Food Research International 170 (2023) 112962

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

# Pasta fortified with *C*-glycosides-rich carob (*Ceratonia siliqua* L.) seed germ flour: Inhibitory activity against carbohydrate digesting enzymes

Francesco Siano [a], Gianfranco Mamone [a], Ermanno Vasca [b], Maria Cecilia Puppo [c], Gianluca Picariello [a,*]

[a] Istituto di Scienze dell'Alimentazione, Consiglio Nazionale delle Ricerche, Via Roma, 64, 83100 Avellino, Italy
[b] Dipartimento di Chimica e Biologia "A. Zambelli", Università degli Studi di Salerno, Via Giovanni Paolo II, 132, 84084 Fisciano, SA, Italy
[c] Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA - UNLP - CONICET), Calle 47 y 116, 1900 La Plata, Argentina

ARTICLE INFO

*Keywords:*
Carob seed germ flour
Apigenin 6,8-C-diglycosides
Fortified pasta
α-Amylase inhibition
α-Gycosidase inhibition
*In vitro* starch digestion

ABSTRACT

Carob (*Ceratonia siliqua* L.) seed germ flour (SGF) is a by-product resulting from the extractionextraction of locust bean gum (E410), which is a texturing and thickening ingredient used for food, pharmaceutical and cosmetic preparations. SGF is a protein-rich edible matrix and contains relatively high amounts of apigenin 6,8-C-di- and poly-glycosylated derivatives. In this work, we prepared durum wheat pasta containing 5 and 10 % (w/w) of SGF and carried out inhibition assays against type-2 diabetes relevant carbohydrate hydrolysing enzymes, namely porcine pancreatic α-amylase and α-glycosidases from jejunal brush border membranes. Nearly 70–80% of the SGF flavonoids were retained in the pasta after cooking in boiling water. Extracts from cooked pasta fortified with 5 or 10% SGF inhibited either α-amylase by 53% and 74% or α-glycosidases by 62 and 69%, respectively. The release of reducing sugars from starch was delayed in SGF-containing pasta compared to the full-wheat counterpart, as assessed by simulated oral-gastric-duodenal digestion. By effect of starch degradation, the SGF flavonoids were discharged in the water phase of the chyme, supporting a possible inhibitory activity against both duodenal α-amylase and small intestinal α-glycosidases *in vivo*. SGF is a promising functional ingredient obtained from an industrial by-product for producing cereal-based foods with reduced glycaemic index.

## 1. Introduction

Carob (*Ceratonia siliqua* L.) is an evergreen tree of the Fabaceae family native to the Mediterranean region. It has been cultivated for centuries especially in Spain, Italy, Portugal and Morocco because of its edible fruits (Ayaz et al., 2009). Carob ripe pods can be eaten raw as a sweetmeat due to their high sugar content, mainly sucrose, but more often they are used as animal feed. Owing to their excellent flavouring properties, the non-fleshy carob pods are utilized in many countries of the Mediterranean area to produce popular beverages, homemade cakes, pastries and confectionery (Ibrahim et al., 2020; Yousif & Alghzawi, 2000). Ground roasted carob pods constitute natural sweeteners, commonly employed as caffeine- and theobromine-free cacao and coffee surrogates (Bengoechea et al., 2008). Carob pod flour has been evaluated as a functional ingredient for wheat bread (Salinas et al., 2015) and successfully used as a suitable ingredient to produce gluten-free baked products as well (Tsatsaragkou et al., 2014). Despite its interesting properties, nowadays carob is considered a neglected or underutilized crop, which is maintained only in semiarid and marginal soils (Batlle & Tous, 1997). Currently, the main interest for the cultivation of carob is precisely in the seeds, which are discarded in the traditional uses. Seeds are exploited to extract locust bean gum (LBG) that is a galactomannan used as a natural food additive (E410) with excellent texturing and thickening properties and as an emulsifier and gelling ingredient in food, pharmaceutical and cosmetic preparations (Avallone et al., 1997; Srivastava & Kapoor, 2005). LBG is in great demand especially for its functional properties, neutral flavour and toxicological safety, which also make it suitable for the formulation of foods suitable for infants and individuals suffering from celiac diseases or food allergies. Estimated world production of LBG is about 315 thousand tons *per* year and it is sharply growing (Barak & Mudgil, 2014).

Germ is the by-product of the gum production and can be mechanically separated from the gummy endosperm. Seeds account for 10–15 % in weight of the carob pods, germ representing one third-to-half of the seed dry weight (Sciammaro et al., 2016a). Carob seed germ flour (SGF) is a protein-rich edible matrix, containing 48–55 % protein with a

* Corresponding author.
*E-mail address:* picariello@isa.cnr.it (G. Picariello).

https://doi.org/10.1016/j.foodres.2023.112962
Received 23 January 2023; Received in revised form 22 March 2023; Accepted 10 May 2023
Available online 13 May 2023

balanced content of essential amino acids (Fidan et al., 2020) and a nutritional profile similar to other *Leguminosae* such as soy (Bengoechea et al., 2008). The structural and functional properties of SGF proteins have been investigated (Dakia et al., 2007; Smith et al., 2010). Simulated *in vitro* digestion experiments have demonstrated that the carob germ proteins are substantially digestible (Mamone et al., 2019). SGF also contains other functionally and nutritionally interesting components, including unsaturated lipids, phospholipids and phenolic compounds (Custódio et al., 2011; Sciammaro et al., 2016a, 2016b; Siano et al., 2018). By virtue of the exclusive composition and nutritional properties, SGF has potential to be used as an ingredient for special food formulations (Bengoechea et al., 2008; Dakia et al., 2007; Sciammaro et al., 2016b). The idea of using SGF as an ingredient for pasta or baked products is ancient, as it was proposed for increasing the level of gluten-like proteins in a US patent dating back to 1935 (Bienenstock et al., 1935). Later on, SGF was investigated as an ingredient for baked products suitable for diabetics because of the high protein-to-carbohydrates ratio (Plaut et al., 1953). However, SGF is particularly interesting for its polyphenols, which have been recently characterized by our group (Picariello et al., 2017). The predominant polyphenols of SGF are apigenin 6,8-*C*-di-glycoside isomers, namely schaftoside (apigenin 6-*C*-glucosyl-8-*C*-arabinoside) and isoschaftoside (apigenin 6-*C*-arabinosyl-8-*C*-glucoside), and other variously glycosylated forms of apigenin, overall accounting for more than 8 g $kg^{-1}$ SGF. Flavone *C*-arabinosides are one of the less common classes of flavonoid glycosides occurring in nature (Chen et al., 2021). Nevertheless, they have been detected in a variety of taxonomically different species although at much lower concentration than in SGF (Wang et al., 2020). Like many other polyphenols, several flavones, including apigenin *C*-glycosides, have been described as potent inhibitors of carbohydrate hydrolysing enzymes, such α-amylase and α-glycosidase (Tadera et al., 2006; Takahama & Hirota, 2018). Salivary and pancreatic α-amylase isoforms catalyse starch degradation into malto-oligosaccharides, while α-glycosidases anchored at the brush border membranes of the small intestinal enterocytes catalyse the hydrolysis of upstream produced oligosaccharides into absorbable monosaccharides. Since α-amylase and α-glycosidase inhibitors delay glucose absorption and reduce postprandial glycemia, they have been proposed as possible alternatives to the pharmaceutical treatment of insulin-resistance and type-2 diabetes (Barrett et al., 2013; Rohn et al., 2002). Potent α-glucosidase and α-amylase inhibitors, such as acarbose, miglitol, and voglibose, are used as drugs for reducing postprandial hyperglycaemia in individuals with overt or at risk of type-2 diabetes. However, the excessive inhibition potential of these compounds could be undesired because large amount of indigested starch may induce abnormal fermentation in the gut, which is cause of commonly reported adverse side effects, including symptoms of serious gastrointestinal discomfort (Fujisawa et al., 2005; Kumar et al., 2021). Sound *in vitro*, animal and in humans evidence demonstrates that naturally occurring flavonoids can contribute to prevent diabetes and its complications (Al Duhaidahawi et al., 2021; Al-Ishaq et al., 2019). The mechanisms underlying enzymatic inhibition by polyphenols are complex and not fully elucidated (Zhu et al., 2020). The presence of other components in real foods can alter the flavonoids-enzymes interplay (Sun & Miao, 2020). For example, several types of soluble polysaccharides affect binding and reduce inhibitory activity of tea polyphenols against porcine pancreatic α-amylase (Sun et al., 2018). For these reasons, the determination of effective α-amylase and α-glycosidase inhibitory properties of real foods prepared with ingredients such as SGF deserves investigation.

