaudiana*, rich in antioxidant compounds, and thus evaluate its antioxidant and antidiabetic potential in vitro. The Stevia UEM-13 leaves, the fraction in ethyl acetate, the microencapsulated fraction (maltodextrin 1:4 (w/w)), the membrane concentrate, and a commercial concentrate were submitted to physicochemical analyses for their characterization (proteins, lipids, ash, moisture, fiber, carbohydrates, glycosides, lactose, phenolic compounds, flavonoids, and antioxidant activity; also solubility, microencapsulation efficiency, and stability). In addition, the fractions, concentrates and also two formulations of microencapsulated fraction added to the membrane concentrate were tested for their potential to inhibit the enzyme α-amylase (in vitro antidiabetic activity). The microencapsulated fraction and the formulation containing 20g/100g of membrane concentrate showed inhibitory potential for the enzyme, not significantly differing from the official inhibitor (acarbose). Thus, this work developed a *Stevia rebaudiana* fraction, microencapsulated it, obtained a protein concentrate by membrane separation process, characterized them, and also showed that the microencapsulated fraction increases the solubility and keeps the compounds of interest more stable for twice the time. Still, it showed that this fraction presents great potential to fortify the antioxidant and antidiabetic activity of products such as Whey protein.

Introduction

This study aimed to obtain and characterize the Stevia fraction with greater solubility, sensory profile and stability (microencapsulation with maltodextrin); the development of a whey protein concentrate by membrane separation process and also the production of the supplement with the addition of the microencapsulated fraction to this protein concentrate, evaluating its potential inhibitor of the α-amylase enzyme, and thus its antidiabetic potential.

Materials and methods

The whey was supplied by Vidativa dairy, in the city of Terra Boa - PR. The obtaining of whey protein concentrate by membrane separation processes (CM) and the ethyl acetate fraction (FAE), from the leaves of the Stevia UEM-13 variety, were performed according to MILANI et al., (2017). The FAE was microencapsulated (FAEM) in a 1:4 ratio with maltodextrin according to the methodology of ZORZENON et al., (2019). Physicochemical analyses were performed on FAE, FAEM, CM and on a commercial whey protein concentrate (CC).

Two formulations were made to fortify the protein supplement with FAEM, the first with 0.016g of the fraction in 1g of CM (F1) and the second in 20g/100g (F2). The products and the two formulations were also evaluated for the ability to inhibit the enzyme α-amylase, for antidiabetic activity in vitro.

Results

Table 1 shows the content of glycoside compounds, phenolic compounds, flavonoids, and the in vitro antioxidant capacity of the EAF. The EAF showed an important concentration of antioxidant compounds (higher than 50%), which was reflected in the antioxidant activity analyses.

Table 1. Evaluation of glycosides concentration, phenolic compounds, flavonoids and antioxidant activity of the Ethyl Acetate Fraction (FAE).

Analysis	FAE Concentration g/100g
Glycosides	0.5 ± 0.5
Phenolic compounds (gEAG/100g)	40.07 ± 0.04
Flavonoids (gEQ/100g)	12.18 ± 0.01

ANTIOXIDANT ACTIVITY

Antioxidant activity gET/100gFAE	gEAG/100gFAE	% Inibição/mgFAE
(Method)		
DPPH	82,7 ± 0,01	82.3± 0,01	97.32
ABTS	-	81.0 ± 0,01	81.59

ET = TROLOX Equivalents; EAG = Gallic Acid Equivalents; EQ = Quercetin Equivalents; FAE = Ethyl Acetate Fraction

The microencapsulation process obtained a mass of 2.897g of FAEM, with an efficiency of 83.5%. These data are also in agreement with the study of Zorzenon et al., 2019 who obtained 84% efficiency. Regarding solubility, microencapsulation increased it by 75%. Table 2 shows the stability test results of MEF compared to EAF after 30, 45, and 60 days.

Table 2. Stability of phenolic content and antioxidant capacity over a period of 60 days.

Days	FAE (g/100g)	FAEM (g/100g)
0-30	40.07±0,004^a	12.39±0,06^a
45	30.20±0,0001^b	13.73±0,0024^a
60	16.93±0,004^c	8.07±0,002^b
Antioxidant activity (DPPH)		
	FAE (% inhibition/mg)	**FAEM (% inhibition /mg)**
0-30	82.7±0,01^d	7.41±0,2^f
60	30.37 ±0,002^e	10.12 ±0.004^f

Data express the mean ± value standard error of the mean (epm). Significant differences between groups were analyzed by ANOVA, analysis of variance. Different letters in the same column show significant difference at 5% probability level by Tukey's test (p <0.05).

