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# Plasma lipid lowering effect by a novel chia seed based nutraceutical formulation

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ABSTRACT

Atherosclerotic cardiovascular diseases (ASCVD) are preferential targets of preventive medicine partially through therapies to improve atherogenic lipid profiles. The aim of the present work was to test a novel nutraceutical formulation (CSN) for its potential effects on plasma triglyceride levels of healthy subjects with moderate dyslipidemia. A cohort of 52 individuals were administered daily, for 8 weeks, with four gastro-resistant capsules of CSN, each one containing 500 mg of cryo-micronized chia seeds and 15 mg of vitamin E, according to a single centre, randomized, placebo controlled, 16 weeks trial. Data showed the following mean lipid changes: triglycerides, −27.5% ($P = .0095$); total cholesterol, −8.0% ($P = .0019$); High Density Lipoprotein cholesterol, +5.7% ($P = .0042$); Low Density Lipoprotein cholesterol, −10.2% ($P = .0021$). CSN may be regarded as a novel complementary and/or alternative safe remedy with clinical relevance in the primary cardiovascular disease prevention.

## 1. Introduction

The importance of dietary polyunsaturated ω-3 fatty acids (FA) is well recognised, as concerns, particularly, their positive influence on the cardiovascular system. It has been reported that ω-3 FA intake can reduce triglyceride plasma levels and platelet aggregation, and stabilizes the cardiac rhythm (Harris, 2009). Fish and seafood products are the main dietary source of ω-3 FA, mainly represented by docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). The protective effects are still appreciable even in case of low consumption levels: at least 30 g fish/day can support a significant prevention against cardiovascular diseases; interestingly, an increase of 20 g/day in fish consumption would favour a decrease by 7% of death risk incidence for cardiovascular disorders in individuals who occasionally consume fish (Mozaffarian et al., 2003). A ω-3/ω-6 FA ratio of 1:6 is considered to be appropriate for the nutritional requirements of most healthy subjects. Such ratio would exert an important influence on plasma lipids and serve cardiac and endothelial functions to impact the prevention and treatment of coronary heart diseases (CHD) (Wijendran & Hayes, 2004; Makni et al., 2010). Today, this balance in the current Western diet has been strongly shifted in favour of ω-6 FA (from 1:10 to even 1:30), due to a very low consumption of seafood products, and a large use of vegetable oils (the main source of ω-6 FA). Over the last few years, this imbalance has been considered as one of the main causes of the dramatic increase of cardiovascular disorders (Wijendran & Hayes, 2004).

Apart sea products, the vegetable world represents an important alternative source of ω-3 FA, mainly under the form of alpha-linolenic acid (ALA; 18:3 ω-3). The estimated average ALA intake in the United States and most European countries is 1.3–1.7 g/day (Gebauer, Psota, Harris, & Kris-Etherton, 2006; Zatónski, Campos, & Willett, 2008; Hulshof et al., 1999). The Institute of Medicine (IOM) of the National Academies established for ALA an Adequate Intake (AI) of 1.6 g/day for men and 1.1 g/day for women (IOM, 2002). The IOM noted that intakes of ALA above the AI may confer additional health benefits, especially with respect to cardiovascular health. Many advisory boards consider ALA intakes greater than 1.5 g/day important for human health (Gebauer et al., 2006). The most important vegetable sources of ALA are the seeds of *Dracocephalum moldavica*, *Perilla frutescens*, and *Aleurites moluccana* (Stuchlík & Žák, 2002). However, among more common vegetable sources, flax (*Linum usitatissimum*) seeds and chia (*Salvia hispanica*) seeds are of major interest, not only as regards their high ALA contents, but also for their ω-3/ω-6 FA ratio of about 4:1 (Ciftci, Przybylski, & Rudzińska, 2012). Nevertheless, chia seeds are more valuable than flax seeds in terms of nutritional values, specifically

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regarding their higher amounts (for 100 g seeds) of calcium (631 mg *vs* 255 mg), fiber (34.4 mg *vs* 27.3 mg), phosphorus (860 mg *vs* 642 mg), their superior levels of antioxidants (mainly polyphenols) and proteins characterised by a high biological value (https://ndb.nal.usda.gov), and, of no secondary importance, their much lower contents in compounds of toxicological concern (Choi, Kim, Pyo, Jo, & Han, 2007).

