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# Innovative protein bar - obtaining and evaluating of some physico-chemical and nutritional characteristics

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#### Abstract

The first objective of this paper was to obtain an innovative type of protein bar, sugar free, in two variants: one vegan with soy flour (PBAS) and the second with whey powder (PBAW). The common basis for the two types of protein bars was composed of peanut butter, dried leaf powder of *Stevia rebaudiana*, carob, oat flakes and inactive yeast flakes. A second objective of this paper was to determine the total polyphenol content (Folin-Ciocalteu assay), the antioxidant capacity (CUPRAC method), of the protein bars obtained, compared to the raw materials, and to determine the proximate composition and energy value of the finished products. Among the raw materials, the highest total polyphenol content was found in *Stevia rebaudiana* (37.22±0.94 mg gallic acid/g), followed by carob powder (16.41±0.0.70 mg gallic acid/g), and the lowest concentration of these compounds was found in whey powder (0.20±0.01 mg gallic acid/g). Regarding the antioxidant activity, the highest value was also recorded in the case of Stevia rebaudiana (114.23±1.34 mg Trolox/g). Of the two finished products obtained, the vegan protein bar (PBAS) had the highest content of total polyphenols (9.64±0.33 mg gallic acid/g) and the best antioxidant activity (55.62±0.98 mg Trolox/g). Vegan protein bar (PBAS) had no cholesterol and had a higher caloric intake than whey protein bar (PBAW) which had more protein (25.94 g/100g compared to 23.78 g/100g in. PBAS).

Keywords: protein bar, sugar free, whey, soybean, antioxidant activity, polyphenols.

1. Introduction

Protein bars are widely consumed by athletes, adults- in recreational purpose, and soldiers who generally believe that combining extra protein intake with exercise will promote weight loss, increase of lean muscle mass, and improved physical performance [1-3]. Numerous scientific studies show that protein supplements increase gains in muscle mass and muscle strength in both young and older adults [4-9].

Whey powder is a rich source of high quality protein, rich in essential amino acids (EAA) and branched chain amino acids (BCAA) that are important in tissue growth and regeneration.

EAA and BCAA from whey proteins are present in higher concentrations compared to other proteins such as soy, meat and wheat; they are also absorbed and used effectively. Whey protein also has some important benefits such as: it reduces the symptoms of chronic fatigue and its major use is in HIV and viral diseases because it increases immunity [10]. Whey proteins contain mainly beta-lactoglobulin (β-LG), alpha-lactalbumin (a-LA), bovine serum albumin (BSA), immunoglobulins and protease peptone, as well as some minor proteins, including lactoferrin [11]. Advances in processing technologies have allowed the purification and separation of whey proteins that are sold as concentrate (WPC) or isolate (WPI) containing 35-80% and> 90% protein, respectively [12].

Both WPC and WPI have important antioxidant properties [13].

Soy protein has received increasing attention in recent years from consumers, researchers and the media [14-16]. Several studies have shown that consuming soy protein instead of animal protein reduces the plasma levels of total cholesterol, lowdensity lipoprotein (LDL) and triglycerides. Soy protein has also been found to contribute to weight loss, to control hyperglycaemia, hyperlipidemia and hyperinsulinemia [16-18]. The predominant soy proteins are storage proteins, namely 7S globulin (conglicinin) and 11S globulin (glycine), which make up about 80% of the total protein. Other storage proteins are 2S, 9S and 15S, which are present in much smaller amounts in soy protein [19]. Soybeans as well as soy protein isolates are also known for their antioxidant properties [20].

Stevia rebaudiana is a sweet plant, belonging to the genus Stevia and the family Asteraceae [21]. Stevia is enriched with many nutrients, such as 80 to 85% water, amino acids, proteins, fiber, lipids, essential oils, free sugars, vitamins and organic acids. The plant is considered a good source of calcium, magnesium, potassium, iron, phosphorus, sulfur, sodium. Steviol glycosides are responsible for the sweetening properties of plants. The most representative form of steviol glycosides in the leaves are steviosides followed by rebaudiosides. The rest of the sweet compounds are present in very small quantities. The sweetening components of stevia make up 14% of the dried leaves by weight. Toxicological studies have shown that the secondary metabolites present in stevia are not mutagenic, teratogenic or carcinogenic and no allergic reactions have been observed after consumption as a sweetener. The high nutritional profile of stevia leaves, the richness of phenolic compounds and other antioxidants, makes it superior to other sugar substitutes [22-23].

