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Centesimal composition and bioactive compounds in African *mustards* used as condiments in Ivory Coast

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The aim of this research was to evaluate the potential of African locust bean *mustard*, produced with fermented *Parkia biglobosa* seed and *Glycine max* seed, as functional food, focusing on its incorporation into the diet. These *mustards* contained in iron and zinc are respectively 12.16±0.63 mg/100 g and 8.38±0.3 mg/100 g in African locust bean *mustard*, and respectively 7 87±0.45 mg/100 g and 4.26±1.07 mg/100 g in soy *mustard*. Polyphenols and flavonoids were contained in large amounts. Ascorbic acid content was, respectively, 19.31 mg/100g of African locust bean *mustard* and 13.26 mg/100 g of soybean *mustard.* However, total carotenoids were 60.72±5.06 mg/100 g of African locust bean *mustard* and 100.86±8.45 mg/100g of soybean *mustard*. The lipid fraction contained unsaturated fatty acids in large amounts. Based on the results obtained, it can be said that West African *mustards* are excellent sources of vegetable proteins, iron, zinc, ascorbic acid, total carotenoids and oils rich in unsaturated fatty acids. These condiments may contain bioactive compounds with functional activities, but further research is needed to assess such potential.

Key words: Mustard, functional food; bioactive compounds.

INTRODUCTION

In the West African region, highly concentrated plant biodiversity sources such as the *Parkia biglobosa* (African locust bean) with innumerable functional properties can be found. However, many of those sources are still unknown.

Parkia biglobosa is a multipurpose fodder tree up to 20 m tall. The fruit pods are dark brown and contain up to 30 seeds called African locust bean. Many vernacular names (nere tree, ahwa, ewé, igba, igiougba, ogba) are used by the local populations. All of the different parts of this plant are used by traditional healers to cure many disorders like hypertension, hemorrhages and dermatosis (Odetola et al., 2006; GrØnhaug et al., 2008; Udobi and Onaolapo, 2009).

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Phytochemical studies have demonstrated the presence of known sterols and triterpenes from the petroleum extract (Tringali et al., 2000). The fermented seeds are well appreciated as a condiment in cooking under various names (for example,, afitin in Benin, nététou in Senegal, dawadawa in Nigeria), being rich in proteins, sugars and vitamin B2 (Azokpota et al., 2006). Instead of nutritional interest of African locust bean *mustard*, soybean (*Glycine max*) was used as an interesting legume eligible for production of the *mustard*, as a way of diversification and to cater for the unavailability of *Parkia biglobosa* seeds. Soy characteristics are indisputable today. Its proteins are well balanced in amino acids whose amounts are very close for standards recommended by Food and Agriculture Organization (FAO) (Lamboni et al., 1999). African locust beans can be substituted by soybean to manufacture *mustard*.

However, being an important supplement to human diet and/or for their fundamental role in the prevention and treatment of some diseases, the nutritional quality of African locust bean *mustard* is very important. However, diet particularly rich in vegetables and fruits is also recognized as part of a healthy lifestyle, and can lead to reduction of some diseases, and some types of cancer including lung, colon, esophagus, and stomach cancer. Although the mechanisms associated with the reduction of the incidence of these diseases are still not completely clarified, it is known that these diets are usually poor in saturated fats and rich in fibers and various vitamins and minerals. The foods which either prevent or minimize chronic and degenerative diseases, in addition to their role to a good nutrition, are called functional foods (Pourchet-Campos, 1998). They have drawn the public opinion attention due to the health benefits they bring. In the interest of preserving food identity and enhancement of African locust bean *mustards* from Ivory Coast, this study was conducted to evaluate, by analytical determinations, the proximate composition and bioactive compounds of the African locust bean *mustard* and soybean *mustard*. Providing nutritional information about these can be an incentive to the population to include these products in their diets which can help prevent or minimize the incidence of certain diseases.