Although many types of oily seeds are indicated as a good source of ω-3 FA, and strongly suggested to be consumed daily for a proper intake of such precious cardioprotective nutrients, some of them, such as flax seeds and chia seeds, are characterised by a very small size, so can be quite difficult, or almost impossible, to be properly chewed and, thus, crushed. This aspect could be at the basis of a very low bioaccessibility of seed ω-3 FA. What is more, some oily seeds are characterised by a very high capacity of hydration: chia seeds, for example, can absorb up to 27 times their weight in water, forming a gelatinous mass which, on one side, usefully contributes to stimulate the intestinal peristalsis, but, on the other hand, provides a very low, or even no, bioaccessibility of ω-3 FA (Tha Goh et al., 2016). A preventive grinding before the consumption of such oily seeds could be expected to favour the bioaccessibility of ω-3 FA. Actually, previous authors have clearly demonstrated that the administration of ground chia seeds both to animals and humans favours a significant increase of ALA plasma levels and exerts a positive influence on some cardiovascular risk factors, respect to what can be achieved with whole chia seeds (Ayerza & Coates, 2007; Nieman et al., 2012; Nieman et al., 2009; Ayerza & Coates, 2005; Pereira da Silva, Morais Dias, de Castro Moreira, Toledo, & Pinheiro-Sant'Ana, 2016; Jin et al., 2012). Nevertheless, it is extensively reported that the effects of gastrointestinal digestion on vegetable and animal oils, not only lead to a significant degradation of ω-3 FA, thus, much decreasing their actual intestinal bioaccessibility (Nieva-Echevarría, Goicoechea, & Guillén, 2017; Domoto, Koenen, Havenaar, Mikajiri, & Chu, 2013; Cofrades et al., 2017), but, more importantly, also favour the development of oxidation products which would be absorbed along the duodenal tract (Maestre, Douglass, Kodukula, Medina, & Storch, 2013). Thus, the aim of the present work was to develop a novel nutraceutical formulation, based on gastro-resistant (GR) micronized chia seeds and antioxidative co-formulants. Specifically, this formulation was tested for its potential effects on human plasma triglyceride levels through a randomised clinical trial.

## 2. Materials and methods

### 2.1. Reagents and standards

All chemicals and reagents used were analytical-reagent. The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA, USA) before use. Chemicals and reagents used to simulate the gastrointestinal digestion were: potassium chloride (KCl), potassium thiocyanate (KSCN), monosodium phosphate ($NaH_2PO_4$), sodium sulphate ($Na_2SO_4$), sodium chloride (NaCl), sodium bicarbonate ($NaHCO_3$), urea, α-amylase, hydrochloric acid (HCl), pepsin, pancreatin, bile salts (Sigma Chemical Co., St. Louis, MO, USA). Vitamin E (DL-alpha-tocopheryl acetate) was purchased from Farmalabor Srl (Canosa di Puglia, Italy).

### 2.2. Chia seed based nutraceutical formulation

The nutraceutical formulation used in this study consisted of GR capsules containing cryo-micronized chia seeds (500 mg/cps) and vitamin E (15 mg/cps). Cryo-micronized chia seeds were purchased by MB-Med Company (Turin, Italy). The product was formulated by the Department of Pharmacy, University of Naples "Federico II" (Naples, Italy) and indicted as CSN.

