

Revista Española de Nutrición Humana y Dietética

Spanish Journal of Human Nutrition and Dietetics

RESEARCH - *post-print* version

This is the peer-reviewed version accepted for publication. The article may receive changes in style and format

Development of functional dietetic snack using black carob flour (*Prosopis nigra*) and discarded blueberries (*Vaccinium corymbosum* L.)

Desarrollo de un snack dietético funcional utilizando harina de algarroba negra (*Prosopis nigra*) y arándanos de descarte (*Vaccinium corymbosum* L.)

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Assigned Editor: Édgar Pérez Esteve, Universitat Politècnica de València, España.

Received: 23/05/2022; accepted: 06/06/2022; published: 01/07/2022

CITA: Miranda VN, Gonçalvez de Oliveira E, Olivares La Madrid AP, Lotufo Haddad AM, Ramón AN, Villalva FJ. Development of functional dietetic snack using black carob flour (*Prosopis nigra*) and discarded blueberries (*Vaccinium corymbosum* L.). Rev Esp Nutr Hum Diet. 2022; 26(3):e1686 doi: 10.14306/renhyd.26.3.1686

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ABSTRACT

Introduction: Snacks have become an important part of the daily diet, however, their nutritional profile could be improved by adding ingredients with health benefits. The aim of this work was to develop a functional dietetic snack using black carob flour and discarded blueberries, and to evaluate its chemical and functional composition.

Methods: Three formulations were prepared using different proportions of wheat flour and black carob flour: 60:40; 50:50; and 40:60, respectively. Proximal chemical composition was determined by the procedures of AOAC methods and functional properties were also analyzed.

Results: Three dietary snacks were obtained, which showed a caloric reduction of 20 - 23%. In all snacks, an increase in protein content of more than 60% was achieved and a higher total dietary fiber content (DS2) was observed compared to commercial snacks. All formulations showed a reduction in bioactive compound content during processing.

Conclusions: The snacks showed functional properties, higher protein content and a reduced total caloric value compared to commercially available products. DS2 was the snack with the highest antioxidant activity and, according to fiber values, is a product with "high fiber content". Despite the losses caused by the cooking process in the content of bioactive compounds, the antioxidant activity was greater than 50% in all the snacks produced.

Keywords: dietetic snack, black carob flour, discarded blueberries, bioactive compounds.

RESUMEN

Introducción: Los snacks se han convertido en una parte importante de la dieta diaria, sin embargo, su perfil nutricional podría mejorarse añadiendo ingredientes con beneficios para la salud. El objetivo de este trabajo fue desarrollar un snack dietético funcional utilizando harina de algarroba negra y arándanos de descarte y evaluar su composición química y funcional.

Metodología: Se prepararon tres formulaciones utilizando diferentes proporciones de harina de trigo y harina de algarroba negra: 60:40; 50:50; y 40:60, respectivamente. Se determinó la composición química proximal mediante los procedimientos metodológicos de la AOAC, así como también se analizaron las propiedades funcionales.

Resultados: Se obtuvieron tres snacks dietéticos, éstos mostraron una reducción calórica del 20 - 23%. En todos los snacks se logró un aumento del contenido proteico de más del 60% y se observó un contenido mayor de fibra dietética total (DS2) en comparación con los snacks comerciales. Todas las formulaciones mostraron una reducción en el contenido de compuestos bioactivos durante el procesamiento.

Conclusiones: Los snacks mostraron propiedades funcionales, mayor contenido en proteínas y un valor calórico total fue reducido en comparación con los productos comerciales disponibles. El DS2 fue el snack con mayor actividad antioxidante y, según los valores de fibra, es un producto con "alto contenido en fibra". A pesar de las pérdidas causadas por el proceso de cocción en el contenido de compuestos bioactivos, la actividad antioxidante fue superior al 50% en todos los snacks elaborados.

Palabras claves: snack dietético, harina de algarroba negra, arándanos de descarte, compuestos bioactivos.

KEY MESSAGES

- The use of discarded blueberries as a potential functional ingredient was revalued.
- A serving of SD2 covers 16% of the recommended daily intake of dietary fiber.
- The formulated snacks cover approximately 30-50% of the estimated daily intake of polyphenols, based on a healthy diet.

