



Intake of soluble fibre from chia seed reduces bioaccessibility of lipids, cholesterol and glucose in the dynamic gastrointestinal model simgi®



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ABSTRACT

The role of soluble fibres on hypoglycemic and hypocholesterolemic effects has been widely documented, but the effect on glucose and cholesterol binding capacity of soluble fibre extracted from chia seed mucilage has not been studied until now. In the present research, dynamic gastrointestinal model simgi® combined with absorption static techniques have been used to explore the effect of chia seed mucilage at 0.75 and 0.95% w/w on the bioaccessibility of glucose, dietary lipids and cholesterol along the gastrointestinal tract.

Glucose bioaccessibility was reduced when 0.95% of chia mucilage was present in sugar food models. The total reduction of glucose bioaccessibility reached a maximum of 66.7% while glucose dialysis retardation index presented its maximum of 53.4% at the end of small intestine digestion. The in vitro studies with lipid food models, showed that the presence of both, 0.75 and 0.95% of chia seed mucilage caused substantial reductions on the bioaccessibility of free fatty acids (16.8 and 56.1%), cholesterol (18.2 and 37.2% respectively) and bile salts (4.8 and 64.6%), revealing a clear dependence on fibre concentration. These innovative results highlight the potential functionality of the soluble fibre extracted from chia seeds to improve lipid and glycemic profiles and suggest the dietary health benefits of this new soluble fibre source as an ingredient in functional foods designed to reduce the risk of certain non-communicable diseases.

1. Introduction

During the last years, the role of diet in non-communicable diseases has been widely evidenced through epidemiological and social studies. Within these diseases, the progressive increase in overweight and obesity is especially relevant. Global overweight, considered as adults with body mass index (BMI) ≥ 25 kg/m², increased from 20.2% of average on 1975 until 39.1% on 2016 (Fleischer, Diez Roux, Alazraqui, & Spinelli, 2008; Ng et al., 2014). Lifestyle and diet changes related to economic development and globalization have favoured the consume of foods with high energy, fats, free sugars and sodium at the expense of the decrease in fruits, vegetables and whole grains intake, which implies lower levels of dietary fibre ingestion (WHO, 2015; WHO/FAO, 2003). A balanced diet is a critical factor in a healthy lifestyle. Therefore, the reduction of high fat, high cholesterol and high sugar foods would have a positive effect on health, particularly for subjects that have a predisposition for non-communicable diseases. However, as Minekus et al. (2005) described, it is also possible to reduce the adverse

effects of over-ingestion by adding to the meals compounds that are able to reduce these components absorption.

Dietary fibre has been recognized as an essential component in a healthy diet. In fact, fibre can promote several beneficial physiological effects such as reduction of the risk of cardiovascular disease (Theuwissen & Mensink, 2008), prevention or reduction of metabolic syndrome (Aleixandre & Miguel, 2008; Weickert & Pfeiffer, 2008) and prevention of gastrointestinal disorders (Müller, Canfora, & Blaak, 2018) among others. Several studies have reported that the presence of any dietary fibre in the upper gastrointestinal tract (GIT) will result in a decreased rate of intestinal nutrient absorption (Adams, Sello, Qin, Che, & Han, 2018; Gidley, 2013; Lattimer & Haub, 2010; Tungland & Meyer, 2002) and, as it was previously exposed by Brownlee (2011), this reduction can be beneficial but also concerning; because of the possibility of reducing the absorption of essential micronutrients such as minerals and vitamins or phytochemicals.

Regarding the effects related with over-ingestion, high-fibre diets, especially those with high presence of dietary fibre, are associated with

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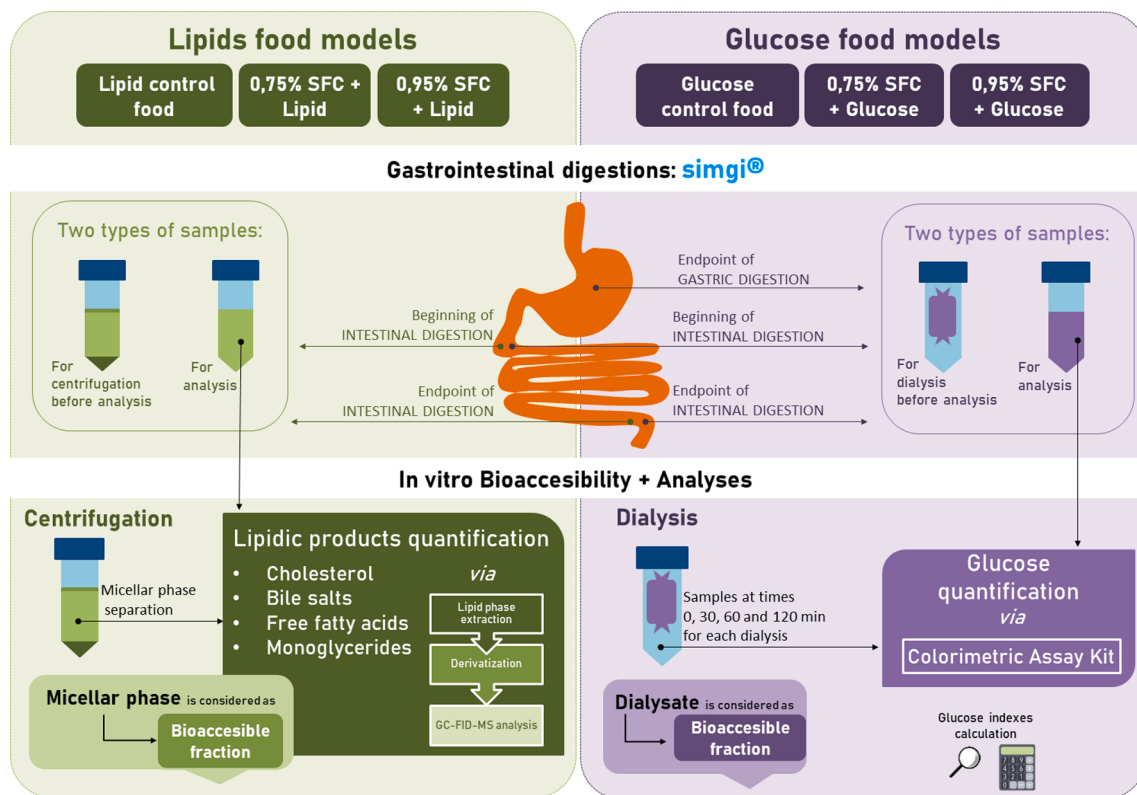