The purpose of this paper was to make an innovative protein bar, sweetened with *Stevia rebaudiana*, in two variants: one vegan with soy flour and the other with whey powder, as well as to determine the proximate composition, energy value, but also protective qualities by analyzing the content of total polyphenols and antioxidant activity.

#### 2. Materials and Methods

Two variants of protein bars were obtained, using ingredients from the local market, according to the recipes presented in table 1.

**Table 1.** Recipes used to obtain the two varieties of protein bars

Raw and auxiliary materials	PBAS	PBAW
Soy flour (g)	10	-
Carob powder (g)	1	- -
Oat flakes (g)	30	31
Innactive yeast flakes (g)	1	1
Whey powder (g)	-	10
Peanuts butter (g)	50	50
Stevia rebaudiana powder (g)	0.5	0.5

#### 2.1.Protein bars obtaining method

The oat flakes was crushed with the help of a food processor, after which the necessary quantities of each raw and auxiliary material needed for the two recipes were weighed. The components for each variant of protein bar were mixed and homogenized well in a kitchen bowl, then the bars were formed and packaged. From each type of bar, as well as from the raw materials, were taken samples to determine the total polyphenol content and antioxidant activity.

### 2.2.Determination of total polyphenol content and antioxidant activity

In order to determine the content of total polyphenols as well as the antioxidant activity for the raw materials and the finished products obtained, the methods Folon Ciocalteu and CUPRAC were used as described by Dumbrava *et al.* (2016) [24]. All analyzes were performed in triplicate, and the results were expressed as a mean and standard deviation (mean  $\pm$  SD).

## 2.3. Determining of the proximate composition and energy value

The proximate composition and energy value of the two assortments of protein bars obtained were determined by calculation, using "USDA Food Compozition Databases" [25].

#### 3. Results and discussion

#### 3.1. Total polyphenol content analysis

The total polyphenolic compounds analysis (by the Folin-Ciocalteu method) from the two finished products obtained and from the raw materials, led to the results presented in table 2.

**Table 2.** Total polyphenols content in the finished products and in the raw materials

Sample	Total polyphenols content (mg gallic acid/g)
PBAS	9.64±0.33
PBAW	8.14±0.23
Oat flakes	2.83±0.08
Soy flour	0.85±0.03
Whey powder	0.20±0.01
Carob powder	16.41±0.70
Peanut butter	0.31±0.02
Stevia rebaudiana dry leaf powder	37.22±0.94
Inactive yeast flakes	0.41±0.05

From the experimental data on the content of total polyphenols in the raw materials and protein bars obtained, it can be observed that, of the raw materials used, *Stevia rebaudiana* powder was the richest in these compounds (37.22±0.94 mg gallic acid/g), followed of carob powder (16.41±0.70 mg gallic acid/g) and oat flakes (2.83±0.08 mg gallic acid/g). All other raw materials used had a content of total polyphenols below 1 g gallic acid/g.

Khiraoui et al., (2018) [26] determining the total polyphenols content in the dried leaves of Stevia rebaudiana, found values between 37.13-67.85 mg gallic acid/g, Shulca et al., (2012) [27] found values of 56.74 mg gallic acid/g and Myint et al., (2020) [28] values of 77.89 mg GAE/g DW - so that the values we found are at the lower end of the range in the studied literature data. For carob powder, have been found some studies on the content of total polyphenols in extracts obtained with other types of solvents: Benchikh and Louailèche, (2014) [29] for hexane extracts, found values of total polyphenols between  $11.31 \pm 0.49$  and  $9.70 \pm 0.46$  mg GAE/g, and Petkova et al., (2017) [30] for aqueous extract found values of  $8.11 \pm 1.15$  mg GAE/g dw. The results of this paper for ethanolic extract of carob indicate higher values of total polyphenols.

Regarding the two protein bars obtained, it was found that the PBAS protein bar had a slightly higher value of the total polyphenol content  $(9.64\pm0.33 \text{ mg gallic acid/g})$  than that of the PBAW protein bar  $(8.14\pm0.23 \text{ mg gallic acid/g})$ .

