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Sugar, total phenolic content and antioxidant activity of integral carob (*Ceratonia siliqua L.*) powder during the simulated gastrointestinal tract digestion as related to the particle-size effect

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Introduction

Carob (*Ceratonia siliqua L.*) fruit is obtained from the evergreen carob tree, native to the Mediterranean region [1,2] and produced mainly in Portugal, Italy, Morocco and Turkey. [3–5] Portugal has a high-cultivated extent with relevant carob fruit production and at low prices. Carob products containing phenolic substances exhibit antioxidant capacity and can promote human health and aid in preventing chronic diseases. [6,7] Currently, carob powder (CP) production is mainly achieved after the pulp separation despite having been demonstrated that seeds improve the extraction efficiency of bioactive compounds like polyphenols. [4]

Objectives

This study aimed to produce an integral CP through an innovative process and assess its physicochemical and bioactive properties in different particle sizes throughout a simulated gastrointestinal tract (GIT) digestion.

Methods

The carob pods were obtained from a regional producer in Faro, Portugal. The pods were harvested in September 2020. Initially, the carob pods were washed with hot running water and placed in an oven at 45 ° C for 72 h so that they were free of dirt and moisture and eased the grinding process. The milling process is presented in **Figure 1**. Briefly, the carob pods were crushed in a knife mill without a sieve, followed by an 8000 µm sieve and a 6000 µm sieve. Then the obtained milled product was transferred to a hammer mill and milled with a sieve of 3000 µm followed by a 1500 µm sieve. The knife milling step was repeated in the same previous parameters. Finally, the integral carob powder was milled using an ultracentrifugal mill with a 500 µm sieve. The final CP product was stored and further analyzed for its nutritional and physicochemical properties.

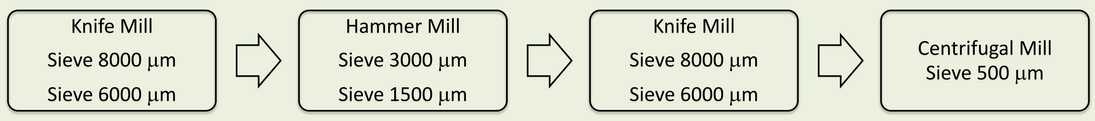

Figure 1 - Schematic representation of carob milling process.

Carob powder's particle-size distribution was determined with an automatic sieve shaker with circular oscillation using 50 g of flour and a 5 min sifting time and 1.85 amplitude. Sieves, 20 cm in diameter, with mesh sizes of 100 and 250 µm were used. These different fractions underwent a modified INFOGEST digestion protocol and analyzed in each phase in terms of sugar (HPLC) and total phenolic content (Folin-Ciocalteu) and antioxidant activity (ABTS, DPPH and ORAC).

Results and Discussion

The integral carob powder (CP) was analyzed for its nutritional and physicochemical composition (**Table 1**). Results show a high content in total carbohydrates and fibers, namely insoluble fibers which are present in higher quantities than soluble fibers. The nutritional and physicochemical analysis are in agreement with previous studies made on carob flours, processed by milling approaches, in terms of ash, protein, lipid, carbohydrate and starch content [4,19].

Table 1 - Nutritional and physicochemical composition of carob powder.

Proximate Composition (g/100 g dry basis)	Carob Powder
Moisture	6.35 ± 1.24
Ashes	2.41 ± 0.02
Carbohydrates	86.70 ± 1.13
Proteins	4.31 ± 0.06
Lipids	0.23 ± 0.12
Energy [kcal/100 g]	366.14 ± 5.60
Total Dietary Fibers	14.64 ± 1.81
Insoluble Dietary Fibers	14.12 ± 1.48
Soluble Dietary fibers	0.52 ± 0.52
Total Starch	0.96 ± 0.11

These comparisons allow us to understand that the innovative process used in this study for obtaining an integral carob powder is efficient and produces similar nutritional and physicochemical profiles to those obtained for other carob-based products, described in the literature, which undergo much harsher and costly treatments.

Table 2 - Size distribution of the different carob powders fractions.

Particle size (µm)	Fraction weight (%)
≤ 100	70.30 ± 0.31
100 < x < 250	20.18 ± 0.76
≥ 250	9.52 ± 0.43

The particle-size distribution of CP is presented in **Table 2**. The results show that 70% of the particles are ≤100 µm, which is expected as most of the carob pod is composed of pulp, grinding easier than the seed portion, possibly most present in the >100 µm fractions. The different size fractions were assessed regarding their digestion throughout the GIT and antioxidant potential, to determine the best-suited fraction for incorporation in food products and health benefits.

The sugar content profile obtained throughout GIT digestion indicates that the highest present sugar in undigested carob powders is digested and broken into simple sugars like glucose and fructose, potentiating CPs as a functional and within healthy food intake recommendations ingredient to use.