Fig. 1. Experimental setup carried out to investigate the effect of two different concentrations (expressed as % of w/w) of soluble fibre from chia seed (SFC) on the bioaccessibility of glucose, fats and cholesterol by using the dynamic gastrointestinal simulator simgi®.

an improvement in serum lipid and glucose concentrations; showing that the physicochemical factors of dietary fibres are important in these roles (Shah et al., 2009). For example, concerning cholesterol absorption, viscous or gel forming water-soluble fibres can reduce absorption rate more than low molecular weight and low viscosity fibres (Brownlee, 2011). The potential health benefits of each type of fibre also depend on their water-soluble and water-insoluble composition (Eswaran, Muir, & Chey, 2013). Water-insoluble fibre is mainly responsible for increasing stool bulk, which helps to regulate bowel movements and reduce transit time through the gut (Vuksan, Rogovik, Jovanovski, & Jenkins, 2009). Alternatively, water-soluble fibre effects are mainly related to gel-forming capabilities, viscosity and fermentation rate by gut microbiota (Weickert & Pfeiffer, 2018). Rideout, Harding, Jones, and Fan (2008) also reported that soluble fibre produces larger hypocholesterolemic and hypoglycemic responses compared with insoluble fibre.

Different mechanisms whereby fibre reduces the bioavailability of lipids, cholesterol and glucose have been previously described (Fernandez, 1995; Galisteo, Duarte, & Zarzuelo, 2008; Papathanasopoulos & Camilleri, 2010). The main effects are associated with viscous and gel-forming properties of soluble fibre (Brownlee, 2014; Chawla & Patil, 2010). Thus, a promising strategy to prevent or reduce non-communicable diseases could be to add soluble fibre to decrease the intestinal absorption of glucose, dietary lipids and cholesterol. In this sense, mucilages are an example of soluble fibre that form high viscosity dispersions at low concentrations, which seems to partially explain their several health benefits such as modulation of postprandial glycemic and insulinemic responses, hyperlipidemia counteracting, satiety enhancement or regulation of gut microbiota function (Soukoulis, Gaiani, & Hoffmann, 2018).

Mucilages can be found in different natural sources, being seeds the richest. Chia seeds (*Salvia hispanica* L.) are a well-known example of mucilage source (de Falco, Amato, & Lanzotti, 2017; Ullah et al., 2016). The seed is an important source of omega-3 fatty acids, proteins and

carbohydrates, including soluble and insoluble fibre, that offer enormous potential as functional ingredients. Some of its healthy benefits have been summarized in a previous work by Muñoz, Cobos, Diaz, and Aguilera (2013). The chemical structure of chia seed mucilage has not been elucidated yet, but a tentative structure was proposed by Lin, Daniel, and Whistler (1994) consisting of a tetrasaccharide with 4-O-methyl- α -D-glucuronopyranosyl residues occurring as branches at O-2 of some β -D-xylopyranosyl residues in the main chain of (1 $\rightarrow$ 4)- β -D-xylopyranosyl-(1 $\rightarrow$ 4)- α -D-glucopyranosyl-(1 $\rightarrow$ 4)- β -D-xylopyranosyl units. Although previous studies about the effects of soluble fibre from chia seed at the gastrointestinal level are scarce, a recent study where soluble fibre from chia seed was digested using the dynamic digestion simulator simgi® showed that viscosity was not reduced through the different stages of the GIT (Tamargo, Cueva, Laguna, Moreno-Arribas, & Muñoz, 2018). This behaviour might indicate that soluble fibre from chia seed could influence dietary compounds bioaccessibility. To the best of our knowledge, detailed information about chia seed soluble fibre effect on gastrointestinal behaviour, glucose, lipids or cholesterol bioaccessibility, has not been reported till the moment.

Nowadays, it is well known that in vitro gastrointestinal models of digestion are a useful approach for obtaining preliminary and valuable information concerning the digestion process of either isolated compounds or complex mixtures. Diverse in vitro gastrointestinal models of digestion can be found in the scientific literature trying to simulate physiological conditions. In this sense, some of the most interesting models are those of dynamic nature which include most of the physicochemical changes that take place along the GIT: rhythmic peristaltic contractions, gradual bolus flow between the different compartments, progressive incorporation of digestive secretions or intestinal absorption (Dupont et al., 2019; Li, Yu, Wu, & Chen, 2020). Therefore, considering that viscosity and gel-formation properties of soluble fibre might interfere with the physicochemical changes of the digestion process, in vitro dynamic models could be a useful tool to test soluble fibres interaction with dietary compounds bioaccessibility.

The aim of the present study was to investigate for the first time the effect of soluble fibre from chia seed (SFC) on the bioaccessibility of glucose, dietary lipids and cholesterol by using the dynamic gastrointestinal simulator simgi®. After the intake of glucose or lipid food models with or without SCF supplementation, glucose, lipid products, cholesterol and bile salts were analysed in simgi® stomach (ST) and simgi® small intestine (SI) digested samples.

2. Materials and methods

The experimental plan is schematized in Fig. 1.

2.1. Materials

Chia seeds were provided by Benexia (Functional Products Trending S.A., Chile). D-glucose used for preparing glucose food model was purchased from Difco™ (BD, USA). Cholesterol, high refined olive oil and L- α -Phosphatidylcholine used for lipid food model, were purchased from Sigma-Aldrich (Merk, USA). Materials of Gut Nutrient Medium (GNM) formulation were purchased as follow: arabinogalactan, pectin from citrus peel and mucin from porcine stomach from Sigma-Aldrich; xylan from Carbosynth (UK), yeast extract and special peptone from Oxoid (ThermoScientific, USA); soluble starch from Difco™ and L-cysteine from Panreac (Spain). Pepsin and pancreatin from porcine pancreas powder were purchased from Sigma-Aldrich, and Oxgall Dehydrated Fresh Bile was purchased from Difco™. Other reagents as NaCl, NaHCO₃, HCl or NaOH were purchased from VWRchemicals (USA). D-Glucose Assay kit (GOPOD Format) used for glucose quantification, was purchased from Megazyme (Ireland).