### 2.3. In vitro gastrointestinal (GI) digestion

The assay was performed according to the procedure described by Raiola, Meca, Mañes, and Ritieni (2012), with slight modification. GI digestion was divided into salivary, gastric and duodenal digestive steps. The following samples were submitted to GI digestion: sample 1, 500 mg chia seeds; sample 2, 500 mg cryo-micronized chia seeds; sample 3, one GR capsule containing cryo-micronized chia seeds (500 mg); sample 4, one GR capsule containing cryo-micronized chia seeds (500 mg) and vitamin E (15 mg). For the salivary digestion, the samples were mixed with 6 mL of artificial saliva composed of: KCl (89.6 g/L), KSCN (20 g/L), $NaH_2PO4$ (88.8 g/L), $Na_2SO_4$ (57.0 g/L), NaCl (175.3 g/L), $NaHCO_3$ (84.7 g/L), urea (25.0 g/L) and 290 mg of α-amylase. The pH of the solution was adjusted to 6.8 with HCl 0.1 N. The mixture was introduced in a plastic bag containing 40 mL of water and homogenised in a Stomacher 80 Microbiomaster (Seward, Worthing, UK) for 3 min. Immediately, 0.5 g of pepsin (14,800 U) dissolved in HCl 0.1 N was added, the pH was adjusted to 2.0 with HCl 6 N, and then incubated at 37 °C in a Polymax 1040 orbital shaker (250 rpm) (Heidolph, Schwabach, Germany) for 2 h. After the gastric digestion, the pancreatic digestion was simulated as follows: the pH was increased to 6.5 with $NaHCO_3$ 0.5 N and then 5 mL of a mixture pancreatin (8.0 mg/mL) and bile salts (50.0 mg/mL) (1:1; v/v), dissolved in 20 mL of water, were added and incubated at 37 °C in an orbital shaker (250 rpm) for 2 h. After each step of digestion, 10 mL of the obtained extract were centrifuged at 4000 rpm and 4 °C for 1 h: before each following step, the digestion procedure was started over again. To determine the peroxide values and the polyunsaturated fatty acids (PUFA) quali-quantitative profile, the intestinal digestive solution was freeze-dried and, then, subjected to lipid extraction according to AOAC (1995) method 948.16, by using a 6-place units Extraction Unit E-816 Soxhlet (Buchi, Flawil, Switzerland). After centrifugation at 3000*g* for 5 min, supernatants were transferred into a pre-weighed scintillation vial, and dried under nitrogen.

### 2.4. Peroxide value determination

Peroxide values were measured by treating each lipid extract sample (5 ± 0.05 g) with 30 mL acetic acid-chloroform (3:2, v:v) and 0.5 mL of saturated potassium iodide solution, followed by titration with 0.1 N sodium thiosulphate (AOCS 1998).

### 2.5. Analysis of fatty acid composition

Lipid extracts were dissolved in 2 mL of *n*-eptane and treated with 0.2 mL of 2 N potassium hydroxide methanolic solution (11.2 g of potassium hydroxide in 100 mL methanol). The mixture was shaken energetically for 1 min at room temperature and then centrifuged (3000*g* for 5 min). Supernatants were collected and analysis of fatty acid methyl esters was performed by gas chromatography using a DANI GC instrument (DANI Instruments, Milan, Italy) coupled to a flame ionization detector (FID) and equipped with a HP-5 capillary column (Agilent, Milan, Italy). The temperature program started at 150 °C (10 min), increased by 2 °C/min to 180 °C and then increased again by 3 °C/min to 240 °C (20 min).

### 2.6. Study population and protocol

Study participants were recruited by the Samnium Medical Cooperative (Benevento, Italy). Patients were enrolled in February 2017. Patients aged 18–83 years were eligible for enrolment if they had the following values of serum parameters at baseline: TC, 200–260 mg/dL; HDL-C, 31–45 mg/dL; LDL-C, 179–205 mg/dL; glucose, 90–125 mg/dL; TG, 170–280 mg/dL. The subjects were asked to keep their dietary habits unchanged throughout the entire study: to this regard, they were provided with a food diary on which annotate their daily dietary habits.