In this work, we prepared pasta on a laboratory scale incorporating 5 and 10 % (w/w) of SGF in wheat (*Triticum durum*) flour and assessed the capability of the phenolic extracts from pasta to inhibit porcine pancreatic α-amylase and intestinal α-glycosidases. Pasta samples were subjected to an oral-gastric-duodenal *in vitro* simulated digestion to assess the effects of incorporating SGF on starch digestion.

## 2. Material and methods

### 2.1. Chemicals

HPLC-grade solvents and high purity chemicals, including 2,2-diphenyl-1-picrylhydrazyl (DPPH), 3,5-dinitrosalicylic acid, acarbose, *p*-nitrophenol and pure standards of schaftoside and isoschaftoside (purity > 99 %), were purchased from Merck-Sigma-Aldrich (St. Louis, MI, USA).

### 2.2. Carob seed germ flour (SGF)

Carob (*Ceratonia siliqua*) SGF was a kind gift by Carob Ingredients Co., Casablanca, Morocco (https://www.carobingredients.com). SGF was shipped in a refrigerated box and stored at 4 °C in vacuum-packed plastic bags until used. SGF had a certified protein concentration of 54 % (w/w), 7 % (w/w) lipids and 9 % (w/w) moisture. SGF has long been used for animal and human consumption and it should be considered as toxicologically safe.

### 2.3. Preparation of pasta containing SGF

Pasta was prepared on a laboratory scale (500 g) by replacement of commercial durum wheat (*Triticum durum*) semolina with 5 % or 10 % (w/w) of SGF. A control sample with 100 % durum wheat semolina was prepared as the control. Tap water was added with a flour-to-water ratio 2.5:1 (w/w) until a homogeneous and workable dough was obtained. The doughs were kneaded manually for 5 min and then flattened, pressed into 4 mm thick foils, and finally extruded into a spaghetti-like shape, using a domestic machine for handmade fresh pasta equipped with pairs of both rolling and cutting rollers. Raw pasta samples were air dried at 25 °C in a chamber with 20 % humidity for 16 h and then stored in vacuum-packed plastic bags at −20 °C until used for analyses.

One-hundred grams aliquots of pasta samples were cooked in 1 L of boiling water containing 1 % (w/v) NaCl for 6 min and then drained, storing the cooking water. Cooked pasta samples were weighed to assess the incorporation of water and immediately used for polyphenol extraction or for experiments of simulated digestion. Hereinafter, raw pasta containing 5 and 10 % of SGF will be referred to as R-5 and R-10, samples, while the corresponding cooked pasta samples will be referred to as C-5 and C-10, respectively. Pure semolina raw and cooked pasta samples will be referred to as R-CTR and C-CTR, respectively. Coking water from pasta containing 5 and 10 % SGF will be indicated as W-5 and W-10, respectively.

### 2.4. Polyphenol extraction

To maximize the extraction yield, polyphenols were extracted with aqueous methanol 80 % (v/v) in ultrasonic bath (Oniszczuk, 2016). More in detail, SGF and pasta samples were suspended in aqueous methanol 80 % (v/v) with a 1-to-10 (w/v) flour or pasta dry weight-to-solvent ratio. The suspensions were magnetically stirred 1 h at room temperature, kept in an ultrasonic bath for 30 min and then centrifuged at 5500 *g* for 20 min at 4 °C. The supernatants were filtered through 0.45 μm nylon disposable syringe membranes (Merck-Millipore, Darmstadt, Germany) and stored at −20 °C for subsequent analyses. Before enzymatic assays, the methanol extracts were tenfold diluted in phosphate saline buffer (PBS, pH 6.9), without observing macroscopic formation of opacity or precipitate.

### 2.5. Total polyphenol and DPPH radical-scavenging capacity

Total polyphenols were determined with the Folin-Ciocalteu method (European Directorate, 2007). Briefly, in a 3 mL plastic cuvette it was combined consecutively: 2300 μL of deionized water, 50 μL of the Folin-Ciocalteu reagent (Merck-Sigma) diluted 1:2 with water, 50 μL of SGF or

pasta extracts. The suspension was mixed for 3 min and then 100 μL of a saturated sodium carbonate solution was added. After 90 min of incubation in the dark, the absorbance was determined at 765 nm (GE-Heathcare Ultrospec 2100 UV–Vis; Uppsala, Sweden). Total phenolics were quantified against a calibration curve ($R^2 > 0.99$) built with gallic acid (≥99 % purity). Results were expressed as mg of gallic acid equivalent (GAE) *per* kg of SGF or dry pasta.

The DPPH radical-scavenging activity was determined by spectrophotometric assay, combining 100 μL of extracts from SGF, pasta samples or cooking water with 2.4 mL of a freshly prepared ethanol solution of 0.1 mM DPPH radical and the mixture was incubated for 30 min at room temperature in the dark. The absorbance was measured at 517 nm using the Ultrospec 2100 UV–Vis (Uppsala, Sweden) spectrophotometer against pure ethanol used as the blank. DPPH radical-scavenging activity was calculated as the % inhibition (% I) with the following formula: % $I_{DPPH} = [(A_{DPPH} - A_s)/A_{DPPH}] \times 100$, where $A_{DPPH}$ was the absorbance of the DPPH radical solution and $A_s$ was the absorbance after radical inhibition with sample solutions.

### 2.6. RP-HPLC and HPLC-MS/MS analysis of polyphenols

The 80 % methanol extracts from SGF or from pasta samples were fiftyfold or tenfold diluted with 0.1 % trifluoroacetic acid (TFA), respectively, and 100 μL of the resulting solutions was separated by reversed phase-high performance liquid chromatography (RP-HPLC) using a modular HP 1100 chromatographer (Agilent, Palo Alto, CA, USA) equipped with a diode array detector (DAD). The stationary phase was a C18 reversed-phase column 250 × 2.1 mm i.d., 4 μm particle diameter (Jupiter Phenomenex, Torrance, CA, USA), kept at a 40 °C using a thermostatic oven. Phenolic compounds were separated applying a 5–65 % gradient of the organic modifier (solvent B: acetonitrile/TFA 0.1 %) in 5–65 min, following 5 min of isocratic elution at 5 % B, at a 0.2 mL $min^{-1}$ constant flow rate. Solvent A was 0.1 % TFA in HPLC-grade water. Separations were monitored at λ = 280, 320, 340, 360 nm wavelengths and peaks were integrated using the HPLC ChemStation software vers. A.07.01 (Agilent). Assignment of individual compounds was confirmed by high resolution tandem mass spectrometry, using a Q Exactive Orbitrap instrument (ThermoFisher Scientifics, San Jose, CA, USA) operated with previously detailed parameters (Picariello et al., 2017). Phenolic compounds were quantified using calibration curves built with a standard solution of pure schaftoside or isoschaftoside ($R^2 = 0.99$), which were prepared at six different concentrations in the 0.01–15 mg $mL^{-1}$ range in methanol and diluted with 0.1 % TFA prior to injection. Quantification was performed with chromatograms recorded at 340 nm. Analyses of samples were carried out in triplicate and values were averaged.