2.2. Formulation of glucose and lipid food models

The amounts of SFC and the doses of glucose and cholesterol used for the food models development were selected based on the recommendations for dietary fibre, glucose and cholesterol intake from the European Food Safety Authority (EFSA), Food and Drug Administration of the United States (FDA) and World Health Organization (WHO) (EFSA, 2010).

2.2.1. SFC suspensions

Crude mucilage from chia seeds was extracted by using the methodology proposed by Muñoz, Aguilera, Rodríguez-Turiénzo, Cobos, and Diaz (2012). In brief, samples of chia seeds were soaked in distilled water 1:40 w/v seed:water ratio during 2 h at 20 °C under constant stirring. After that time, the hydrated seeds were put on a drying tray and exposed to a temperature of 50 °C for 10 h in a forced air oven (Biobase Model BOV-V70F, China). Then, the dried SFC was separated from the seed by rubbing over 40 mesh screen. SFC was kept at room temperature until its use.

Right before composing food models, SFC suspensions at 0.75 and 0.95% w/w of extracted chia mucilage on 80 mL of Gut Nutrient Medium were prepared at 37 °C under constant stirring and 1200 rpm for 2 h and according to Lazaro, Puente, Zúñiga, and Muñoz (2018). Modified GNM was used instead of GNM to prepare SFC suspensions used with glucose food model.

2.2.2. Glucose food model

Two different kinds of glucose food models were prepared: i) glucose control food (0% of SFC) and ii) glucose + SFC foods. Glucose control food was prepared by adding 1 g of D-glucose to 80 mL of modified GNM. Glucose + SFC foods were prepared by adding 1 g of D-glucose to 80 mL of 0.75 or 0.95% SFC suspensions. The prepared food models were homogenized at 37 °C and 1200 rpm for 3 min before gastrointestinal digestion or sampling. The final concentration of D-glucose was maintained for the two kinds of glucose food models.

2.2.3. Lipid food model

Two different kinds of lipid food models were prepared: i) lipid control food (0% of SFC) and ii) lipid + SFC foods. Lipid control food was prepared by mixing 100 mg of cholesterol with 1 g of refined olive oil at 50 °C using a vortex for 2 min. Then, the lipid mixture was homogenized with GNM by Ultra-Turrax® (IKA, Deutschland) at 50 °C and 7000 rpm during 2 min. The formation of the emulsion was favoured by including L- α -Phosphatidylcholine at 4% (w/w of oil content) during the homogenization. Lipid + SFC foods were prepared following the above method but homogenizing the lipid mixture (100 mg of cholesterol and 1 g of refined olive oil) with 0.75 or 0.95% SFC suspensions. The prepared food models were kept at 37 °C and 1200 rpm for 3 min before gastrointestinal digestion or sampling. The final concentration of cholesterol, olive oil and L- α -Phosphatidylcholine was maintained for the two kinds of lipid food models.

2.3. Gastrointestinal digestions

Eight complete gastrointestinal digestions were carried out by using the dynamic gastrointestinal model simgi®. Glucose control food and lipid control food were digested in duplicate, while [glucose + 0.75% SFC food], [glucose + 0.95% SFC food], [lipid + 0.75% SFC food] and [lipid + 0.95% SFC food] were digested once.

Simgi® is a computer-controlled gastrointestinal in vitro model designed to simulate the physiological processes taking place during digestion in the stomach and small intestine, while also reproducing the colonic microbiota responsible for metabolic bioconversions in the large intestine (Cueva et al., 2015). The flexible and modular design of the system allows continuous or staged simulation on its five compartments: stomach, small intestine, ascending, transverse and descending colon. Gastrointestinal digestions on this study were performed by using just ST and SI compartments operating continuously.

2.3.1. Gut nutrient medium

Standard media used in simgi® is called Gut Nutrient Medium. This media acts as a support for digestion and a complete matrix for added ingredients. GNM contains, arabinogalactan (1 g/L), pectin from citrus peel (2 g/L), xylan (1 g/L), soluble starch (3 g/L), D-glucose (0.4 g/L), yeast extract (3 g/L), special peptone (1 g/L), mucin from porcine stomach (4 g/L) and L-cysteine (0.5 g/L). All compounds were dissolved in distilled water and pH was adjusted to pH = 6 by using NaOH 10 M before the solution was sterilized at 121 °C for 21 min. In the case of glucose studies, the GNM medium was exceptionally prepared without D-glucose; this is referred as modified GNM.

2.3.2. Simulated gastrointestinal fluids

Simulated gastric juice is a solution that consisted of NaCl (9 g/L) and pepsin (2000 U/mL). This solution was kept at 4 °C until gastric digestion. Simulated pancreatic juice consisted of NaHCO₃ (12 g/L), Oxgall Dehydrated Fresh Bile (6 g/L) and pancreatin from porcine pancreas powder (0.9 g/L). All compounds were dissolved in distilled water and the solution was filtered by polyethersulfone (PES) 0.45 μ m pore membrane.

2.3.3. Simulation set-up on simgi®

Simgi® digestion parameters were selected according to those previously reported by Tamargo et al. (2018), where they were widely described. The system was tempered before each food model intake. ST compartment was prefilled with 95 mL of pH = 1.8 GNM and SI vessel was prefilled with 55 mL of pH = 7.0 GNM. Modified GNM was used to prefill the system on Glucose food model digestions instead of GNM.

An acute intake of each studied food model (80 mL) flowed to the ST where the peristaltic movements mixed it with the fasting ST content. Then, 15 mL of simulated gastric juice flowed to the ST. During gastric digestion, HCl was added gradually until the pH of the gastric content decreased from the food intake value to 1.8. Elashoff function (Elashoff,

Reedy, & Meyer, 1982) was used to determine gastric emptying flow to SI in order to simulate physiological gastric emptying. On SI, chemo was mixed gradually with 40 mL of simulated pancreatic juice and the fasting SI content. Anaerobic conditions, 150 rpm stirred movement, 37 °C and pH 7.0 ± 0.2 were maintained during the 120 min of intestinal digestion. All digestions were performed under identical conditions.