INTRODUCTION

Eating patterns have undergone worldwide changes throughout the 20th century, characterized by a sedentary lifestyle and diets with high fat, sugar, and sodium contents, and low in fiber. This modification has been associated to the emergence of chronic, non-communicable diseases such as type II diabetes, obesity, heart conditions, cancer, and respiratory illnesses, representing a strong impact in population morbidity and their quality of life.¹⁻³

Nutritional science studies on the issue have been increasingly associating feeding as a modulator in the processes related with degenerative diseases, aging, oxidative stress (which generates reactive oxygen species that also lead to damage and cellular death).^{1,4} In this context, nutritional science and the food industry play a key role in the development of healthy and functional foods with the purpose of preventing illnesses.^{5,6}

The growth of the functional food market is currently on the rise since consumers nowadays look for not only safe or nutritious products, but they also demand natural, organic, or healthy food made from natural, innovative and non-traditional ingredients.^{7,8}

Fruit and vegetable demand has increased considerably, this also entails an increase in their losses and waste because of inadequate handling methods and infrastructure. However, those discarded products are a rich source of bioactive compounds like natural pigments, phenolic compounds, fiber, minerals, and other components with health benefits.⁹

In this context, Salta province in northwestern Argentina, produces export-only blueberries (*Vaccinium corymbosum* L.). Only a low proportion is industrially used in the production of juices and derivatives, in which around 20-30% of solid waste is produced. Therefore, the agricultural wastes from blueberry juice processing and discarded export fruit could be incorporated into products using entire fruit, minimizing waste generation, and contributing to the development of new foods.¹⁰ Blueberries are rich in phenolic compounds, such as anthocyanins, which can play an active role in the body against reactive oxygen species. This is attributed to their potential antioxidant properties.⁹

Currently, different types of legume flours have been incorporated into bakery products not only for their nutritional properties but also for the sensory characteristics they contribute to the final product. *Prosopis nigra* flour would be an interesting ingredient to give an added value to bakery

products, since it stands out for its dietary fiber and phenolic compound contents, as well as its purple color.¹¹

Snacks have become an important part of the daily diet in Western cultures. However, people generally opt for unhealthy products high in calories, fats, sugars, and sodium.¹³ Based on this, the nutritional profile of snacks could be improved with the addition of healthy ingredients such as blueberries and alternative flours like *Prosopis nigra*, thus granting an added value to them.

Considering that, the food industry seeks to incorporate food waste into food matrices, to grant their functional properties, reduce agro-industrial losses and offer consumers healthy products based on non-traditional ingredients. The aim of this work was to develop a functional dietetic snack using black carob flour and discarded blueberries, and to evaluate its chemical and functional composition.

MATERIALS AND METHODS

Raw Materials

Dietetics snacks (DS) were prepared with the following ingredients: black carob flour (*Prosopis nigra* Grisebach Hieronymus) obtained in Sabores Andinos S.A., Buenos Aires, Argentina; wheat flour; fresh egg white; high oleic sunflower oil; chocolate essence and flavoring from a local Salta, Argentina market. The filling was made with discarded for-export Emerald blueberries (*Vaccinium corymbosum* L.). Sucralose was provided by Saporiti S. A., potassium sorbate and low methoxy pectin obtained from Gelfix S.A. and calcium lactate was obtained from Todo Droga, Córdoba, Argentina.

All the analytical reagents used for chemical determinations were obtained in MERCK S.A and were analytical grade.

Snack formulation

DS were performed following a traditional recipe. Three formulations were prepared using different proportions of wheat flour (WF) and black carob flour (BCF): 60:40; 50:50; and 40:60, respectively. Ingredients and proportions used are shown in **Table 1**.