### 2.7. α-Amylase and α-glycosidase inhibition assays

The activity of α-amylase and α-glycosidases in the presence or in the absence of inhibitors was assayed using the amylase activity assay kit (cod. MAK009, Merck-Sigma) and the α-glycosidase activity assay kit (cod. MAK123, Merck-Sigma), respectively, according to the manufacturer's instruction. Acarbose (Merck-Sigma), which is a well-established inhibitor of both α-amylase and α-glycosidase, was used as the positive control. Assays were carried out preincubating the enzymes with 20 μL of extracts from SGF or from pasta, prior to including the substrates. The substrates were ethylidene-4-nitrophenyl-α-D-maltoheptaoside (pNP-G7) and *p*-nitrophenyl-α-D-glucopyranoside (α-NPG), for α-amylase and α-glycosidase, respectively. Extracts from SGF were tenfold diluted in PBS pH 6.9, while pasta samples were not diluted but concentrated in speed vac and then reconstituted in the original volume of PBS, pH 6.9. To determine the activity of the non-inhibited enzymes, preincubation was done with 20 μL of PBS. Acarbose, pure schaftoside and isoschaftoside were dissolved in methanol at 1 mM concentration and then tested after tenfold dilution in PBS, pH 6.9 (0.1 mM final concentration).

For α-amylase activity assay, samples were pre-incubated with 2 μL of a 0.1 mg $mL^{-1}$ solution of porcine pancreatic α-amylase (E.C. 3.2.1.1, >10U $mg^{-1}$, Merck-Sigma). To assay the α-glycosidase activity, samples were pre-incubated with 5 μL of tenfold diluted (in PBS) brush border membrane vesicles (α-glycosidase activity 90 mU/mL), which were purified from pig jejunum as previously described (Picariello et al., 2015). After reactions, the absorption of free *p*-nitrophenol was determined at 405 nm using a 96-well Synergy HT microplate reader (Bio-Tek Instruments, Winooski, VT, USA). In the case of the α-amylase assay the absorbance was measured at time zero ($T_0$) and every 3 min, until the value of not-inhibited α-amylase exceeded the absorbance of the most concentrated standard solution ($T_{final}$), and it was referred to a standard curve built with six varying concentrations of *p*-nitrophenol (2–20 nmol *per* well).

In contrast, the enzymatic reaction of α-glycosidases was carried out for a given time period (20 min) at 37 °C and the absorbance of free *p*-nitrophenol was referred to an opportune "calibrator" solution, furnished with the kit. In both cases, the absorbance of the blank, which was prepared as the corresponding test wells but replacing the enzymes solution with PBS, was subtracted. Absorbance values are the average of three replicate measurements.

The α-amylase activity was determined as nmol $min^{-1}$ $mL^{-1}$ (milliunits) using the following equation:

$$\alpha - \text{amylase activity} = B \times \text{sample dilution factor}/\text{reaction time} \times V$$

In which:

B = amount (nmol) of *p*-nitrophenol generated between $t_0$ and $t_{final}$ (after blank subtraction).

reaction time = $t_{final} - t_0$ (minutes).

V = sample volume (mL) added to well.

The α-glycosidase activity (U $L^{-1}$) was determined according to the following equation:

$$\left(A_{final} - A_{initial}\right)/\left(A_{calibrator} - A_{water}\right) \times 250 \text{ U L}^{-1}$$

Where $A_{final}$ and $A_{calibrator}$ are the absorbance at 405 nm of the test well and of the calibrator, respectively, after 20 min reaction.

One unit of α-glycosidase is the amount of enzyme that catalyzes the hydrolysis of 1.0 μmol substrate per minute at pH 7.0.

Results are reported as percent inhibition (% inhibition) according to the formula below:

$[(A_{n.i.} - A_{test}) / A_{n.i.}] \times 100$, where $A_{n.i.}$ is the absorbance relevant to not inhibited enzymes and $A_{test}$ is the absorbance of the test solutions.

### 2.8. Determination half-maximal inhibitory concentration ($IC_{50}$)

The half-maximal inhibitory concentration ($IC_{50}$) against α-amylase and α-glucosidase activities was obtained for pure schafoside and isoschaftoside as well as for the SGF extract, in comparison to acarbose, by determining the inhibitory activity of their solutions in the $0.1–10^{-5}$ mM range of concentrations. For molar calculation polyphenols in SGF were calculated as schaftoside equivalents (molecular weight 564.5 g $mol^{-1}$). The $IC_{50}$ value, expressed as μg $mL^{-1}$, was calculated from the least-squares regression line of the plot of inhibition % versus $\log_{10}$ concentration of the test samples, using the ED50plus v1.0 open-source software.

### 2.9. In vitro simulated digestion of pasta samples

Simulated salivary, gastric and intestinal fluids were prepared according to the harmonized protocol of *in vitro* digestion (Brodkorb et al., 2019). Cooked pasta was drained, and 2 g of each sample was gently crashed in a mortar for 2 min in the presence of 2 mL of simulated salivary fluid (pH 7.0) containing human salivary amylase (75 U $mL^{-1}$) to reproduce the oral phase of digestion. Afterwards, the samples were suspended in 4 mL of simulated gastric fluid (pH adjusted to 3.0)

containing porcine pepsin (3300 U $mg^{-1}$, final concentration of 12 mg $mL^{-1}$) and lecithin liposomes (0.17 mM final concentration) and incubated 2 h at 37 °C with mild stirring. At the end of the simulated gastric phase, the pH was raised to 7.0 using a saturated solution of sodium bicarbonate. To reproduce the duodenal digestion, the gastric digests were added with 4 mL of simulated intestinal fluid (pH 7.0) containing bile salts (10 mM in the final mixture, calculated as cholic acid) and porcine pancreatin with 100 U $mL^{-1}$ trypsin (determined with TAME assay). The chyme was incubated at 37 °C for 2 h. During the duodenal phase, 0.25 mL aliquots of the digesting samples were withdrawn at several timepoints, and enzymes were inactivated by addition of 0.25 mL of methanol. Samples were quickly refrigerated in an ice-cold bath and centrifuged at 5500 *g* for 20 min at 4 °C. The clear supernatants were used for the determination of maltose and for HPLC analysis of polyphenols. For the HPLC analysis, 20 μL of the mixture was tenfold diluted with 0.1 % TFA and analyzed according to the above-described procedure.

### 2.10. Determination of reducing sugars

Reducing sugars released during the duodenal phase of digestion of pasta were determined by adapting the spectrophotometric assay based on the coupling reaction with 3,5-dinitrosalicylic acid (DNSA) (Miller, 1959). To this aim, 20 mL of 96 mM DNSA in deionised water was combined with 8 mL of 5.31 M sodium potassium tartrate in 2 M NaOH and the solution was diluted up to 40 mL with deionized water (colour reagent solution). The colour reagent solution (100 μL) was combined with 100 μL of the supernatants obtained from duodenal chyme at several timepoints or with water for the blank and reacted in a boiling water bath for 15 min. After cooling on ice to room temperature, 900 μL of deionized water was added, and the absorbance was read at 540 nm. A standard curve was built with 0.1–2 mg $mL^{-1}$ of maltose prepared from a 0.2 % stock solution in deionized water.

### 2.11. Statistical analysis

All parameters were determined at least in triplicates and results were expressed as means ± SD.

The calibration curves, graphs and figures were generated using the OriginPro 2016 software (OriginLab, Inc., Northampton, MA, USA).