The whey provided by Vidativa dairy presented 7.0°BRIX, pH 6.5 and a fat content of 0.25%. A volume of 4.3L of whey was used to obtain WM in an ultrafiltration (UF) system. The UF was started using a 10-kD polystersulfone membrane (cut-off), 50cm^2 area, from Koch. During the process of obtaining concentrate, qualitative tests were performed for protein and lactose in the concentrate and in the permeate at each diafiltration, and was terminated when the ºBRIX of the permeate was equal to 0 and the apparent lactose content was not identified by the qualitative method, and the protein content was higher in the concentrate. Table 3 shows this parameters.

Table 3. DF parameters and qualitative analyses of soluble proteins and lactose in the permeate and concentrate (retained)

DF	Membrane entry pressure (psi)	Brix of permeate output (°)	Permeate flow rate (mL/min)	Concentrate Brix (°)	Temp. (°C)
6th DF	3-4	0	18	4,0	23

Análises de proteínas solúveis e lactose

	Permeate Lactose	Concentrate Lactose	Permeate Protein	Concentrate Protein
4th DF	-	-	-	+

Table 4 shows the quantitative results for lactose and protein in the membrane concentrate obtained by ultrafiltration, six diafiltration processes, and after spray drying.

Table 4: Lactose and total protein content of membrane concentrate (CM) and commercial concentrate (CC).

Sample	membrane concentrate protein (g/100g)	commercial concentrate protein (g/100g)
Lactose	2.38 ± 0,10^a	2.8 ± 0.07^b
Total protein	77.8 ± 0,08^a	85.5 ± 0.5^b

Data express the mean ± value standard error of the mean (epm). Significant differences between groups were analyzed by ANOVA, analysis of variance. Different letters in the same row show significant difference at 5% probability level by Tukey's test (p <0.05).

The results presented in figure 1 showed that FAEM and F2 reduced the enzyme activity, acting as inhibitors, by 66.23% and 28.56%, respectively. Thus, further studies should be conducted in order to produce a protein supplement fortified with stevia antioxidant fraction so that it can act as an adjuvant in the treatment of DM.

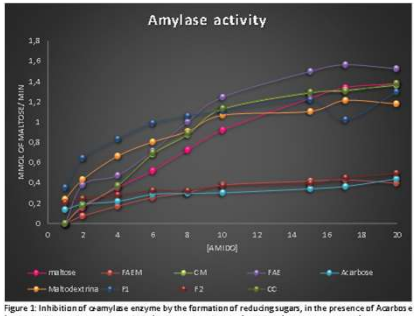

Figure 1: Inhibition of α-amylase enzyme by the formation of reducing sugars, in the presence of Acarbose (main inhibitor of a-amylase, FAE (free acetate fraction), FAEM (microencapsulated), membrane concentrate (CM) and commercial concentrate (CC) and the final product formulations (F1= 16 mg of FAEM/100mg of CM; F2=4 mg of FAEM/20mg of CM). Data express the mean and significant differences between groups were analyzed by ANOVA, analysis of variance and Tukey's test (p <0.05). *FAE differs from Maltodextrin (p<0.05); **F1 differs from Maltose and FAE (p<0.05); #Acarbose, F2 and FAEM differs from the others (p<0.05).

Conclusion

The membrane concentrate (whey protein) obtained in this work without the addition of chemical inputs, solvents or preservatives showed lactose and total protein contents very similar to the commercial concentrate. The microencapsulation of FAE, fraction in ethyl acetate, showed better solubility and stability of the antioxidant compounds contained therein, and showed important antioxidant and antidiabetic activity in vitro, and its incorporation into whey protein, fortified beneficial effects already attributed to these supplements. The protein supplement formulated with 20g FAEM/100g whey protein showed ability to inhibit the action of pancreatic amylase, demonstrating that this concentration may have beneficial effects to act as an adjuvant in the prevention and/or treatment of diabetes.

Recommendations

1 MILANI, P.G.; FORMIGONI, M.; LIMA, Y.C., et al. Fortification of the whey protein isolate antioxidante and antidiabetic activity with fraction rich in phenolic compounds obtained from *Stevia rebaudiana* (Bert.). Bertoni leaves**. J Food Sci Technol,**v.54, n.7, p.2020-2029, 2017.

2 ZORZENON, M.R.T.; HODAS, F.; MILANI, P.G.; FORMIGONI, M.; DACOME, A.S.; MONTEIRO, A.R.G.; MAREZE-COSTA, C.E.; COSTA, S.C. Microencapsulation by Spray-Drying of Stevia Fraction with Antidiabetics Effects. Chemical Engineering Transactions,v.75, p.307-312, 2019.