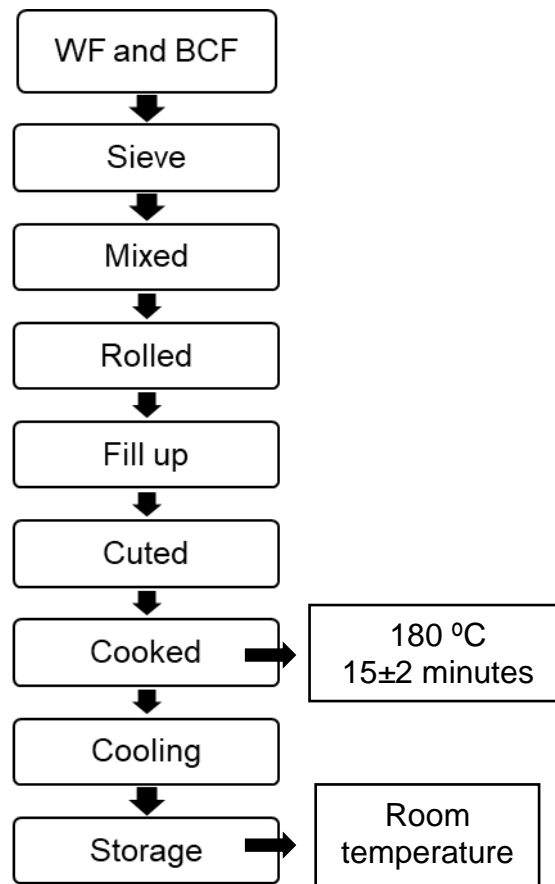
Table 1. Dough ingredients and proportions

Ingredients (g/100g)	DS1 60:40	DS2 50:50	DS3 40:60
WF/BCF	29.3	24.2	19.5
WF	19.5	24.2	29.3
Egg white	24.4	24.4	24.4
Sunflower oil	26.8	26.8	26.8
Sucralose	0.015	0.015	0.015
Potassium sorbate	0.05	0.05	0.05

WF:wheat flour, BCF: Black carob flour.

The snacks' filling was prepared using discarded Emerald blueberries, sucralose (0,01%), calcium lactate (0,055%), potassium sorbate (0,05%) and low methoxy pectin (1%). Blueberries (BB) were previously washed by immersion and crushed in a blender for thirty seconds, sweetener was then added and mixed with a spoon. The mixture was cooked during 10 min with constant agitation. The blueberry filling was allowed to cool for 1h at room temperature. The complete process of the snack's development is indicated in the **Flowchart 1** below

Flowchart 1. Dietetic snack production process



Chemical composition analysis

Proximal chemical composition (moisture, proteins, lipids, carbohydrates, ashes, and sodium) was determined by the procedures of AOAC methods. All analyses were performed in triplicate.¹²

The total caloric value (TCV) was determined considering all the ingredients used in the formulation. Nutritional information was calculated using 4, 9 and 4 calories per gram of protein, fat, and carbohydrate, respectively.

For the comparison of the proximate composition, a commercial product with similar characteristics was taken as a reference, whose chemical composition per 100 g is detailed below: caloric value 476 kcal, carbohydrates 77 g, proteins 8 g, total fats 15 g, dietary fiber 5 g, sodium 100 mg.

Functional compound determination

We studied total polyphenols, anthocyanins, proanthocyanidins and antioxidant activity. All bioactive compounds were analyzed in the BCF, the BB and DS to evaluate changes produced by the processes applied (crushing, kneading, cooking). Dietary fiber was quantified in the product with the highest antioxidant activity.

The fractions of bioactive compounds were quantified using colorimetric methods measured in extracts prepared according to Sciammaro (2015) with some modifications.¹³ A solid-liquid extraction was performed using 50% diluted acetone in a 1:2 ratio (sample/solvent). The sample was stirred at 800 rpm for 40±1 minutes, filtered with Whatman paper N° 4 and kept in refrigerator temperature (2-8 °C) in amber bottles until used.

Total polyphenols (TPF) were identified according to Singleton and Rossi's (1965) technique with some modifications.¹⁴ Extracts (50µl) were oxidized with Folin-Ciocalteu reagent (100µl) and mixed with distilled water (2350µl). Finally, the neutralization was made with sodium carbonate concentrate to 20% (50µl). The absorbance was measured at 765 nm. Results were expressed on calibration curve as milligram of gallic acid equivalents per 100g (mg GAE/100 g).