## 3. Results and discussion

### 3.1. Polyphenol composition of carob SGF

When suspended in 80 % (v/v) aqueous methanol, SGF induces the formation of an intense yellow colour in the solution, despite the creamy white colour of the flour. This observation prompted us to investigate the nature of pigments contained in SGF. Thus, in a previous work we characterized the hydroalcoholic extracts of SGF produced in southern Spain, demonstrating that pigments were predominantly *C*-glycoside derivatives of apigenin (Picariello et al., 2017). Apigenin di-*C*-glycosides have been described in a large variety of higher plants, including cereal crops such as rice, maize, wheat and sorghum, *Citrus* spp., liquorice root (*Glycyrrhiza* spp.) and *Leguminosae* (Wang et al., 2020). As involved in plant defense, these compounds have been found in variable amount in several parts of the plants, and are specially abundant in embryos or cotyledons of some species, such carob. However, the polyphenol composition of SGF might vary significantly with the carob origin. The SGF used in this work was of different origin compared to the previously analyzed sample (Picariello et al., 2017), as it was from Morocco. Thus, we checked preliminarily the consistence of the polyphenol fraction of the SGF with the previous one using RP-HPLC and HPLC-ESI-MS/MS. The RP-HPLC-UV (λ = 340 nm) separation of the methanol (80 %, v/v) extracts from the SGF used in this study is shown in Fig. 1A, which is qualitatively consistent with the one previously obtained. Based on the

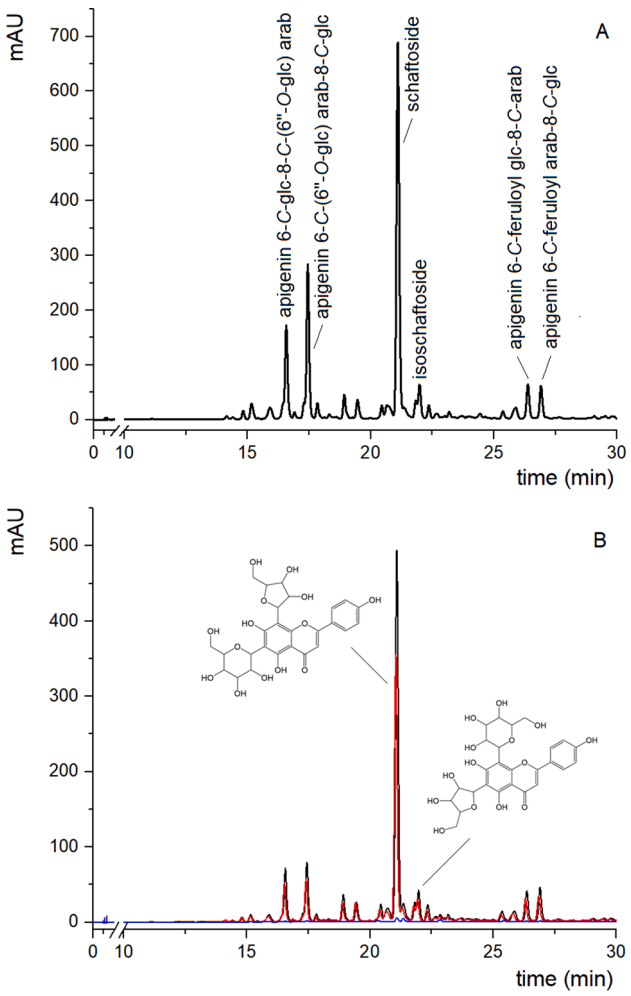


**Fig. 1.** RP-HPLC chromatograms (λ = 340 nm) of 80 % methanol (v/v) extracts from (A) carob SGF; and (B) raw pasta samples (blue line: R-CTR; red line: R-5; black line: R-10). Most abundant peaks were assigned by HPLC-MS/MS as previously described (Picariello et al., 2017), while the elution orer of schaftoside and isoschaftoside was confirmed with authentic standard compounds, as detailed in the text.nd isoschaftoside was confirmed with authentic standards, as detailed in the text.

elution order reported in the literature (Ferreres et al., 2003; Pérez et al., 2014), in our previous research we assigned the most abundant peaks of the SGF extracts to isoschaftoside, that is apigenin 6-*C*-arabinosyl-8-*C*-glucoside, while a low-abundance peak eluting at higher retention time had been identified as schaftoside (apigenin 6-*C*-glucosyl-8-*C*-arabinoside). Using the authentic standards, in this work we ascertained that the previous assignments were wrong and that the elution of the two compounds is reversed. Thus, the most abundant polyphenol component of SGF is schaftoside (retention time, Rt = 21.1 min), while isoschaftoside (Rt = 21.9 min) occurs at much lower amount. The dominant polyphenols of SGF are assigned in Fig. 1A, also based on the high-resolution MS and MS/MS assessments. The couple of peaks at lower Rt (Rt = 16.5 and 17.4 min) have been putatively identified as further glycosylated apigenin derivatives, namely apigenin 6-*C*-glucosyl-8-*C*-(6″-O-glucosyl) arabinoside and its isomer apigenin 6-C-(6″-O-glucosyl) arabynosyl-8-C-glucoside, while those at higher Rt are the feruloyl esters of schaftoside and isoschafotside, as indicated in Fig. 1A. The polyphenol extracts of SGF also contain minor amount of mono-*C*-glycosylated apigenin derivatives, as assessed by HPLC-MS/MS analysis (Picariello et al., 2017).

### 3.2. Preparation of pasta fortified with SGF and cooking stability of polyphenols

Pasta samples were prepared on a laboratory scale using 100 % commercial durum wheat semolina (CTR) and semolina blended with 5 and 10 % (w/w) of SGF (Fig. 2). The RP-HPLC chromatograms (340 nm) of polyphenol extracts from raw pasta samples (*i.e.*, R-CTR, R-5 and R-10) are shown in Fig. 1B. The profiles of R-5 and R-10 extracts were dominated by SGF polyphenols, while no peaks of significant intensity were recorded for R-CTR. Aliquots of the pasta samples were cooked in boiling salted water, according to the conventional procedures. The thermal-induced decomposition of polyphenols and/or their partial leaching in the cooking water could limit the health-promoting properties of pasta fortified with polyphenol-containing ingredients. For this reason, we preliminarily checked the persistence of SGF polyphenols in cooked pasta (C-5 and C-10) after draining, in comparison to the dry raw (non-cooked) counterparts (R-5 and R-10) by RP-HPLC. The RP-HPLC analyses of extracts from C-CTR, C-5 and C-10 are shown in Supplementary material, Fig. S1. In C-10 pasta, the schaftoside was reduced by 29.7 ± 0.5 % compared to R-10, which means that more than 70 % of its original content was retained in pasta after cooking in an extractable form (Supplementary material, Fig. S2). The HPLC analysis of the freeze-dried water resulting from cooking C-10 (*i.e.*, W-10) demonstrated that 12.1 ± 0.4 % of the schaftoside had been discharged (Supplementary material, Fig. S3) and, hence, ~ 17.6 % of its original content was likely decomposed or chemically bound to the starchy matrix of the pasta and was no longer extractable. In cooked pasta, a slight relative reduction of the species at higher glycosylation degree (Rt = 16.5 and 17.4 min) was observed, due to their increased water solubility (cfr. Figs. S1 and S3), which corresponded to their slight relative increment in cooking water. Aside from that, the qualitative HPLC profiles of polyphenol extracts from cooked and raw pasta were substantially conserved (Supplementary material, Fig. S2), indicating that the degree of retention in pasta was similar for the various polyphenol compounds.