MATERIALS AND METHODS

All samples were obtained from the “Grand Marché d’Adjamé” (Abidjan, Ivory Coast). African locust bean seed based “mustard” samples used were produced in Korhogo (in the northern, Ivory Coast). Three batches of these “mustard”, 507.66±10.34 g average weight were purchased from each of eight sellers in this market. All soybean based “mustard” used came from Burkina Faso. Three batches of samples of these soybean based “mustard”, 457.16±13.58 g average weight, were purchased from each of the six wholesale sellers. Each batch of “soumbara” cost 500 CFA. Samples purchased on the same day, were brought to the Food Analysis and Processing laboratory at Nangui Abrogoua University (Abidjan, Ivory Coast), where they were subsequently divided into three portions for each sample type (nere seed based “mustard or soybean based “mustard”), with batches purchased from each of the sellers. Each portion, after being homogenized and hermetically sealed, was stored at -20 °C until analysis.

Analysis

All determinations were carried out in triplicate. Moisture content was determined gravimetrically in an oven at 105°C until a stable weight was obtained (AOAC, 1995). The results were shown in grams of moisture per 100 g of fresh sample. The extraction of the lipid fraction was carried out using a Soxhlet Tecator in accordance to Association of Official Agricultural Chemists (AOAC) method (AOAC, 1995). The results were shown in grams of total lipids per 100 g of fresh sample. The total nitrogen determination was carried out using the Kjeldahl method (AOAC, 1995). Total protein was calculated by multiplying the total nitrogen by 6.25, the conversion factor calculated from the amino acid of total sample. The results were expressed in grams of total protein per 100 g of fresh sample. The total carbohydrate content was obtained by the difference of protein, moisture, lipid and expressing the sum in grams of total carbohydrates/100 g of fresh sample.Total ash content was determined by previous carbonization of the dry samples followed by incineration in an oven at 550 °C (AOAC, 1995). The results were expressed in grams of total ash/ 100 g of sample. The total mineral content was determined from dry samples oxidised in a muffle furnace at 550°C from a minimum period of 2 h followed by acid digestion (HCl, 2 mol/L), and analysis by mass spectrophotometry with a plasma inductively connected in the semi-quantitative mode using a Perkin Elmer-Sciex ELAN 6000 equipment (AOAC, 1995). Phosphorus content was obtained by determination of orthophosphate by the method using ascorbic acid and a combined reagent (APHA, 1995). The results were expressed in milligrams of the corresponding mineral/100 g of sample.

The total energy value (TEV) was calculated using the traditional conversion factors for proteins (4 kcal/gram), lipids (9 $kcal.gram^{-1}$), and carbohydrates (4 $kcal.gram^{-1}$) according to FAO (2006). The results were expressed in kcal/100 g of fresh sample. Ascorbic acid was extracted using metaphosphoric / acetic acid as solvent and assayed by 2, 6-dichlorophenol indophenol calibrated using a standard vitamin C (Pongracz et al., 1971). The results were expressed in milligrams of ascorbic acid/100 g of fresh sample. The polyphenols assay was performed following the spectrophotometric method using the Folin Ciocalteu (Cicco et al., 2009) in which gallic acid was adopted as standard polyphenol. The results were expressed in milligrams of gallic acid/100g of sample. Flavonoids determination was carried out after triple extraction with acetone / water / acetic acid (70/28/2: v / v / v) as solvent (Zhishen et al, 1999; Kim et al, 2003). The assay was performed in the presence of sodium nitrite $NaNO_2$ (5%) (w / v) and aluminum trichloride $A1C1_3$ (10%) (w / v). Quercetin was adopted as standard flavonoid.

Chemical analysis of fats

Total fatty acids composition was determined by gas chromatography (GC) (Chrompack CP9001 (FID) gas chromatograph, equipped with a CP - Sil 88 FAME fused silica WCOT 0.2 µm × 50 m × 0.25 mm capillary column (Chrompack catalog n.7488)) after cold extraction and transesterification of fatty acids. The operating conditions: temperature of the

Table 1. Proximate composition of "mustards".