Total anthocyanins (TA) were determined using pH-differential method Giusti-Wrolstald (2000).¹⁵ An aliquot was diluted (1:2 v/v) with two buffers, pH 1.0 (0.025 M potassium chloride) and pH 4.5 (0.4 M sodium acetate). After 15 minutes of incubation at room temperature, the absorbance was measured at 510 and 700 nm. TA was expressed as Cyanidin-3-glucoside/100g according to the following equations:

Equation 1:

$$\text{Absorbance (A)} = (A_{510\text{nm}} - A_{700\text{nm}})_{\text{pH}1.0} - (A_{510\text{nm}} - A_{700\text{nm}})_{\text{pH}4.5}$$

Equation 2:

$$\text{Total Anthocyanins (mg/100g)} = \frac{A \times \text{Mw} \times \text{DF}}{(\epsilon \times 1)} \times 100$$

A is $(A_{510\text{ nm}} - A_{700\text{ nm}})_{\text{pH}1.0} - (A_{510\text{ nm}} - A_{700\text{ nm}})_{\text{pH}4.5}$; Mw is the molecular weight (449.20 g/mol); DF is the dilution factor; ϵ is the molar extinction coefficient (26,900 L/M/cm for cyanidin-3-glucoside); and l is the pathlength (1cm).

Proanthocyanidins (PAT) were quantified by vanillin-HCl method described by Price et al., (1978).¹⁶ First, 20 μ L of extract were reacted with 180 μ L of methanol, secondly 1.2 mL of vanillin (at 4% w/v in methanol) was incorporated and stirred. Finally, 600 μ L of concentrated HCl was added and protected from light for 30 minutes. Samples were measured at 500 nm and values obtained were calculated in catechin calibration curve. PAT were expressed in milligram catechin equivalent per 100g (mg CE /100g).

Antioxidant activity (AA) Free radical scavenging ability of black carob flour was measured using radical cation ABTS method described by Re et al., (1999).¹⁷ Radical cation was prepared by incubating the ABTS solution with a 2.45 mM potassium persulfate for 16 h in darkness at room temperature. Subsequently was diluted with methanol to a final absorbance of 0.7 at 734 nm. For AA determination, 30 μ L of sample were added to a cuvette containing 3 mL of the ABTS solution. The absorbance was measured at 734nm, and results were expressed as μ mol Trolox equivalent per 100g (μ mol TE/100g) using Trolox curve as standard.

Total dietary fiber (TDF), soluble dietary fiber (FDS) and insoluble dietary fiber (FDI), were determined by the gravimetric enzymatic technique.²⁰

Statistical analysis

An analysis of variance (one-way ANOVA) was performed to evaluate any significant differences between formulations in their chemical composition and possible variation in bioactive compounds. Multiple comparisons were also evaluated by Tukey's post-hoc test using the software INFOSTAT student Version 2018.

RESULTS

Chemical composition

The proximal composition of DS is presented in **Table 2**.

Table 2: Proximal composition of DS (100g)

	DS1	DS2	DS3
TCV ¹ (Kcal)	383 (SD 2 ^a)	395 (SD 3 ^a)	367 (SD 2 ^a)
Cho ² (g)	45.36 (SD 1.17 ^a)	47.91 (SD 0.99 ^a)	48.48 (SD 2.40 ^a)
Prot ³ (g)	13.63 (SD 0.28 ^a)	13.05 (SD 0.73 ^a)	13.13 (SD 0.25 ^a)
TF ⁴ (g)	15.89 (SD 0.04 ^{ab})	16.61 (SD 0.59 ^b)	14.87 (SD 0.46 ^a)
MS ⁵ (%)	16.87 (SD 0.40 ^a)	18.56 (SD 0.11 ^b)	22.83 (SD 0.85 ^c)
AS ⁶ (g)	1.98 (SD 0.02 ^a)	1.98 (SD 0.02 ^a)	1.97 (SD 0.02 ^a)
SDM ⁷ (mg)	166.82 (SD1.00 ^a)	161.58 (SD 7.41 ^b)	130.33 (SD 8.96 ^b)

SD mean standard deviation (n=3). ¹ Total caloric value; ² Carbohydrates; ³Proteins; ⁴Total fat; ⁵Moisture; ⁶Ashes; ⁷Sodium. DS=Dietetic Snack. Values with different superscript characters within a row are significantly different ($p < 0.05$).