2.4. In vitro bioaccessibility and analyses

The evolution of the bioaccessibility was determined along the GIT to evaluate the effect of SFC on glucose and lipid food models. Digested model foods of glucose or lipids without SFC were considered controls on the corresponding digestion stage.

2.4.1. Glucose in vitro bioaccessibility

For glucose food model digestions, the glucose bioaccessibility was evaluated i) at the endpoint of gastric digestion (EGD), ii) at the beginning intestinal digestion (BID) and iii) at the endpoint of the intestinal stage (EID).

To determinate bioaccessibility of glucose, samples of 5 mL of each digestion stage were taken and glucose gastrointestinal absorption was simulated by using dialysis sacks according to the methodology proposed by Ou, Kwok, Li, and Fu (2001). Each dialysis simulation was carried out immediately after sampling and the dialysate samples were taken by triplicate at 0, 30, 60 and 120 min on every dialysis simulation. Dialysis sacks with size pore 12 KDa were purchased from Sigma-Aldrich (Merck, USA).

The % of glucose bioaccessibility reduction after 120 min of dialysis was calculated as it is described on Eq. (1):

$$\% \text{ Bioaccessibility reduction} = \left(\frac{\frac{\text{Absorbable glucose of SFC food models}}{\text{Glucose concentration on SFC food models}}}{\frac{\text{Absorbable glucose of 0\% SFC}}{\text{Glucose concentration 0\% SFC}}} - 1 \right) \cdot 100 \quad (1)$$

In the Eq. (1), the absorbable glucose of SFC food model corresponds to the glucose concentration (mM) in EID dialysate at 120 min of dialysis; the glucose concentration on SFC food model is referred to the total glucose concentration (mM) in the corresponding food model formulation.

The glucose dialysis retardation index (GDRI) was determined as described by Rodríguez-Gutiérrez, Rubio-Senent, Lama-Muñoz, García, and Fernández-Bolaños (2014) as:

$$\text{GDRI} = 100 - \left(\frac{\text{Total glucose diffused sample}}{\text{Total glucose diffused control}} \right) \cdot 100 \quad (2)$$

Considering “samples” as the food models that include soluble fibre from chia seed (0.75% SFC and 0.95% SFC food models) and “control” the food models with 0% SFC. The “total glucose diffused” is calculated as the increase from time 0 min of glucose concentration (mM) present in the dialysate prorated by the retained fraction in the respective digestion stage at the start of dialysis. All calculated at the same corresponding time and digestion stage. GDRI was calculated at times 30, 60 and 120 min for EGD, BID and EID stages.

2.4.2. Determination of glucose

Total glucose content was monitored during the whole digestion of glucose model foods: (i) before the intake, (ii) at the endpoint of gastric digestion (EGD), (iii) at the beginning of intestinal digestion (BID) and (iv) at the endpoint of the intestinal digestion (EID). For dialysis simulations glucose content of the dialysate was determined at 0, 30, 90 and 120 mins.

A colorimetric D-Glucose Assay kit was used to quantify glucose concentrations. The method is based on the oxidase/peroxidase reaction that forms a coloured compound, which can be measured at 510 nm. Samples were diluted to adequate its estimated glucose concentration to the measure range of the kit. Analyses were carried out by

triplicate.

2.4.3. Lipid in vitro bioaccessibility

For lipid food model digestions, the bioaccessibility of cholesterol and lipid products (free fatty acids (FFA) and monoglycerides (MG)) was evaluated i) before the intake and ii) at the endpoint of the intestinal digestion (EID). For this last point (ii) the bioaccessibility of bile salts was also evaluated. To determinate bioaccessibility of all these compounds, the aqueous micellar phase, which would contain the bioaccessible fraction of cholesterol, lipid products and bile salts was isolated from the total digestion medium by centrifugation at 4000 rpm during 40 min (Martin et al., 2016). The bioaccessibility of each compound was determined according to Martin et al. (2016) as Eq. (3):

$$\% \text{ Lipid compounds bioaccessibility} = \frac{\text{mg of compound in micellar phase}}{\text{mg of compound in digestion medium}} \cdot 100 \quad (3)$$

2.4.4. Determination of lipid products, cholesterol and bile salts

The quantification of lipid products, cholesterol and bile salts before the intake and after intestinal digestion (EID) was carry out as described on Martin et al. (2016). Previously, the lipid phase of each sample was extracted with hexane:methyl *tert*-butyl ether (50:50 v/v) with a ratio of 3:1 (v/v) of solvent to sample. This mixture was vortexed for 1 min and centrifuged for 10 min at 3000 rpm. The upper organic phase was collected and the aqueous phase was extracted again with chloroform:methanol (2:1 v/v) at ratio 3:1 (v/v). The two organic phases obtained were mixed, and the solvent was removed by rotary evaporator. The obtained lipid extract was derivatized using bis(trimethylsilyl) trifluoroacetamide (BSTFA) at 75 °C for 1 h. The derivatized samples were analysed by gas chromatography-flame ionization-mass spectrometry (GC-FID-MS) (Agilent 7890A, Agilent Technologies, USA) including a split/splitless injector, an electronic pressure control, a G4513A autoinjector, and a 5975C triple-axis mass spectrometer detector. The column used was an Agilent HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm phase thickness). Helium was used as carrier gas at 2 mL/min. The analysis was performed as described on Herrera, Navarro del Hierro, Fornari, Reglero, and Martin (2019). Briefly, mass spectrometer ion source and interface temperatures were 230 and 280 °C, respectively. The sample injections (1 µL) were performed in splitless mode. The oven temperature at 50 °C was held for 3 min and increased at a rate of 15 °C/min to 310 °C, being held for 25 min. The mass spectra were obtained by electronic impact at 70 eV. The scan rate was 1.6 scans/s at a mass range of 30–700 amu. Identification of compounds was performed by the NIST MS Data library, the mass spectra according to literature, or according to those of pure commercial compounds whenever possible. Quantitation of cholesterol, FFA, MG and bile salts was performed by the FID signal and using calibration curve of commercial standards of cholesterol, oleic acid, monopalmitin and bile salts, which were derivatized under the same conditions as samples.