Parameter	***Nere mustard***	**Soy *mustard***
Moisture (g/100 g FW)	14.57±2.01[b]	25.97±3.93[a]
Proteins (g/100 g FW)	33.98±3.06[a]	32.38±3.52[a]
Lipids (g/100 g FW)	34.53±3.64[a]	20.12±1.76[b]
Carbohydrates (g/100 g FW)	13.87±3.27[b]	17.14±2.19[a]
Ash (g/100 g FW)	3.05±0.42[b]	4.39±0.81[a]
pH	7.22±0.37[a]	6.078±0.15[a]
Energy (kcal/100 g FW)	502.17±10.96[a]	373.24±22.93[b]
Iron (mg/100 g FW)	12.16±0.63[a]	8.38±0.3[b]
Zinc (mg/100 g FW)	7.87±0.45[a]	4.26±1.07[b]
Phosphorus (mg/100 g FW)	506.33±29.39[b]	582±9.58[a]
Calcium (mg/100 g FW)	316.7±70.89[a]	232.45±11.90[b]
Potassium (mg/100 g FW)	509.41±10.81[b]	1268.21±9.37[a]
Sodium (mg/100 g FW)	184.03±43.99[a]	74.71±5.96[b]

Means with the same superscript along the same line are not significantly different are 5% level.

injector = 250°C; temperature of the detector (FID) = 250°C; column initial temperature = 160°C (32 min), with rise of 3°C per minute until 200°C; column final temperature = 200°C (30 min); split of 1:100; carrier gas = hydrogen, at 70 KPa; and injected sample quantity = 0.2 µL. Each sample was injected only once (Huang et al., 2006). The software Alltech Allchrome More Chromatography Data System Version 1.4.2.1 (Alltech Association Inc., Lokeren, Belgium) was used to read the data. The peaks were identified by their retention times and by comparison with standard (Supelco 37 Component FAME Mix, Sigma-Aldrich, Bornem, Belgium). The results were expressed as the fatty acid composition in total lipid content.

Carotenoids determination

With regard to the analysis of carotenoids, the samples were submitted to lipid extraction and saponification steps. The determination of total carotenoids was carried out by high performance liquid chromatography (HPLC) operating under the following conditions: mobile phase: gradient of t-butyl methanol/methyl ether - 80:20 to 10:90 in 28 min; flow: 0.8 mL/min; detector: Photodiode Array (DAD) 300 500 nm; column: C30 3 µm× 250 mm - YMC Carotenoid waters; temperature of the column: 30°C; and injected sample volume: 0.2 µL, one injection for each sample (Rodriguez-Amaya, 2001). All analysis steps were conducted protected from light and the carotenoids was covered with aluminum foil. The extraction of total carotenoids followed by Hitachi-U 3200 spectrophotometer readings used a wave length of 449 nm (Rodriguez-Amaya and Kimura, 2001). The peaks identification was done by comparison with the carotenoids retention times from the standard used. The results were converted to wet basis and expressed in milligrams of total carotenoids/100 g of sample.

Statistical analysis

Results were expressed as mean ± standard deviation. One way ANOVA (SPSS 20.0 for windows, SPSS Inc. Chicago IL, USA) was used to analyse data. The difference between groups of each parameter was determined using the Duncan test and statistical significance was claimed at $P < 0.05$.

RESULTS

Chemical composition of "*mustards*", food condiments from fermented *P. biglobosa* seeds and *Glycine max* seeds are shown in Table 1. Except protein content (32.38±3.52 g/100 g of sample and 33.98±3.06 g/100 g sample), results showed that there were significant differences between these two varieties of *mustards*. According proximate composition, moisture and lipid are second greatest components. Total lipids ranged from 20.12±1.76 g/100 g in soy *mustard* to 34.53±3.64 g/100 g in African locust bean *mustard.* Nevertheless, moisture ranged from 14.57±2.01 g/100 g in African locust bean *mustard* to 25.97±3.93 g/100 g in soy *mustard*. However, energy content was significantly different and was higher in African locust bean *mustard* (502.17±10.96 kcal/100 g) than in soy *mustard* (373.24±22.9 kcal/100 g). Total carbohydrates, ash content and the pH in the two varieties of *mustards* were given almost in the same amounts.

Mustards content minerals (iron, zinc, calcium, phosphorus, sodium and potassium) were in considerable amounts. According to microminerals, iron and zinc are respectively higher in African locust bean *mustard* (12.16±0.63 g/100 g and 7.87±0.45 g/100 g) than in soy *mustard* (8.38±0.3 g/100 g and 4.26±1.07 g/100 g). Amongst the macrominerals presented in Table 1, potassium and phosphorus were found in highest concentration in the soy *mustard* (respectively 1268.21±9.37 g/100 g and 582±9.58 g/100 g) than in African locust bean *mustard* (respectively 509.41±10.81 g/100 g and 506.33±29.39 g/100 g).The oils of *mustards*

Table 2. Fatty acids composition of *mustards* (g/100 g of total fatty acids).