TCV in snacks showed no significative differences. Calories were lower than commercial snack available (476 Kcal/100g). According to these results, we can confirm that snacks have a reduction of 20%, 17% and 23% of the total caloric value in DS1, DS2 and DS3 respectively.

No significative differences were found in carbohydrate content in the snacks formulated. However, a reduction of 41%, 38% and 37% of this nutrient was achieved in the snacks, respectively.

Protein content showed no significant difference throughout the different DS formulations. Besides, levels were also higher than commercial snacks available (8g/100g), that means an increase of 70.37%, 63.12% and 64.12% of total protein content for SD1, SD2 y SD3 respectively.

Significant differences were found with respect to fat and moisture. No significant differences in ash content were observed.

Functional compounds

Values obtained for bioactive compounds fractions in DS are expressed in **Table 3**.

Table 3. Bioactive compounds in DS (100g)

Sample	TPF (mg GAE)	TA (mg Cyanidin-3- glucoside)	PAT (mg CE)	AA (μ mol TE)
DS1	266.27 (SD 4.54 ^a)	110.90 (SD 34 ^a)	11.77 (SD 54 ^a)	202.55 (SD 2.47 ^a)
DS2	185.04 (SD 1.00 ^b)	97.67 (SD 12 ^b)	9.35 (SD 43 ^b)	245.11 (SD 4.66 ^b)
DS3	150.81 (SD 1.42 ^c)	127.08 (SD 25 ^c)	23.29 (SD 10 ^c)	194.39 (SD 2.47 ^a)

TPF: Total Polyphenols; TA: Total anthocyanins; PA: Proanthocyanidins; AA: Antioxidant activity. SD: standard deviation (n=3). Values with different superscript characters within a row are significantly different ($p < 0.05$).

Significative differences were found in **TPF**. Results were lower than the initial quantity in BCF+BB (900.05 SD57.29 mgEAG/100g). Losses represent the 70%, 79% and 83% of the compounds in DS1, DS2 and DS3 respectively.

TA content was lower in the final products in comparison with BCF+BB at initial quantity (151.54 SD12,54 mg Cyanidin-3 glucoside/100g). Decreasing was 26.83%, 35.54% and 16.14 % for DS1, DS2 and DS3, respectively.

PAT values obtained were lower than the original matrix (236.34 SD16.75 mg CE/100g) presenting losses of 95.02%, 96.04% and 90.14%.

AA in this case showed a reduction compared with BCF+BB (284.63±3.78 µmol TE) approximately 28.83%, 13.88% and 31.70% for DS1, DS2 and DS3 respectively.

As we mentioned before, TDF was determined in the snack with higher antioxidant activity, DS2 was selected for this analysis. Values of TDF, IF and SF are indicated in **Table 4**

Table 4. Fiber fractions content in DS2 (100g)

Total dietary fiber (g)	13.77 (SD 1.59)
Insoluble fiber (g)	10.78 (SD 1.23)
Soluble fiber (g)	2.99 (SD 0.36)

SD: standard deviation

DISCUSSION

The snacks developed had an improved nutritional profile compared to commercially available snacks and added value due to the ingredients used.

Discrepancy in caloric values of each snack could be related to fill nature and dough proportions used in each one.

Values obtained, with respect to carbohydrates, were lower to Macías et al. (2013) who studied cookie formulations with a partial substitution of white carob flour (80:20), reporting 66.67g/100g.¹⁸ It was also lower than Zavala Chingay (2016), who investigated the development of cookies with the replacement of white carob flour in different proportions (4, 8 and 12%)¹⁹ reporting 72.52; 70.42 and 68.30 g/100g, respectively. Also, the values obtained were lower than those compared with the commercial product (77g/100g). Differences found could be attributed to ingredient proportion and flour variety as well as the fact that no white sugar was used in the formulation.