3. Results and discussion

3.1. Effect of SCF on the bioaccessibility of glucose

To perform these experiments the amounts of SFC were determined based on the recommendations of EFSA, FDA/WHO who recommend a total dietary fibre daily intake of 25 g, of which about the 25% should correspond to soluble fibre (around 6 g). Considering that soluble fibre content in the mucilage is 95% ± 1.5% (Goh et al. (2016)), in this study, 10–13% of the daily soluble fibre requirement was supplied. Furthermore, the doses of 1 g of glucose on each glucose food model intake were selected based on the dietary recommendations from EFSA who advice to maintain a value sugars ingestion < 10% of the total energy intake (EFSA, 2010).

The bioaccessibility of glucose in food models with 0% (as control),

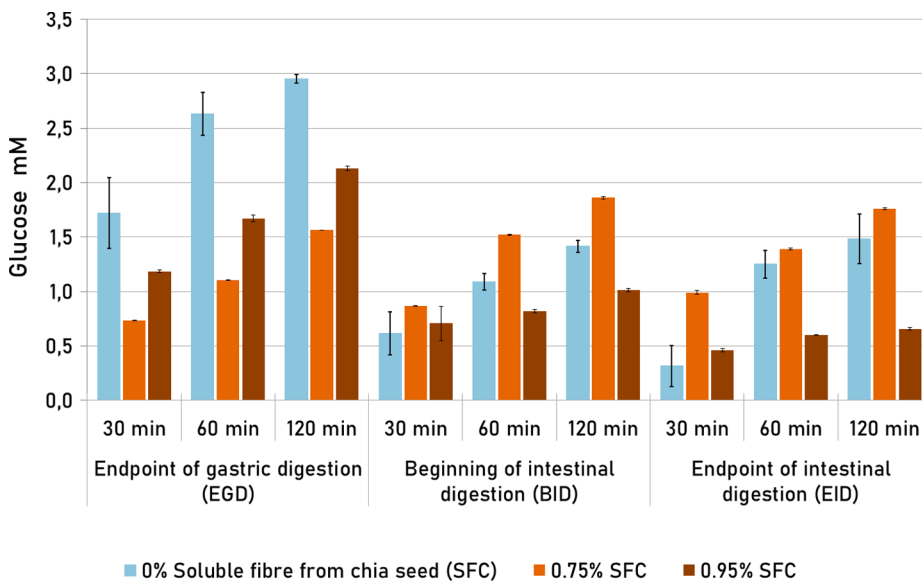


Fig. 2. Glucose diffusion through dialysis membranes of three glucose food models (0% w/w soluble fibre from chia seed (SFC) as control, 0.75% w/w SFC and 0.95% SFC) at the different gastrointestinal digestion stages (EGD, BID and EID). Mean values $\pm$ standard deviation of 0% SFC, 0.75% SFC and 0.95% SFC are expressed as mM of glucose on the dialysate.

0.75% and 0.95% of SFC was determined in terms of glucose diffusion across the dialysis membranes, % of bioaccessibility reduction and GDRI at EGD, BID and EID. Glucose concentration in the dialysates was monitored at 0, 30, 60 and 120 min in each stage.

As expected, glucose concentration in the dialysate increased from 0 to 120 min for 0%, 0.75% and 0.95% SCF food models within each digestion stage. Further, at the same time points of dialysis and food models, a general decrease of glucose content was observed within the stages EGD > BID $\approx$ EID (Fig. 2).

Glucose concentration reduction on the dialysate was not observed for 0.75% SFC at EID; indeed, 0.75% SFC presented higher glucose concentrations than the control glucose food models at BID. This result could be explained because lower concentrations of SFC produce weaker 3D networks, thus lower viscosity gels. This behaviour was previously observed with SFC and explained on Tamargo et al. (2018). The lower viscosity gels, due to its weaker 3D network, could favour the action of pH and digestive enzymes that could cause partial hydrolysis of the polysaccharide itself and for hence the release of glucose from the matrix. This effect of fibre concentration has been explained as an irreversible structural breakdown due to a lower molecular alignment and decrease of molecular chain entanglement in the dispersion (Zhang, Zhou, & Hui, 2005). Otherwise, 0.95% SFC food reduced the glucose content in the dialysate after 60 min of dialysis time at EGD, BID and EID; reaching a value of reduction (calculated as GDRI) of 53.4% at the endpoint of the small intestine.

In agreement with López et al. (1996), GDRI is a useful in vitro index to predict the effect of fibres in glucose absorption retardation at the GIT. Accordingly, the effect of % SFC referred to dialysis time and digestion stage is showed as GDRI in Table 1.

Maximum GDRI of 0.75% SFC food model was reached during the first 60 min of dialysis in EGD. Whilst GDRI values were higher during intestinal digestion (BID and EID) for 0.95% SFC. However, the major site of absorption of glucose and other end-products of the digestive process is the small intestine (Chater, Wilcox, Pearson, & Brownlee, 2015), therefore, SI should be the focus of bioaccessibility indexes.

The highest GDRI value for 0.95% SFC food model was reached at 120 min of EID being around 50%, although more studies with SFC would be necessary to corroborate these results. The GDRI data obtained for 0.95% SFC food models are higher than the GDRI obtained for other fibres as psyllium with 38.81% soluble fiber with similar composition, *Citrus limetta* peel with 32.5% composed by pectic substances or cellulose with 28.75% which also has similar composition (Flores-Fernández, Barragán-Álvarez, Díaz-Martínez, Villanueva-

Table 1

Glucose dialysis retardation indexes for glucose food models with 0.75% w/w soluble fibre from chia (SFC) and 0.95% w/w SFC expressed as mean values $\pm$ standard deviation at different dialysis time of each gastrointestinal digestion stage. Values with no retardation index are indicated as “nr”.

	Dialysis time (min)	Glucose dialysis retardation index (GDRI)	
		0.75% Soluble fibre from chia seed (SFC)	0.95% Soluble fibre from chia seed (SFC)
Endpoint of gastric digestion (EGD)	30	36.2 $\pm$ 1.2	27.0 $\pm$ 0.05
	60	34.7 $\pm$ 0.1	29.4 $\pm$ 1.9
	120	14.0 $\pm$ 0.2	16.6 $\pm$ 1.9
Beginning of small intestine digestion (BID)	30	nr	nr
	60	1.5 $\pm$ 0.4	28.3 $\pm$ 0.3
	120	6.5 $\pm$ 0.6	31.7 $\pm$ 0.4
Endpoint of small intestine digestion (EID)	30	nr	nr
	60	16.0 $\pm$ 2.7	49.9 $\pm$ 0.5
	120	9.0 $\pm$ 2.5	53.4 $\pm$ 0.5

Rodríguez, & Padilla-Camberos, 2017; Huang, Ma, Tsai, & Chang, 2019). In accordance with Edwards et al. (1987), GDRI seems to be related to soluble dietary fibre and uronic acids content, which are present in SFC. Therefore, 0.95% SFC with higher soluble fibre concentrations showed the highest values of GDRI, which involves higher reductions on glucose bioaccessibility during the digestion.