Total polyphenols were determined in raw and cooked pasta samples and in cooking water using the Folin-Ciocalteu spectrophotometric assay as reported in Table 1. This assay confirmed that SGF is a polyphenol rich matrix, as this class of compounds accounts for 4.58 g $kg^{-1}$ (dry weight), although this content was almost half of the previously analyzed SGF sample (Picariello et al., 2017). Nearly 82 % of the extractable polyphenols were retained in C-10 after cooking, which is roughly in agreement with the HPLC based determination, taking into account the possibility that the Folin-Ciocalteu assay can be biased by a number of interfering compounds (e.g., reducing sugars, amino acids). Based on the Folin-Ciocalteu assay, the concentration of polyphenols in R-CTR was relatively high, whereas only a few peaks of relatively low intensity were detected in the HPLC chromatograms of the relevant extracts at 280 nm (Supplementary material, Fig. S4).

The values of % DPPH radical inhibition by raw and cooked pasta extracts as well as of the corresponding cooking water were consistent with the polyphenol content of the samples, as summarized in Table 1 and in the histogram of Supplementary material, Fig. S5. Extracts from raw pasta inhibited DPPH radical more than the corresponding cooked pasta samples, and extracts from R-10 and C-10 had a higher inhibitory activity than R-5 and C-5, which in turn exhibited a stronger antioxidant activity than the corresponding all-wheat control pasta.

### 3.3. α-amylase and α-glucosidase inhibition assay

The results of the α-amylase activity assay are summarized in the histogram of Fig. 3A, % inhibition being 100 - % activity. As expected, acarbose 0.1 mM strongly inhibited α-amylase and the residual activity was only 2 % of the not-inhibited enzyme (98 % inhibition).

Both pure schaftoside (0.1 mM) and 1/10 diluted SGF significantly inhibited α-amylase with inhibition of 77 and 90 %, respectively. Extracts from pasta samples containing SGF demonstrated α-amylase inhibitory activity, which increased with the relative content of SGF and was higher for raw than cooked samples. The % inhibition activity of extracts from R-5 (97 %) and R-10 (99 %) was partly reduced for C-5 (53 %) and C-10 (74 %), in line with the partial release of polyphenols during cooking. Importantly, extracts from all wheat pasta (R-CTR and C-CTR) also inhibited α-amylase, as a consequence of the solubilization of small amounts of phenolic substances (barely detectable by RP-HPLC), or more likely due to the release of inhibiting oligosaccharides.

The inhibitory effects of schaftoside and isoschaftoside against α-glycosidases was less evident compared to α-amylase (Fig. 3B). Residual activity of the enzyme preincubated with 0.1 mM acarbose was 28.9 %, which indicated 71.1 % inhibition. Pure schaftoside and isoschaftoside, both at 0.1 mM, exhibited 50.5 and 54.8 % inhibition, respectively. The α-glycosidase inhibition of tenfold diluted SGF extracts was 57.4 %. These values indicate a remarkable inhibitory potential of apigenin *C*-di-glucosides against intestinal α-glycosidases, although


**Fig. 2.** Pasta samples: all-wheat control (R-CTR) and pasta prepared with 5% (R-5) and 10% (R-10) of SGF (w/w).

**Table 1**
Parameters determined for pasta samples and $IC_{50}$ inhibition activity for pure compounds and carob SGF.

| Sample | Umidity (%) | Total phenolics (Folin-Ciocalteu) (mg $kg^{-1}$, DW) | DPPH inhibition (%) | schaftoside-HPLC (mg $kg^{-1}$, DW) | isoschaftoside-HPLC (mg $kg^{-1}$, DW) |
|---|---|---|---|---|---|
| R-CTR | 33.3 | 503 | 8.0 | – | – |
| R-5 | 30.3 | 616 | 17.4 | 211 | 20 |
| R-10 | 31.4 | 700 | 26.5 | 393 | 32 |
| C-CTR | | 193 | 2.5 | – | – |
| C-5 | | 340 | 12.2 | 108 | 12 |
| C-10 | | 574 | 23.6 | 315 | 27 |
| W-CTR | | 17 | 6.8 | | |
| W-5 | | 23 | 6.1 | | |
| W-10 | | 55 | 9.3 | | |
| carob SGF | | 4582 | 48.1 | 3083 | 79 |
| | $IC_{50}$ (μg $mL^{-1}$) | | | | |
| **α-amylase inhibiting activity** | | | | | |
| acarbose | 3.1 | | | | |
| schaftoside | 7.3 | | | | |
| isoschaftoside | 7.6 | | | | |
| carob SGF extract* | 6.3 | | | | |
| **α-glycosidase inhibiting activity** | | | | | |
| acarbose | 22.6 | | | | |
| schaftoside | 27.2 | | | | |
| isoschaftoside | 25.7 | | | | |
| carob SGF extract* | 24.5 | | | | |

* Evaluated as schaftoside equivalents, after 1/10 dilution. DW: dry-weight. Values are the average of triplicate determinations at least. The variability, evaluated as relative standard deviation, was lower than 5% in all the cases and it is not reported.

lower than acarbose, in line with previous observations (Li et al., 2009). It should be emphasized that brush border vesicles employed as a source of small intestinal glycosidases contain an array of enzymes with glycosidase activity. Thus, it is not possible to establish whether the inhibitory activity is directed toward some specific or all these enzymes. Similar to the α-amylase inhibition, the introduction of SGF in pasta induced a reduction of the α-glycosidase activity with reduced effects for the extracts from cooked pasta samples compared to the raw counterparts (inhibition: 70.6 and 79.1 % for R5- and R-10, and 61.7 and 68.6 % for C-5 and C-10, respectively), most likely due to the partial loss of SGF polyphenols into cooking water. The trends of α-amylase and α-glycosidase inhibition activity were consistent with the estimated half inhibition concentration ($IC_{50}$) values (Table 1). The $IC_{50}$ values of α-amylase and α-glycosidase inhibition for SGF were much lower than those described for unfractionated phenolic extracts from other *Leguminosae*, such as *Phaseolus vulgaris* biotypes (Ombra et al., 2018) or *Tamarindus indica* (Buchholz & Melzig, 2016), thus suggesting that SGF is particularly effective to inhibit the carbohydrate digesting enzymes. However, it is difficult to make a comparison of activities determined in different studies, due to the different experimental conditions employed that include origin and activity of the enzymes, incubation time and temperature, concentrations, type of substrate as well as expression of data (Takahama & Hirota, 2018). To this purpose, the published $IC_{50}$ values for acarbose, which is often chosen as a reference inhibitor of α-amylase and α-glycosidase, are not well defined and span over an ample range covering several orders of magnitude (Dirir et al., 2022). Anyway, schaftoside and isoschaftoside exhibited relatively high inhibition efficiency, demonstrating that their activity is slightly lower than acarbose in the same experimental conditions. Polyphenol concentration of the SGF was reduced by tenfold dilution to estimate its inhibition potential. According to previous determinations, *C*-mono-glycoside derivatives of apigenin can inhibit α-glycosidase even more strongly than acarbose, probably acting with combined competitive and non-competitive mechanisms (Şöhretoğlu & Sari, 2020; Takahama & Hirota, 2018; Zeng et al., 2016), while more extensively glycosylated flavonoid derivatives could be milder enzymatic inhibitors (Li et al., 2009). The relatively reduced inhibitory activity of SGF polyphenols compared to acarbose or to mono-*C*-glycoside derivatives of apigenin might have positive implications because a mild inhibition of carbohydrate digesting enzymes can contribute to lowering post-prandial glycaemia while preventing gastrointestinal side effects of acarbose and drugs of the same class (Fujisawa et al., 2005; Moelands et al., 2018).