All values of protein were higher than Escobar et al. (2009), who reported values of 10.7 and 13.3mg/100g in the study of cookie development with carob cotyledon flour and wheat flour mixtures (10:90 and 20:80).²⁰ According to results obtained, we can also claim the snack as "high protein food".²¹

Although the amount of fat was like that found in the commercial product, the DS would have a healthy fat profile due to the fat used. In addition to the various values observed could be associated to the different flour percentage added. Data found was like Macias et al. (2013), for cookies made with wheat flour and BCF, reporting fats values of 15.3, 15.9 g/100g and 17.3 y 16.7 g/100g.¹⁸

In terms of moisture, this parameter increased proportionally to the quantity of BCF added, that could be attributed to flour sugars composition who can absorb the environmental humidity.^{19,21} Protein content also has an influence in product moisture, mayor protein levels produced a more viscous dough with less expansion during the baking process due to the water absorption capacity of proteins when gluten structure formed.²²

In reference to ashes, Macias et al., (2013) and Escobar et, al. (2009) analyzed substitution of carob flour in cookies at different proportions (10-20%), shown quantities ranged up to 0.98 and

0.60 g/100g; 1.2 and 1 g/100g respectively.^{18,20} The variations observed could be associated to variety of legume, portion used, and proportions applied in dough.

The decrease in TPF can be explained because of the heat treatment applied, producing ruptures and degradation of covalent bonds.²⁴⁻²⁶ According to polyphenol intake studies, there is a relationship between the consumption of this compounds and the appearance of non-communicable diseases.^{27, 28, 29} Besides, estimated polyphenol intake is around 500-900mg²⁹, according to these values, the snacks' formulations cover the 53.25%; 37.08% y 30.16% for DS1, DS2 and DS3 respectively.

Changes in TA can be attributed to anthocyanins' susceptibility to high temperature, light, and oxygen. These conditions provoke adverse effects over their structure.^{27,28}

Losses in PAT as anthocyanins could be attributed to heat conditions applied. Besides, PAT determination could be conditioned for non-extractable pigments retained in the original matrix.²⁹

Changes in AA can be explained for the chemical reactions produced during the cooking process like mailoindins from Maillard reaction.²⁶

Dietary fiber values found were lower than Escobar et al. (2009) who studied the same fractions of fiber indicating 2.80; 2.18 and 0.62 g/100g in cotyledon algarroba flour at 20% of substitution.²⁰ Differences found could be attributed to flour origin and variety, proportion used, and the fruit filling added.

In comparison with similar commercial products (5g/100g), TDF was higher with a percentage around 59%. Thus, a snack ration (30g) covers the 16.5% of daily recommendations for dietary fiber. In addition, this product can be claimed as "high fiber content" according to established in Argentinian Food Code.²¹

CONCLUSIONS

The development of snacks with black carob flour and discarded blueberries was possible. The formulations performed had functional properties, were high in protein content and reduced in total caloric value in comparison with commercial products available. DS2 was the snack with the greatest antioxidant activity and, according to fiber values, it is a product "high in fiber content". Despite the losses caused by the cooking process in the content of bioactive compounds, the antioxidant activity was greater than 50% in all the snacks produced.

ACKNOWLEDGEMENTS

We thank Margarita Armada and Claudia Tapia from the Laboratorio de Alimentos de la Facultad de Ciencias Exactas, Universidad Nacional de Salta (UNSA) for their selfless contribution in the use of the equipment.

AUTHOR'S CONTRIBUTION

VNM: bibliographic review, snacks development, chemical analyses, manuscript drafting, results and discussion, EG: co-director, snacks development, chemical analyses, manuscript drafting, results and discussion APOL: polyphenols, flavonoids and antioxidant activities, AMLH: statistical analyses, review and correction of the article, ANR: supervisor, funding, reagents and physical space to carry out the experiments, review and correction of the article, FJV: director, results and discussion, review and correction of the article.

FUNDING

This work was supported by Consejo de Investigaciones de la Universidad Nacional de Salta (CIUNSA) (Project type A N° 2362/0 and Project type A N°2308/0) and by a fellowship (BIEA -

Beca de Investigación para Estudiantes Avanzados) granted to VN Miranda.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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