Fig. 3 shows the % of glucose bioaccessibility reduction respect to the % of SFC on the food models and in accordance with the results showed in Fig. 2, only an addition of 0.95% SFC in the food model showed bioaccessibility reduction. The total reduction of glucose bioaccessibility of 0.95% SFC food model was over 66%, thus this dose of SFC could reduce glucose in vitro absorption: from each 1 g of ingested glucose to less than 0.34 g at the end of dialysis process (120 min) at EID. A similar trend of reduction was found on different fibres on (Ahmed, Sairam, & Urooj, 2011), where all the fibre samples showed inhibitory effects on glucose diffusion through the dialysis membranes. Johnson and Gee (1981) also reported significant reductions in glucose transport by using guar gum and sodium carboxymethylcellulose. Further, Edwards et al. (1987) reported a glucose movement inhibition of guar, xanthan and locust bean gums across the cellulose membrane in vitro.

The mechanism that explains this behaviour has been described as a viscosity increase of the digesta that could change the flow regime from

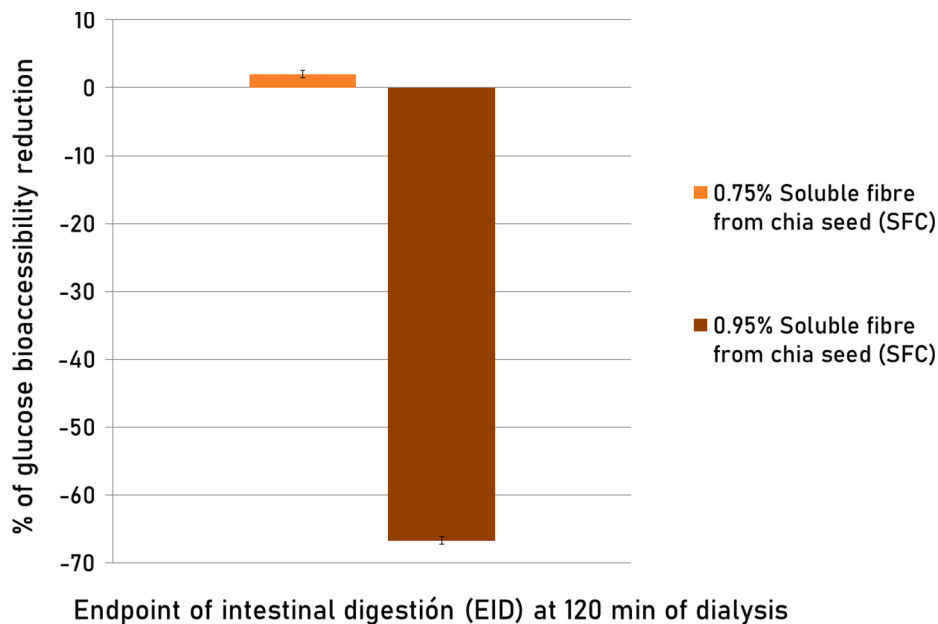


Fig. 3. Mean of values bioaccessibility % reduction of glucose after 120 min of dialysis at the Endpoint of Intestinal Digestion (EID) showed for 0.75% w/w of soluble fibre from chia seed (SFC) and 0.95% w/w of SFC. Values of bioaccessibility reduction are expressed as a % of decrease from the glucose intake and the control (0% w/w of SFC).

turbulent to laminar flow within the GIT, modifying the nutrient transport from lumen to GIT mucosa and delaying glucose diffusion (Capuano, 2017; Zheng et al., 2019). Mackie, Bajka, and Rigby (2016) have also associated this behaviour with viscosity increases and the ability of the fibre to bind endogenous compounds, such as digestive enzymes, leading a lower diffusion of nutrients. Previous studies have shown that SFC produces highly viscous solutions at low concentrations and the viscosity is retained during the digestion so it could explain the decrease of glucose available to be absorbed (Vera, Laguna, Zura, Puente, & Muñoz, 2019).

Further analyses are needed to establish dependence and corroborate the showed trends, but in general terms, results indicated that higher mucilage concentrations decreased glucose bioaccessibility and SFC seems to be able to entrap glucose through the GIT.

3.2. Effect of SFC on the bioaccessibility of lipid products, cholesterol and bile salts

For this study, the amounts of SFC were based on the same criteria than the point 3.1. The cholesterol doses were also selected using the recommendations from FDA/WHO, EFSA and previous studies. According to the FDA, cholesterol intakes should be less than 300 mg per day (FDA; USDA, 2016). Regarding European recommendations, EFSA decided not to propose a reference on cholesterol intake (EFSA, 2010).

Based on this information, the selected doses for this study were 100 mg of cholesterol on each lipid food model intake. According to Moran-Valero, Martin, Torrelo, Reglero, and Torres (2012), the combination of cholesterol with lipids would be closer to a real situation in which cholesterol would be taken as part of a meal containing diverse lipids, mainly under the form of triglycerides. Moreover, cholesterol needs the coexistence of other lipid products in the intestinal lumen to form sufficient surface of the mixed micelles of lipid products (FFA and glycerides) where cholesterol would be included (Moran-Valero et al., 2012). Therefore, 1 g of refined olive oil was added to the lipid food model in order to enhance its representativeness.

In general, the digestion of dietary lipids is known to be taken place at the small intestinal level, due to the main action of pancreatic lipase. This enzyme hydrolyses triacylglycerol to FFA and 2-MG. These final products are included within mixed micelles of bile salts and phospholipids, which are necessary to be formed to enhance the absorption of lipid products by enterocytes. Therefore, only the fraction of these

lipid products dispersed as micelles in the aqueous media of digestion is considered bioaccessible (Martin, Nieto-Fuentes, Señoráns, Reglero, & Soler-Rivas, 2010).