### 3.4. *In vitro simulated digestion*

C-CTR, C-5 and C-10 pasta samples were subjected to *in vitro* simulated oral-gastro-duodenal digestion according to the Infogest harmonized protocol. During the duodenal phase, digesting pasta was sampled at several time points and centrifuged. The clear supernatants were used for monitoring both the formation of reducing sugars and the release of SGF polyphenols. Reducing sugars were determined using the DNSA spectrophotometric assay, against a calibration curve built with pure maltose ($R^2 = 0.99$). The concentration of the DNSA-sugars condensed products, expressed as maltose equivalents, was plotted against the time of duodenal digestion (Fig. 4). During duodenal digestion, the concentration of reducing sugars increased faster for C-CTR than for C-5 and C-10, while at the end of the monitored time it tended to equalize. These data indicated a significant delayed duodenal degradation of starch for SGF-containing pasta compared to the control. Unexpectedly, the concentration of reducing sugars was constantly higher in C-10 compared to C-5 digesting pasta. Most likely, SGF already introduces a small amount of reducing sugars that yielded higher response of C-10 than C-5 in the DNSA assay. However, reducing sugars were released substantially with comparable rate between C-10 and C-5, because the increased amount of SGF in C-10 did not induce appreciable further delay in the kinetics of starch duodenal digestion compared to C-5. Thus, the introduction of 5 % of SGF could be an optimal compromise between healthy advantages in terms of delayed starch degradation and technological instances. In fact, by a technological standpoint, it could be important to minimize the quote of SGF as it could affect textural and other sensory traits of pasta, thus compromising its acceptability (Bianchi et al., 2021).

The release of polyphenols into the water-soluble fraction of the digesting chyme was monitored by RP-HPLC with UV-detection at 340 nm. This analysis demonstrated a progressive increase in the concentration of SGF polyphenols during digestion, which resulted from the breakdown of the starchy-gluten network of pasta. For example, RP-HPLC analysis of constant volumes that were sampled from the

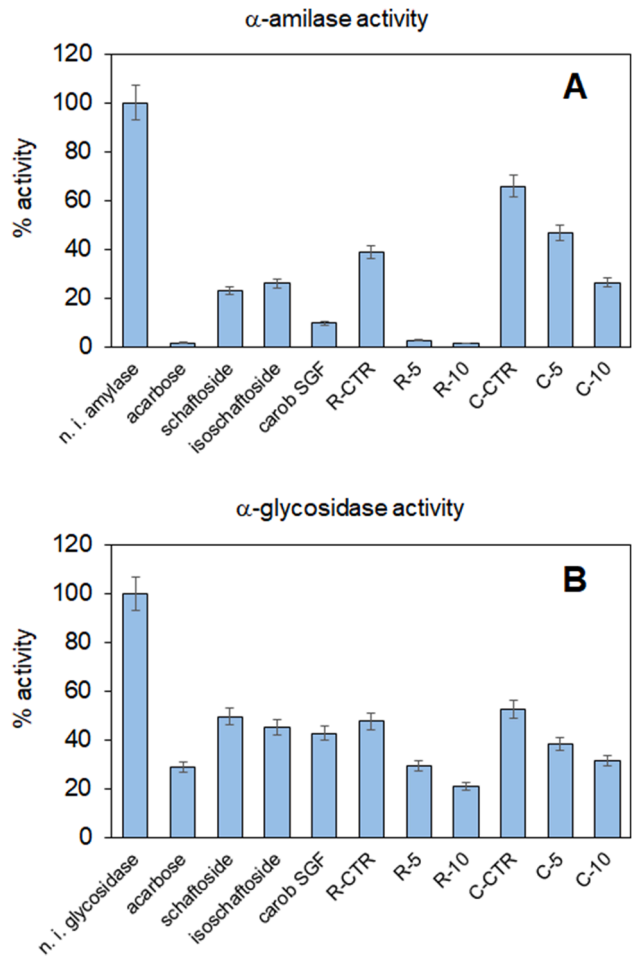


**Fig. 3.** Activity (%) of porcine pancreatic α-amylase (A) and porcine small intestinal α-glycosidases (B). The activity of non-inhibited enzymes (n.i.) was set at 100 %. Samples: activity (%) after inhibition with 0.1 mM acarbose (positive control), 0.1 mM schaftoside and isoschaftoside, extracts from SGF (tenfold diluted) and from raw or cooked pasta samples. Error bars represent the relative standard deviation.

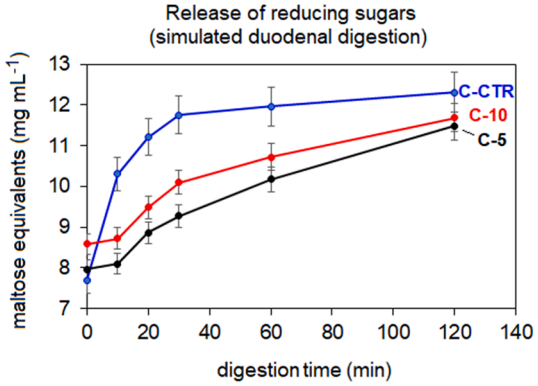


**Fig. 4.** Release of reducing sugars from cooked pasta samples during the duodenal phase of simulated digestion. The concentration of reducing sugars (expressed as mg $mL^{-1}$ maltose equivalents) was determined with the DNSA assay using a calibration curve built with pure maltose.

soluble fraction of C-10 digests at three time points (t = 0, 20 and 120 min) clearly showed the increase in the schaftoside peak area (Fig. 5). These findings demonstrated a significant bioaccessibility of SGF polyphenols that could be released in the intestinal lumen for exerting

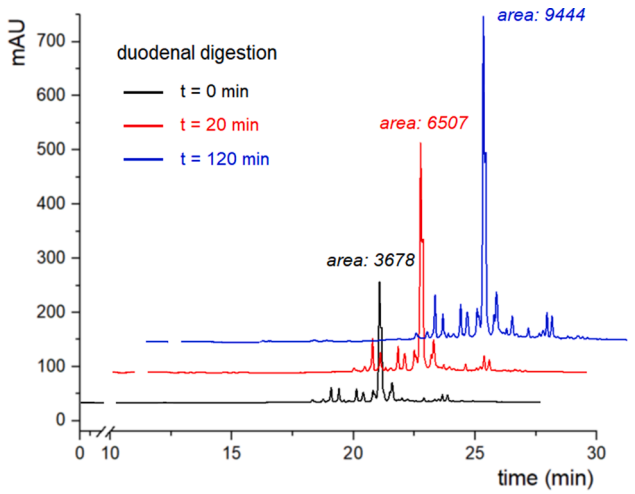


**Fig. 5.** RP-HPLC-UV monitoring (λ = 340 nm) of SGF polyphenols released in the chyme during the duodenal phase of simulated digestion. Chromatograms resulted from the analysis of a constant volume of duodenal digestate of C-10 sampled at 0, 20 and 120 min, and evidenced the progressively increasing concentration of soluble SGF flavonoids.

inhibitory activity against both duodenal α-amylase and small intestinal α-glycosidases. Interestingly, schaftoside, isoschaftoside and vicenin-2 (apigenin 6,8-di-*C*-glucoside) have been described also as potent inhibitors of pancreatic lipase (Fernando et al., 2019), which mediates the digestion and adsorption of acylglycerols. Therefore, SGF could be a source of interesting phytochemical compounds for preventing and treating overweight and metabolic syndrome in general, overall contributing to reduce the risk factors of cardiovascular diseases.