Fatty acid	Nere mustard	Soy mustard	Mustard*	Soy*	Palm*	maize*
Palmitic (C16:0)	10.5[b]	12.34[a]	0.5-4.5	8.0-13.5	8.0-14.0	8.6-16.5
Stéaric (C18:0)	16.94[a]	6.72[b]	0.5-2.0	2.0-5.4	1.0-4.5	-3.3
Oléic (C18:1c)	16.86[b]	24.64[a]	8.0-23.0	17-30	35.0-69	20.0-42.2
Linoléic (C18:2c)	34.41[b]	49.76[a]	10.0-24.0	48.0 -59.0	12.0-43.0	34.0-65.6
Linolénic (C18:3c)	0.24[b]	5.87[a]	6.0-18.	4.5-11.0	0.3	2.0
Arachidic (C20 :0)	4.45	-	1.5	0.1-0.6	1.0-2.0	0.3-1.0
Béhénic (C22 :0)	15.36	-	0.2-2.5	0.7	1.5-4.5	0.5
Saturated fatty acids	47.25[a]	19.06[b]	2.7-10.5	10,8-20.2	11.5-24.5	12.7-21.3
Unsaturated fatty acids	51.51[b]	80.27[a]	24-65	50.3-90	47.3-89.5	56-87.3
Mono-unsaturated fatty acids	16.86[b]	24.64[a]	8.0-23.0	17-30	35.0-69	20.0-42.2
Poly-unsaturated fatty acids	34.65[b]	55.63[a]	16-42	52.5-70	12.3-43.3	36-67.5
Non identified fatty acids	1.24	0.67	-	-	-	-

Means with the same superscript along the same line are not significantly different are5% level *CODEX STAN 210-1999.

Table 3. Antioxidant content present in "mustards" expressed in mg/100 g sample (Average ± s.d.).

Samples	Polyphenols (mg GAE/100 g FW)	Flavonoids (mg Equer/100 g FW)	Ascorbic acid (mg/100 g FW)	Total carotenoids (mg/100 gFW)
Nere mustard	100.01±24.29[b]	2.67±0.23[b]	19.31[a]	60.72±5.06[b]
Soy *mustard*	170.63±31.39[a]	5.28±1.92[a]	13.26[b]	100.86±8.45[a]

Means with the same superscript along the same column are not significantly different are5% level.

were characterized and have been compared to other oils usually used in Ivory Coast. Fatty acids were determined and results presented in Table 2, show significant difference between African locust bean *mustard* oil fatty acids and soy mustard oil fatty acids.

African locust bean *mustard* oil contains saturated acid in very large amounts than *soy mustard* oil. An average of 47.25 g/100 g of African locust bean *mustard* oil and 19.06 g/100 g of *soy mustard* oil was obtained. However, 1686 g/100 g of African locust bean *mustard* oil and 24.64 g/100 g of *soy mustard* oil, for mono-unsaturated, 34.65 g/100 g of African locust bean *mustard* oil and 55.63 g/100 g of *soy mustard* oil, for poly-unsaturated, were obtained. As for saturated acid, nere mustard and soy mustard showed the greatest values than mustard oil, soy oil, olive oil and palm oil. Mono-unsaturated acids in African locust bean *mustard* oil and *soy mustard* oil, were closer to values recommended in mustard oil and soy oil. And then, poly-unsaturated content in African locust bean *mustard* oil was closer to values recommended in mustard oil and palm oil. Poly-unsaturated content in *soy mustard* oil was closer to values recommended in soy oil and maize oil.

Regarding content of nutrients with high antioxidant activity, significant differences between the two varieties of mustard were presented, and very large amounts were found. Values of 100.01±24.29 mg/100 g of African locust bean *mustard* and 2.67±0.23 mg/100 g of African locust bean *mustard*, 170.63±31.39 mg/100 g of soy *mustard* and 5.28±1.92 mg/100 g of soy *mustard*, were found respectively for total polyphenols and flavonoids (Table 3). Average of 9.31 mg/100 g of African locust bean *mustard*, 60.72±5.06 mg/100 g of African locust bean *mustard* 13.26 mg/100 g of soy mustard and 100.86±8.45 mg/100 g of soy mustard, were found respectively for ascorbic acid and total carotenoids.