Concerning cholesterol, its physiological-intestinal absorption is similar to the of most lipids in the sense that it needs previous micellar solubilisation to enhance the transport of cholesterol to enterocytes. However, lipids absorption rate is very efficient (around 70–80%) and dietary cholesterol absorption varies from 40 to 60%, some authors have reported even a wider range of 20–80% absorption (Ros, 2000). This wide range of absorption is a result of the presence of other dietary components that can influence cholesterol availability by either increasing or decreasing its absorption. Moreover, different dietary compounds can also affect the solubility of bile salts by co-crystallisation or co-precipitation, decreasing their ability to form mixed micelles. Therefore, any circumstance that decreases the necessary components to form mixed micelles would lead to a decrease in the bioaccessibility of either lipid products and cholesterol (Moran-Valero et al., 2012). Considering that the related factors simultaneously affect the bioaccessibility of lipid products, cholesterol and bile salts the effect of SFC on the bioaccessibility was tested for all products under in vitro dynamic conditions.

The bioaccessibility of lipid digestion products as cholesterol, FFA and MG, as well as bile salts, after digestion in the presence of 0% (as control), 0.75% and 0.95% of SFC is shown in Fig. 4.

Before describing the effect of SFC, it is important to remark that the control digestions of the lipid model food, in absence of SFC, reproduced physiological results. The bioaccessibility of total lipid products (FFA + MG) was 70%, and cholesterol and bile salts bioaccessibility was 57% and 81%, respectively. As previously explained, these values would be similar to in vivo digestion absorption values, since the bioaccessibility of total lipid products uses to be between 70 and 80% and dietary cholesterol within the range of 40–60% (Ros, 2000). This is an important result because it suggests that simgi® model dynamic parameters are able to reproduce results of lipid bioaccessibility close to those expected in vivo. Therefore, these results would validate simgi® model for lipid studies and would allow rejecting that the digestion method itself or any other artefact would interfere with the data obtained by the experimental trials with SFC.

Concerning the results for SFC, a clear dependence of SFC concentration on the bioaccessibility of all the products was observed. Thus, as the concentration of mucilage increased, the bioaccessibility of FFA, MG, cholesterol and bile salts decreased (Figs. 4 and 5). The most

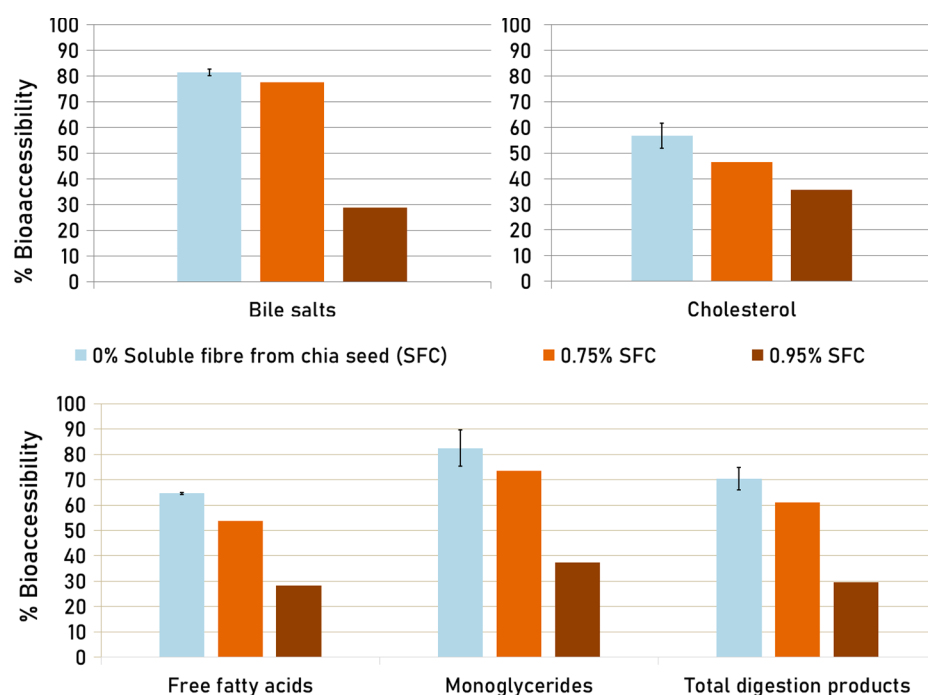


Fig. 4. Bioaccessibility of bile salts, cholesterol and lipid digestion products (free fatty acids and monoglycerides) expressed as % of compound at micellar phase on the endpoint of small intestinal digestion stage (EID). Results are presented for mean values of 0% w/w soluble fibre from chia seed (SFC) and simple values of 0.75% w/w of SFC and 0.95% w/w of SFC.

important bioaccessibility reduction was observed for bile salts, with values of reduction up to 65% at 0.95% of SFC (Fig. 5). As previously explained, bile salts are essential for the aqueous dispersion of dietary lipids and cholesterol, under the formation of mixed micelles. Therefore, the obtained results would suggest that SFC limited the solubility of bile salts in the aqueous media, and this could explain the reduction in the bioaccessibility of FFA, MG and cholesterol due to SFC presence. Specifically, with 0.75% of SFC, the reduction of cholesterol bioaccessibility was 18%. In 0.95% of SCF digestion, the bioaccessibility reduction reached 37%, which was more than twice. In the case of total lipid products (FFA + MG), a reduction of up to 58% was obtained, being similar for FFA and MG.