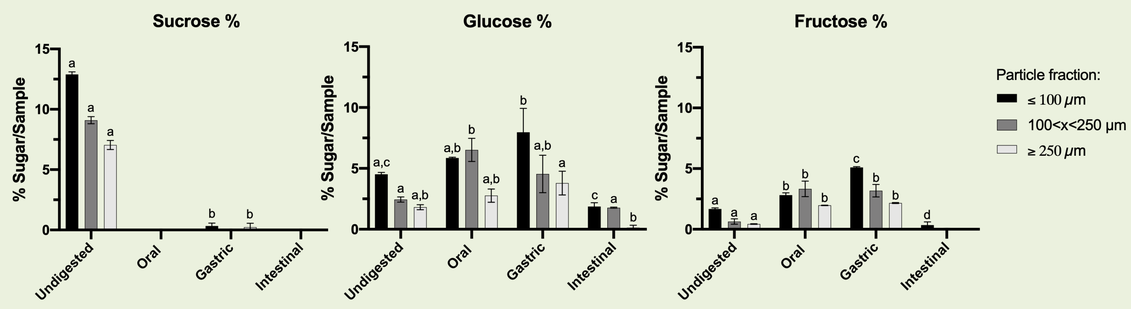

Figure 3 - Sucrose, glucose and fructose content in different particle fractions of CP throughout GIT simulation. The bars represent the standard deviation; for a given fraction, results with different letters differ significantly throughout GIT digestion.

The total phenolic content and antioxidant activity obtained for the ≤100 µm fraction is in correlation and gastric digestion promotes the increase in TPC value compared to the chemical extracted samples (undigested). The >100 µm fractions display a distinct profile from that of ≤100 µm possibly due to higher content in insoluble fibers which hinders the release of these bioactive compounds from the carob matrix.

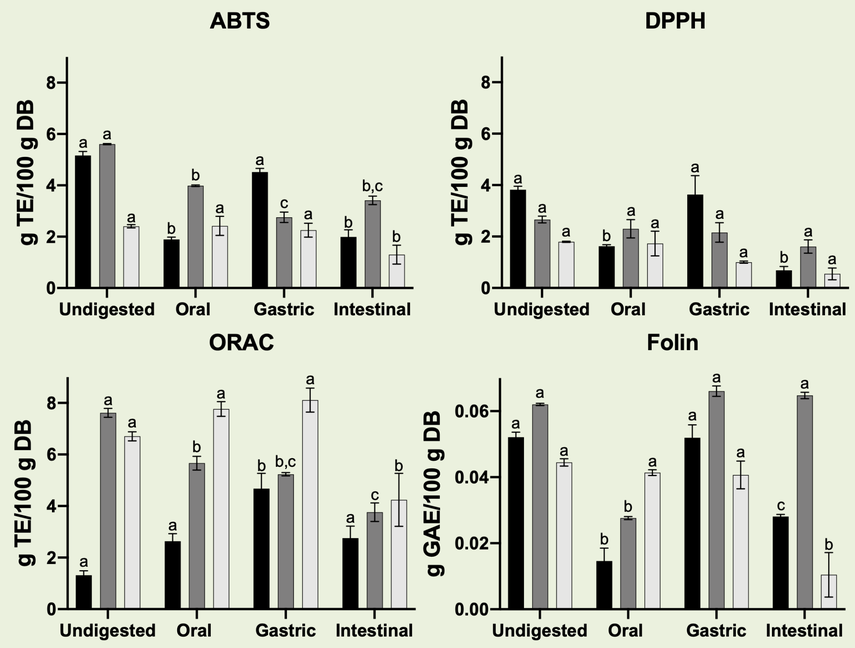

Figure 3 - Evaluation of total phenolic compounds (Folin-Ciocalteu method) and antioxidant capacity (ABTS, DPPH and ORAC assays) of carob powder with different particle sizes in the simulated GIT. All results expressed in g/100 g on dry basis (DB). The bars represent the standard deviation; for a given fraction, results with different letters differ significantly throughout GIT digestion.

These results show not only that the preparation method of the integral carob powder allows for a similar and interesting nutritional profile of that obtained for more common carob products such as carob pulp, but also the particle size affects the bioavailability, stability and absorption of the bioactive compounds throughout the GIT. Ultimately, ≤100 µm fraction exhibited the best suitable profiling for use as a functional food ingredient.

Conclusions

These findings showed that the carob powder production process is effective for the obtention of a functional ingredient and the particle size affects the bioavailability, stability and absorption of the bioactive compounds throughout the GIT. The CPs with ≤ 100 µm of particle size exhibit the best suitable profile throughout GIT digestion.

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