DISCUSSION

The moisture content in the centesimal composition of mustard deserves attention on account of being higher than 12. It was higher than values determined by Lamboni et al. (1999) which ranged from 6.35 to 9% for varieties of mustards (Mna and Mnas) manufactured in their laboratories. *Mustard* used in this study was probably not sufficiently stored after being manufactured, before them to the market.

Attention must be drawn to the high content of protein found in this study, which was 33.98±3.06 g/100 g of African locust bean *mustard* and 32.38±3.52 g/100 g of soy *mustard*. Although African locust bean *mustard* and soy *mustard* can be considered as vegetable protein source, and the consumption of 100 g of *mustards* is capable of supplying significant amount of protein daily according to recommendation. The content of total carbohydrates in mustards was lower than the values (26.33 to 28.5) found in the study of Lamboni (1999). *Mustards* present some minerals in considerable amounts, which is extremely important since they act as co-factors in various metabolic reactions in the human organism. Amongst the macrominerals presented in Table 1, potassium was found in highest concentration in the pulp. This element is very important for its involvement in vital physiological functions, such as the osmotic and acid-base balances, and intra and extracellular concentrations related to the Na/K pump system (Mahan and Escott-Stump, 2002).

The third highest macromineral concentration in these mustards is attributed to calcium, relevant in the prevention of bone problems, such as osteoporosis in adults and rachitis in children, since low calcium consumption is a potential problem in Brazil (Vannucchi, 1990). Sodium as the lower component of the minerals content in these mustards, must be considered since it also operates in the osmotic and acid-base balances although it can cause health problems, such as hypertension, when ingested in high quantities (Shils, 2003**)**.

Microminerals (Table 1) were found in very large amounts. The highest concentration found in *mustards* was that of iron followed by zinc. Iron deficiency anemia has a high incidence in women and children in developing countries, which emphasizes the importance of the presence of this mineral in the pulp studied (Nogueira et al., 1998).

Zinc is essential because it is a co-factor for more than 100 enzymes, and participates in diverse metabolic processes such as cellular growth and multiplication, cicatrization, macrophage and lymphocyte functioning. The presence of these minerals in African locust bean *mustard* and *soy mustard* enriches the nutritional value and the true function in human metabolism, of this condiment even more.

If the bioavailability of these minerals is not considered, 100 g of African locust bean *mustard* and soy *mustard* can supply, comply fully with the daily requirements, both for men and women. Mineral elements are extremely related to human health and diseases since they can induce physiological changes in individuals (Gibson, 1989). From the public health point of view, it is important to assure the population that the ingestion of nere mustard and soy mustard is adequate to a normal diet. At the same time, the diet must not contain toxic elements above acceptable levels, preventing chemical poisoning.

The knowledge of the total energy value of foods is of great interest for the nutrition field since it makes it possible to know the calories ingested by the consumer. Therefore, the present study determined that the total energy value of 100 g of African locust bean nere *mustard* or soy *mustard* was higher to the value on the ENDEF Food Composition Table, which is 144.00 kcal (Instituto., 1999) (Table 1).

Result showed large amounts and significant difference in total lipids between African locust bean *mustard* and *soy mustard*. These mustards lipid must be valued for its oil and fatty acids, which are the main energy sources for the human body, and have been used by industries due to their abilities to dissolve flavor and aromatic compounds to modify various products consistency. The amount of fatty acids found in this study is similar to those found by Babacar et al. (2000) in *netetu* (a food condiment from fermented *Parkia biglobosa* seeds) of different origins available on the senegalian market. Regarding the amount of unsaturated fatty acids, they were found in very large amounts in these mustards.Nere mustard and soy mustard have the most interesting nutritional value because of their richness in unsaturated fatty acids (34.41 g/100 g of African locust bean *mustard* oil and 49.76 g/100 g of *soy mustard* oil of C 18:2 ω-6, and 0.24 g/100 g of African locust bean *mustard* oil and 5.87 g/100 g of *soy mustard* oil of C18: 3 ω-3). It is important to point out that the amount of oleic acid (16.86 g/100 g of African locust bean *mustard* oil and 24.64 g/100 g of *soy mustard* oil), were closer to values recommended in mustard oil and soy oil. Poly-unsaturated content in African locust bean *mustard* oil and in *soy mustard* oil were closer to values respectively recommended in mustard oil, palm oil, soy oil and maize oil, which are also widely used in the prevention of coronary heart disease.