Previous studies about the effect of SFC on bioaccessibility of bile salts, lipid products or cholesterol have not been published until the

moment. Nevertheless, the effect of soluble fibres on these compounds has been mainly related to the increase of viscosity in the digestion medium, which might lead to limit diffusion of bile salts in the aqueous media, causing their excretion (Zacherl, Eisner, & Engel, 2011). Besides, the direct binding between soluble fibres and bile salts or cholesterol has been previously suggested by Zacherl et al. (2011). In addition, the potential mechanism suggested by which the soluble fibre may increase the excretion of bile acids and cholesterol in faeces has been described as a physical effect by entrapment or adsorption of these components (Mudgil, 2017). As an example, Zacherl et al. (2011) reported values of bile acid-binding capacity between 33 and 66% when soluble fibre from psyllium was used at 1 and 3% respectively. In the present study a 65% of the reduction of the bioaccessibility was reached with 0.95% of SFC present on the food model. Concerning cholesterol, the mechanism by

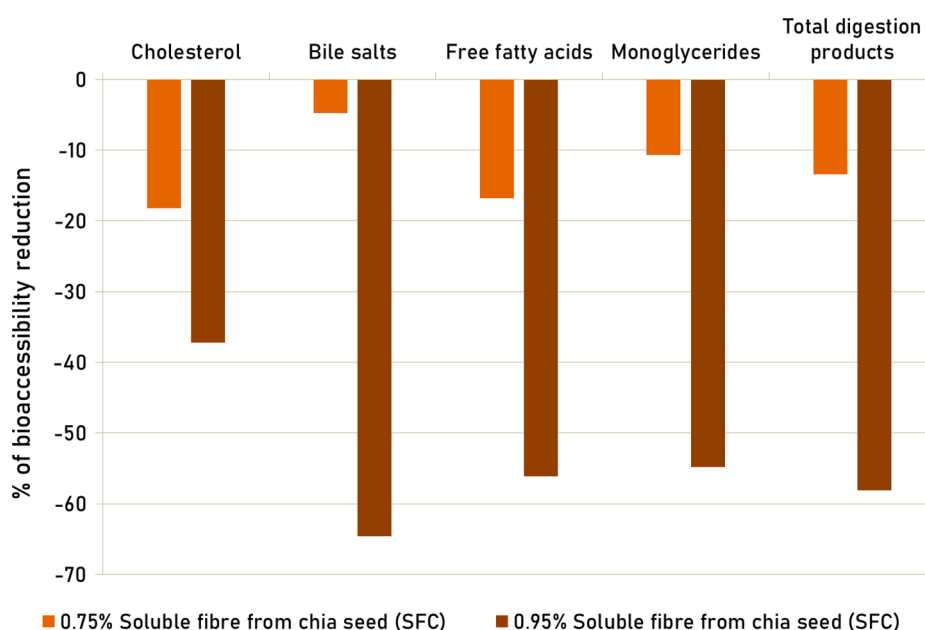


Fig. 5. Bioaccessibility % reduction of lipid digestion components at the endpoint of small intestinal digestion stage (EID). Results for lipid food models with 0.75% w/w of soluble fibre from chia seed (SFC) and 0.95% w/w SFC are expressed in relation to control food (0% w/w of SFC).

which some soluble fibre lower the cholesterol levels has been suggested as a predisposition to bind or absorb bile salts when they pass through the intestinal lumen preventing their normal reabsorption. This effect can increase the content of faecal bile acid, facilitating its elimination and reducing the cholesterol pool (Mudgil, 2017). This approach would be in agreement with the observed results in the present study, where bile salts and cholesterol bioaccessibility values decreased with SFC presence in the lipid food model. In this sense, it is interesting to remark that any mechanism of disruption of the reabsorption of bile acids, as shown for SFC, would force the synthesis of new bile salts from cholesterol by the liver, leading to a cholesterol decrease (Barbana, Boucher, & Boye, 2011). Therefore, SFC might lead to relevant hypocholesterolemic effect due to the combination of two mechanisms, as the reduction in the bioaccessibility of cholesterol as well as the potential stimulation of degradation of endogenous cholesterol in the liver.

Concerning lipid products of oil digestion such as FFA and MG, reduction of serum lipids in vivo have been previously suggested for chia seeds and other mucilages by Ayerza and Coates (2005); Boban, Nambisan, and Sudhakaran (2009); Tavares Toscano, Tavares Toscano, Leite Tavares, Silva, and Silva (2015). Still specific bioaccessibility studies of lipid digestion products influenced by soluble fibre are scarce. In addition, in a study where partially hydrolysed guar gum (PHGG) was used, the values obtained to FFA and cholesterol bioaccessibility reduction were 19.4% and 18% at 6% of PHGG (Minekus et al., 2005), significantly lower than the obtained in the present study and even more considering that lower concentrations of soluble fibre were used.

As a summary, the obtained results in the present study showed for the first time that soluble fibres from chia seeds specifically reduced the bioaccessibility of total lipid products, cholesterol and bile salts simultaneously when they are tested by in vitro dynamic gastrointestinal digestion. Although more studies would be necessary to corroborate the observed effects, they would contribute to the potential use of soluble fibre from chia seeds against chronic diseases related with hypercholesterolemia, hypertriglyceridemia, overweight and obesity.

4. Conclusions

The results shown on this study suggest that the mucilage from chia seed could be used as a novel source of soluble fibre due to its potential effect on reducing the rate of lipids, cholesterol and glucose available to be absorbed on the small intestine. In this study, the 0.95% SFC food model showed the more promising results with respect to both, lipids and glucose absorption reductions. The in vitro hypocholesterolemic and hypoglycemic effects observed by the incorporation of soluble fibre from chia seeds mucilage showed promising results and suggest that this new food product could be used as a functional ingredient. The addition of chia seed mucilage into food matrixes could be considered as a strategy to increase the functionality of foods, especially those targeted to reduce non-communicable diseases as overweight, obesity and type 2 diabetes, between others. Future research about functional properties of chia seeds fibre should include in vivo studies, the incorporation of this new soluble fibre into several food matrixes and the evaluation of the effect of SFC on other nutrients and micronutrients bioaccessibility. As a whole, this study confirmed that simgi® model could be considered a useful in vitro tool to evaluate the effects of chia seed in the intestinal bioaccessibility of glucose, dietary lipids and cholesterol, prior to human studies, and, therefore, suitable to minimise animal testing.

CRedit authorship contribution statement

Alba Tamargo: Investigation, Methodology, Formal analysis, Data curation, Validation, Writing - original draft, Writing - review & editing. **Diana Martín:** Investigation, Methodology, Formal analysis, Data curation, Validation, Writing - original draft. **Joaquín Navarro del**

Hierro: Investigation, Formal analysis, Data curation. **M. Victoria Moreno-Arribas:** Methodology, Conceptualization, Resources. **Loreto A. Muñoz:** Project administration, Conceptualization, Methodology, Writing - original draft, Supervision, 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.

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