## 4. Conclusions

SGF is a food ingredient with unique nutritional and technological potentiality because of the large quantity of hypoallergenic, non-celiacogenic and good biological value proteins, the relative high content of phospholipids and, especially, the abundance of flavonoids, such as apigenin *C*-glycosides. Food flavonoids are among the most intensively investigated polyphenol classes because, in addition to their health-promoting properties associated with the antioxidant, antiaging and anticancer potential, they may potently inhibit carbohydrate hydrolysing enzymes. By inhibiting α-amylase and intestinal α-glycosidases, dietary flavonoids can reduce postprandial glycaemia and enhance the insulin sensitivity, which makes them beneficial for the prevention and management of type-2 diabetes, without associated adverse gastrointestinal effects. Importantly, dietary flavonoids show extremely low acute toxicity (Barber, Houghton & Williamson, 2021).

In this study, we demonstrated that SGF flavonoids significantly inhibits porcine pancreatic α-amlyase and small intestinal α-glycosidases, selected as models of the human carbohydrate digesting enzymes. The presence of 5 and 10 % (w/w) SGF in pasta delays starch digestion and the enzyme inhibiting properties are substantially retained after pasta cooking. Therefore, a partial replacement of wheat flour with SGF could represent a dietary strategy for reducing the onset of insulin resistance and type-2 diabetes. In addition, SGF proteins are high in lysine, and can compensate for the deficiency typical of cereals such as wheat, enabling the preparation of high nutritional value pasta or other products.

SGF is the by-product resulting from the agro-industrial processing of an underutilized and neglected crop, which can be grown in semiarid and marginal soils. For this reason, an extensive exploitation of SGF meets the criteria of the sustainability. In consideration of the promising *in vitro* outcomes, the benefits of SGF and apigenin *C*-glycosydes on human health, specifically antioxidant and glycaemic index lowering potential, still await to be confirmed with purposely designed *in vivo*

studies.

## CRediT authorship contribution statement

**Francesco Siano:** Conceptualization, Formal analysis, Funding acquisition, Data curation. **Gianfranco Mamone:** Conceptualization, Data curation, Investigation. **Ermanno Vasca:** Conceptualization, Data curation, Investigation. **Maria Cecilia Puppo:** Conceptualization, Data curation, Writing - review & editing. **Gianluca Picariello:** Conceptualization, Formal analysis, Funding acquisition, Writing - review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

## Acknowledgements

The Authors gratefully thank Carob Ingredients Co., Casablanca, Morocco (http://www.carobingredients.com), for providing carob seed germ flour used in this research. This work was supported by the ALI-FUN (PON MUR 2018; ARS01_00783) and by the NutrAge FOE projects of Italian National Research Council (CNR).

## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2023.112962.

## References

Al Duhaidahawi, D., Hasan, S. A., & Al Zubaidy, H. (2021). Flavonoids in the treatment of diabetes: Clinical outcomes and mechanism to ameliorate blood glucose levels. *Current Diabetes Reviews, 17*.

Al-Ishaq, R. K., Abotaleb, M., Kubatka, P., Kajo, K., & Büsselberg, D. (2019). Flavonoids and their anti-diabetic effects: Cellular mechanisms and effects to improve blood sugar levels. *Biomolecules, 9*, 430.

Avallone, R., Plessi, M., Baraldi, M., & Monzani, A. (1997). Determination of chemical composition of carob (*Ceratonia siliqua*): Protein, fat, carbohydrates, and tannins. *Journal of Food Composition and Analysis, 10*, 166–172.

Ayaz, F. A., Torun, H., Glew, R. H., Bak, Z. D., Chuang, L. T., Presley, J. M., & Andrews, R. (2009). Nutrient content of carob pod (*Ceratonia siliqua* L.) flour prepared commercially and domestically. *Plant Foods for Human Nutrition, 64*, 286–292.

Barak, S., & Mudgil, D. (2014). Locust bean gum: Processing, properties and food applications - A review. *International Journal of Biological Macromolecules, 66*, 74–80.

Barber, E., Houghton, M. J., & Williamson, G. (2021). Flavonoids as Human Intestinal α-Glucosidase Inhibitors. *Foods, 10*, 1939.

Barrett, A., Ndou, T., Hughey, C. A., Straut, C., Howell, A., Dai, Z. F., & Kaletunc, G. (2013). Inhibition of alpha-amylase and glucoamylase by tannins extracted from cocoa, pomegranates, cranberries, and grapes. *Journal of Agricultural and Food Chemistry, 61*, 1477–1486.

Batlle, I., & Tous, J. (1997). Carob Tree. *Ceratonia siliqua* L. *International Plant Genetic Resources Institute*. Rome. 92 p.

Bengoechea, C., Romero, A., Villanueva, A., Moreno, G., Alaiz, M., Millán, F., Guerrero, A., & Puppo, M. C. (2008). Composition and structure of carob (*Ceratonia siliqua* L.) germ proteins. *Food Chemistry, 107*, 675–683.

Bianchi, F., Tolve, R., Rainero, G., Bordiga, M., Brennan, C. S., & Simonato, B. (2021). Technological, nutritional and sensory properties of pasta fortified with agro-industrial by-products: A review. *International Journal of Food Science and Technology, 56*, 4356–4366.

Bienenstock, M., Csaski, L., Pless, J., Sagi, A., & Sagi, E. (1935). U.S. Patent 2,025,705.

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., … Recio, I. (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature Protocols, 14*, 991–1014.

Buchholz, T., & Melzig, M. F. (2016). Medicinal plants traditionally used for treatment of obesity and diabetes mellitus - Screening for pancreatic lipase and α-amylase inhibition. *Phytotherapy Research: PTR, 30*(2), 260–266.

Chen, Z., Sun, Y., Wang, G., Zhang, Y., Zhang, Q., Zhang, Y., Li, J., & Wang, Y. (2021). De novo biosynthesis of *C*-arabinosylated flavones by utilization of *indica* rice *C*-glycosyltransferases. *Bioresources and Bioprocessing, 8*, 49.

Custódio, L., Escapa, A. L., Fernandes, E., Fajardo, A., Aligué, R., Alberício, F., Neng, N., Nogueira, J. M. F., & Romano, A. (2011). Phytochemical profile, antioxidant and cytotoxic activities of the carob tree (*Ceratonia siliqua* L.) germ flour extracts. *Plant Foods for Human Nutrition, 66*, 78–84.

Dakia, P. A., Wathelet, B., & Paquot, M. (2007). Isolation and chemical evaluation of carob (*Ceratonia siliqua* L.) seed germ. *Food Chemistry, 102*, 1368–1374.

European Directorate for the Quality of Medicines. (2007). Council of Europe. Determination of tannins in herbal drugs. European Pharmacopoeia (6th ed.). France: Strasbourg. p. A286.

Dirir, A. M., Daou, M., Yousef, A. F., & Yousef, L. F.. (2022). A review of alpha-glucosidase inhibitors from plants as potential candidates for the treatment of type-2 diabetes. *Phytochemistry Reviews, 21*, 1049–1079.

Fernando, W. I. T., Attanayake, A. M. K. C., Perera, H. K. I., Sivakanesan, R., Jayasinghe, L., Araya, H., & Fujimoto, Y. (2019). Isolation, identification and characterization of pancreatic lipase inhibitors from Trigonella foenum-graecum seeds. *South African Journal of Botany, 121*, 418–421.

Ferreres, F., Silva, B. M., Andrate, P. B., Seabra, R. M., & Ferreira, M. A. (2003). Approach to the study of *C*-glycosyl flavones by ion trap HPLC-PAD-ESI/MS/MS: Application to seeds of quince (Cydonia oblonga). *Phytochemical Analysis, 14*, 352–359.

Fidan, H., Stankov, S., Petkova, N., Petkova, Z., Iliev, A., Stoyanova, M., Ivanova, T., Zhelyazkov, N., Ibrahim, S., Stoyanova, A., & Ercisli, S. (2020). Evaluation of chemical composition, antioxidant potential and functional properties of carob (*Ceratonia siliqua* L.) seeds. *Journal of Food Science and Technology, 57*, 2404–2413.