Oleic acid contains a double bond on carbon 9, known as an omega-9 fatty acid and has a basic role in hormone synthesis in the human organism, and in the reduction of blood LDL cholesterol levels (Galvão, 2000; Turatti, 2000). Similarly, linoleic and linolenic fatty acids, compounds of the omega-6 (C18:2, ω-6) and omega-3 (C18:2, ω-3) families, respectively, also play important roles in the human organism since they are part of the cell membrane, and have antithrombotic and anti-inflammatory activities. They act as antithrombotic prostaglandin and leukotriene precursors and stimulate immunity, respectively, besides being related to the reduction of coronary heart disease and its risk factors (Chiarello et al., 2005).

Amongst the saturated fatty acids, palmitic acid (C16:0) corresponding to 10.5 g/100 g of African locust bean *mustard* oil and 12.34 g/100 g of *soy mustard* oil, which is in accordance with values determined by Lamboni et al., (1999) which is 12.4 g/100 g of total fatty acids, for varieties of *mustards* (Mna and Mnas) manifactured in their laboratories, but it is one of the villains in blood cholesterol increase (Hartman, 1993).

African locust bean *mustard* and soy mustard oils, present higher values of both unsaturated fatty acids when compared to the oils which are also widely used in the prevention of coronary heart disease and its risk factors. However, it must be considered that these mustards used in foods submitted to heating do not have coronary heart disease risk factors. Therefore, this depending on the time and temperature of the process applied, undesirable alterations in the chemical structures of these fatty acids may occur, which will reduce their benefits to human health.

Regarding carotenoids, the results shown in Table 3 indicate that African locust bean *mustard* contains 60.72±5.06 mg total carotenoids/100 g, and soy mustard contains 100.86±8.45 mg total carotenoids/100 g. The fact that carotenoids exert many functions, for example, antioxidant activity represent vitamin A precursor, which makes this result of utmost importance for human life since African locust bean *mustard* and *soy mustard* could be used for the prevention of various diseases, amongst them eyesight problems caused by vitamin A deficiency, diseases that result from oxidative stress, such as cancer, amongst others (Luciana and Armando, 2011).

Ascorbic acid has various functions which are based mainly on its property as a reversible biological reducing agent.

Thus, it is essential as a co-factor for various biochemical reactions, and as a protective antioxidant that works in the aqueous phase, which can be regenerated in vivo when oxidized; it also affects a variety of factors associated to the risk of heart disease, including the integrity of vascular tissue, vascular tonus, lipid metabolism, and blood pressure (Horrobin, 1996). It can also increase non-heme iron absorption and participate in the formation of collagen. The results showed that the ascorbic acid contents in nere mustard (19.31 mg/100 g) and in soy mustard (13.26 mg/100 g) (Table 3) are much lower than those found in orange (50 to 100 mg/100 g orange) (Andrade et al., 2002).

Ascorbic acid and polyphenols also possess anti-atherosclerotic, anti-inflammatory, antitumor, antithrombotic, anti-osteoporosis and antiviral activities (Nijveldt, 2001). This research evidenced that 100 g of African locust bean *mustard* contained 100.01±24.29 mg of total polyphenols and 2.67±0.23 mg of flavonoids, also 100 g of soy mustard contained 170.63±31.39 mg of total polyphenols and 5.28±1.92 mg of flavonoids. These values are very higher than those found in the literature for carrot and Brussels sprouts (Cunha, 2005) (Table 3).

This characteristic is of great relevance to human health given its relationship with the prevention of diseases caused by oxidative stress, amongst others, as earlier mentioned.

Conclusion

In addition to its high nutritional status and its antioxidant properties conferred by its contents of polyphenols, ascorbic acid and carotenoids, African locust bean *mustard* and soy *mustard* contain bioactive compounds with functional activities due to the high concentrations of oleic acid and carotenoids, and for being a great source of vegetable protein. However, further studies are necessary to confirm the beneficial effects of those functional substances.

Conflict of Interests

The authors have not declared any conflict of interests.

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