#### 3.2. Antioxidant activity analysis

Table 3 shows the values obtained for the antioxidant activity of raw materials and finished products samples (CUPRAC assay).

Among the raw materials, it was found that, Stevia rebaudiana had the strongest antioxidant activity (114.23±1.34 mg Trolox/g), followed by carob powder (79.94±1.42 mg Trolox/g), soy flour (77.84±1.14 mg Trolox/g) and oat flakes  $(60.37\pm1.08 \text{ mg Trolox/g})$ ; peanut butter had the activity antioxidant  $(7.04\pm0.02)$ Trolox/g). Bender et al., (2015) [31] determined for Stevia rebaudiana leaves from different sources, values of antioxidant activity of 69.5 to 267.78 mg Trolox/g, the data obtained by us being included in this range. Ashan et al., (2019) [32] reported lower values of antioxidant activity for carob powder: between  $20.78 \pm 1.45$  and  $29.56 \pm 2.06$  mg TE/g. Brindzova et al., (2008) [33] found for oat flakes much lower values of antioxidant activity using the free radical DPPH:  $3,437 \pm 0.031$  to  $17,802 \pm 0.022$ mg TE/g.

The two protein bars obtained had close values of antioxidant activity, the best value being found in the case of PBAS protein bar (55.62±0.98 mg Trolox/g).

**Table 3.** Antioxidant activity of protein bars samples and of raw materials

Antioxidant activity (mg Trolox/g)
55.62±0.98
53.76±0.86
60.37±1.08
77.84±1.14
22.61±0.44
79.94±1.42
7.04±0.02
114.23±1.34
23.12±0.48

## 3.3.Proximate composition and energy values analysis

The proximate composition and the energy value determined by calculation for the two finished products are presented in figures 1-3.

It can be seen that the PBAS bar (with soy flour and carob powder) had a higher energy value (427.14 kcal/100g) than the PBAW bar (417.43 kcal/100g). Also, the PBAS bar is slightly richer in carbohydrates (35.90 g/100g) and total fat (21.54 g/100g) than the PBAW bar (33.19 g/100g, respectively 20.32 g/100g).

Proteins were found in higher amounts in PBAW (25.94 g/100g) than in PBAS (23.78 g.100g).

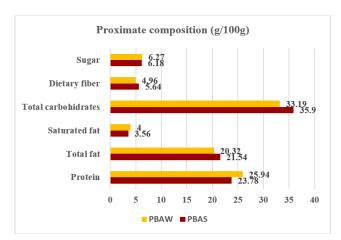


Figure 1. Protein bars proximate composition

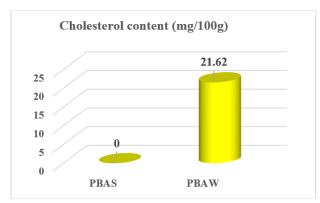


Figure 2. Cholesterol content in protein bars

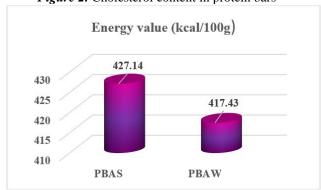


Figure 3. Energy value of protein bars

#### 4. Conclusions

• Two innovative variants of protein bars sweetened with *Stevia rebaudiana* leaf powder were obtained: one vegan with soy flour, peanut butter, oat flakes, carob powder (PBAS) and the other with whey powder, oat flakes and peanut butter (PBAW).

- Analysis of the total polyphenol content for raw materials and finished products showed that Stevia rebaudiana had the highest content, followed by carob powder, and of the two finished products, PBAS had a higher content than PBAW.
- Regarding the antioxidant activity, *Stevia rebaudiana* also had the highest value among the raw materials, followed by carob powder and soy flour; of the finished products, the vegan protein bar (PBAS) had a higher antioxidant activity than whey protein bar (PBAW).
- The two obtained protein bars had a quite similar proximate composition, except that the PBAS bar is free of cholesterol and has more carbohydrates and total fat than PBAW which has a higher protein content. Both finished products have a good protein and dietary fiber content as well as a low amount of sugar.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

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