Fujisawa, T., Ikegami, H., Inoue, K., Kawabata, Y., & Ogihara, T. (2005). Effect of two alpha-glucosidase inhibitors, voglibose and acarbose, on postprandial hyperglycemia correlates with subjective abdominal symptoms. *Metabolism - Clinical and Experimental, 54*, 387–390.

Ibrahim, R., Abdel-Salam, F., & Farahat, E. (2020). Utilization of carob (*Ceratonia siliqua* L.) extract as functional ingredient in some confectionery products. *Food Science & Nutrition, 11*, 757–772.

Kumar, S., Mittal, A., Babu, D., & Mittal, A. (2021). Herbal medicines for diabetes management and its secondary complications. *Current Diabetes Reviews, 17*, 437–456.

Li, H., Song, F., Xing, J., Tsao, R., Liu, Z., & Liu, S. (2009). Screening and structural characterization of α-glucosidase inhibitors from hawthorn leaf flavonoids extract by ultrafiltration LC-DAD-MSn and SORI-CID FTICR MS. *Journal of the American Society for Mass Spectrometry, 20*, 1496–1503.

Mamone, G., Sciammaro, L., De Caro, S., Di Stasio, L., Siano, F., Picariello, G., & Puppo, M. C. (2019). Comparative analysis of protein composition and digestibility of Ceratonia siliqua L. and Prosopis spp. seed germ flour. *Food Research International, 120*, 188–195.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry, 31*, 426–428.

Moelands, S. V., Lucassen, P. L., Akkermans, R. P., De Grauw, W. J., & Van de Laar, F. A. (2018). Alpha-glucosidase inhibitors for prevention or delay of type 2 diabetes mellitus and its associated complications in people at increased risk of developing type 2 diabetes mellitus. *The Cochrane Database of Systematic Reviews, 12*, CD005061.

Ombra, M. N., d'Acierno, A., Nazzaro, F., Spigno, P., Riccardi, R., Zaccardelli, M., Pane, C., Coppola, R., & Fratianni, F. (2018). Alpha-amylase, α-glucosidase and lipase inhibiting activities of polyphenol-rich extracts from six common bean cultivars of Southern Italy, before and after cooking. *International Journal of Food Sciences and Nutrition, 69*, 824–834.

Oniszczuk, A. (2016). LC-ESI-MS/MS analysis and extraction method of phenolic acids from gluten-free precooked buckwheat pasta. *Food Analytical Methods, 9*, 3063–3068.

Pérez, M. J., Cuello, A. S., Zampini, I. C., Ordoñez, R. M., Alberto, M. R., Quispe, C., Schmeda-Hirschmann, G., & Isla, M. I. (2014). Polyphenolic compounds and anthocyanin content of Prosopis nigra and Prosopis alba pods flour and their antioxidant and anti-inflammatory capacities. *Food Research International, 64*, 762–771.

Picariello, G., Miralles, B., Mamone, G., Sánchez-Rivera, L., Recio, I., Addeo, F., & Ferranti, P. (2015). Role of intestinal brush border peptidases in the simulated digestion of milk proteins. *Molecular Nutrition & Food Research, 59*, 948–956.

Picariello, G., Sciammaro, L., Siano, F., Volpe, M. G., Puppo, M. C., & Mamone, G. (2017). Comparative analysis of C-glycosidic flavonoids from Prosopis spp. And Ceratonia siliqua seed germ flour. *Food Research International, 99*, 730–738.

Plaut, M., Zelcbuch, B., & Guggenhem, K. (1953). Nutritive and baking properties of carob germ flour. *Bulletin of the Research Council of Israel, 3*, 129–131.

Rohn, S., Rawel, H. M., & Kroll, J. (2002). Inhibitory effects of plant phenols on the activity of selected enzymes. *Journal of Agricultural and Food Chemistry, 50*, 3566–3571.

Salinas, M. V., Carbas, B., Brites, C., & Puppo, M. C. (2015). Influence of different carob fruit flours (*Ceratonia siliqua* L.) on wheat dough performance and bread quality. *Food and Bioprocess Technology, 8*, 1561–1570.

Sciammaro, L. P., Ribotta, P. D., & Puppo, M. C. (2016). Traditional food products from Prosopis sp. flour. In: K. Kristbergsson & J. Oliveira (Eds.), Traditional foods. General and consumer aspects. ISEKI Food Series. Vol. 10. Traditional foods. General and consumer aspects. ISEKI Food Series (pp. 209–216). Springer.

Sciammaro, L. P., Ferrero, C., & Puppo, M. C. (2016a). Chemical and nutritional proprierties of different fractions of Prosopis alba pods and seeds. *Journal of Food Measurement and Characterization, 10*, 103–112.

Siano, F., Sciammaro, L., Volpe, M. G., Mamone, G., Puppo, M. C., & Picariello, G. (2018). Integrated analytical methods to characterize lipids from Prosopis spp. and Ceratonia siliqua seed germ flour. *Food Analytical Methods, 11*, 3471–3480.

Smith, B. M., Bean, S. R., Schober, T. J., Tilley, M., Herald, T. J., & Aramouni, F. (2010). Composition and molecular weight distribution of carob germ protein fractions. *Journal of Agricultural and Food Chemistry, 58*, 7794–7800.

Şöhretoğlu, D., & Sari, S. (2020). Flavonoids as alpha-glucosidase inhibitors: Mechanistic approaches merged with enzyme kinetics and molecular modelling. *Phytochemistry Reviews, 19*, 1081–1092.

Srivastava, M., & Kapoor, V. P. (2005). Seed galactomannans: An overview. *Chemistry & Biodiversity, 2*, 295–317.

Sun, L., & Miao, M. (2020). Dietary polyphenols modulate starch digestion and glycaemic level: A review. *Critical Reviews in Food Science and Nutrition, 60*, 541–555.

Sun, L., Warren, F. J., & Gidley, M. J. (2018). Soluble polysaccharides reduce binding and inhibitory activity of tea polyphenols against porcine pancreatic α-amylase. *Food Hydrocolloids, 79*, 63–70.

Tadera, K., Minami, Y., Takamatsu, K., & Matsuoka, T. (2006). Inhibition of alpha-glucosidase and alpha-amylase by flavonoids. *Journal of Nutritional Science and Vitaminology, 52*, 149–153.

Takahama, U., & Hirota, S. (2018). Interactions of flavonoids with α-amylase and starch slowing down its digestion. *Food & Function, 9*, 677–687.

Tsatsaragkou, K., Gounaropoulos, G., & Mandala, I. (2014). Development of gluten free bread containing carob flour and resistant starch. *LWT - Food Science and Technology, 58*, 124–129.

Wang, Z. L., Gao, H. M., Wang, S., Zhang, M., Chen, K., Zhang, Y. Q., …, Ye, M. (2020). Dissection of the general two-step di-C-glycosylation pathway for the biosynthesis of (iso)schaftosides in higher plants. *Proceedings of the National Academy of Sciences of the United States of America*, 117, 30816–30823.

Yousif, A. K., & Alghzawi, H. M. (2000). Processing and characterization of carob powder. *Food Chemistry, 69*, 283–287.

Zeng, L., Zhang, G., Lin, S., & Gong, D. (2016). Inhibitory mechanism of apigenin on α-glucosidase and synergy analysis of flavonoids. *Journal of Agricultural and Food Chemistry, 64*, 6939–6949.

Zhu, J., Chen, C., Zhang, B., & Huang, Q. (2020). The inhibitory effects of flavonoids on α-amylase and α-glucosidase. *Critical Reviews in Food Science and Nutrition, 60*, 695–708.