

## IN VITRO AND ANIMAL STUDIES

Impact of micronized starfruit (*Averrhoa carambola* L.) fiber concentrate on lipid metabolism in miceErasmus Herman-Lara<sup>1</sup>, Laura I. Elvira-Torales<sup>1</sup>, Jesús Rodríguez-Miranda<sup>1</sup>, Juan G. Torruco-Uco<sup>1</sup>, Roselis Carmona-García<sup>1</sup>, Patricia G. Mendoza-García<sup>2</sup>, Hugo S. García<sup>2</sup>, Ida Soto-Rodríguez<sup>3,4</sup>, Enrique Sánchez-Valdivieso<sup>4</sup>, and Cecilia E. Martínez-Sánchez<sup>1</sup><sup>1</sup>Instituto Tecnológico de Tuxtepec, Tuxtepec, Oaxaca, Mexico, <sup>2</sup>Instituto Tecnológico de Veracruz, Col. Formando Hogar, Veracruz, Mexico, <sup>3</sup>Facultad de Bioanálisis, Universidad Veracruzana, Veracruz, Mexico, and <sup>4</sup>Escuela de Medicina, Carr, Universidad Cristóbal Colón, Campus Calasanz, Boca del Río, Veracruz, Mexico

## Abstract

The objective of this study was to evaluate the effect of micronized insoluble fiber from starfruit bagasse as an ingredient of a functional food (FF) or as micronized insoluble fiber-rich fraction (IFRF) and its effects *in vivo* on lipids metabolism in a murine model. Experimental animals were divided in four isoproteic (15.8%) treatments differing on the fiber and cholesterol level used. The micronized IFRF particle size ranged from 37.5 to 149 µm. Treatments with added IFRF and those including the FF lowered serum triacylglycerols, total cholesterol (TC), high-density lipoproteins (HDL), and low-density lipoproteins (LDL) concentrations (IFRF: 14.2, 25.4, 55.06, and 12.18%, respectively; FF: 30.18, 39.47, 35.11, and 43.18%, respectively). IFRF produced the overall highest serum hypolipidemic effect and prevented the development of non-alcoholic fatty liver. Both the IFRF and the FF exhibited hypolipidemic effects that suggest a potential role of starfruit insoluble fiber as a component of FFs aimed against cardiovascular diseases.

## Keywords

Functional food, lipid metabolism, micronization, starfruit

## History

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## Introduction

Functional foods (FFs) have taken an important role in the food industry during the past decade. These foods provide basic nutrition as well as health benefits such as disease prevention or may delay the evolution of chronic disorders. Components in these foods can produce physiological benefits or remove compounds that may pose a health risk (Yangilar, 2013). Recent researches have focused on the search for FFs for combating chronic diseases such as cancer, cardiovascular diseases, and type 2 diabetes (Lambert, 2001). Many studies highlighted the functional properties of numerous food components, including ω-3 fatty acids (Hjaltason & Haraldsson, 2006), bioactive peptides (Thomä-Worringer et al., 2006), and fiber (Fernández-López et al., 2009). It is commonly believed that high dietary fiber (DF) intake contributes to attaining a healthy weight, and helps to control diabetes, hypertension and heart disease, as well as to prevent colon cancer and other intestinal diseases (Kendall et al., 2010).

The role of DF in nutrition and health is well established, it is has reported that a minimal recommendable daily intake of soluble and insoluble fiber, respectively, could prevent stroke in certain populations (Casiglia et al., 2013). Knowledge of the

beneficial effects of high DF diets toward the prevention of cardiovascular diseases and several types of cancer, as well as the inclusion of DF supplements in slimming diets, has led to the development of a large and yielding market for DF-rich products. Commonly consumed products include traditional foods (meat, dairy products, breakfast cereals, biscuits, breads, etc.) enriched with different amounts of fiber from various sources, as well as dietary supplements including tablets, capsules, and others (Artiss et al., 2006; Ramos et al., 2008).

DF has received a large attention in nutritional epidemiology. Observational studies have consistently shown that DF intake is associated with reduced cardiovascular risk, including ischemic heart disease (Liu et al., 2002; Mozaffarian et al., 2003). Clinical trials have also suggested that DF supplementation has beneficial effects on risk factors, such as blood pressure, serum lipids, insulin sensitivity, and diabetic metabolic control (Anderson et al., 2000; Stroppel et al., 2005).

Micro- or nanotechnology is the center of intense interest in food research and development. Studies have been focused on the many advantages this technology can offer in terms of changes in particle size, increased surface area, and improved functional properties in different materials, and suggest many possible academic and industrial applications (Masciangioli & Zhang, 2004).

*Averrhoa carambola*, also known as carambola or starfruit, is a popular juicy fruit in several countries. The starfruit juice is usually employed to manufacture confectionary, juice concentrate, and refreshing drinks (Teh et al., 2010). After the juice extraction process, thousands of tons of starfruit bagasse are produced and underutilized as feed. Starfruit was reported to be rich in DF,

especially insoluble DF (Chau et al., 2006). Some authors have pointed out that agricultural by-products from fruits, cereals, and vegetables represent potential sources of fiber and functional compounds (Elleuch et al., 2011; O'Shea et al., 2012).

Starfruit (*Averrhoa carambola* L.) bagasse can be used to produce an insoluble, fiber-rich fraction with desirable functional properties as *in vitro* hypoglycemic and *in vivo* hypolipidemic and hypocholesterolemic effects (Chau et al., 2004a,b). The use of micronization technology with starfruit insoluble fiber results in improving physicochemical properties (Chau et al., 2006) that confers potential applications as a FF ingredient for control of cardiovascular diseases and type 2 diabetes. The physicochemical properties (e.g. swelling property, water- and oil holding capacities, and cation-exchange capacity) of these insoluble fiber-rich fractions (IFRFs) were significantly higher than those of cellulose. Thus, the determination of the abilities of these IFRFs in lowering postprandial serum glucose level and reducing caloric content would be useful for utilization as a promising fiber source in FF applications. The objective of the present study was to evaluate the effect of a micronized insoluble fiber-rich product from starfruit bagasse as a hypolipidemic FF component in a murine model. Chemical characterization on starfruit bagasse was made, its insoluble fiber-rich fraction and a FF containing this fraction as an ingredient was prepared. The use of starfruit insoluble fiber-rich fraction as a FF in combating some chronic diseases currently prevalent world, but would also promote production of healthy foods from starfruit by-products.

## Materials and methods

### Extraction of insoluble fiber-rich fraction

Starfruit bagasse was obtained after juice extraction with a home fruit juicer (heavy duty model, Turmix, Mexico City, Mexico), and dried in a tray dryer at 60 °C for 24 h. After drying the material was ground in a food processor (Masterchef 8000 model, Moulinex, Normandy, France) and filtered through 0.5 mm (No. 32) mesh. Samples were stored in a desiccator until analyzed. The IFRF was prepared by homogenizing a dried bagasse sample with distilled water (fiber:water ratio of 1:10 w/v) in a blender (Sunbeam-Oster, 465-10 model, Niles, IL) and then centrifuged at 1006 × g for 1 min. The IFRF was collected by vacuum filtration, and washed with distilled water (fiber:water ratio of 1:30 w/v) and ethanol (fiber: 70% ethanol ratio of 1:8 w/v) according to Chou et al. (2008). Excess solvent was removed by sun drying for 2 d, followed by drying in a convection oven at 60 °C for 24 h. A FoodSaver<sup>®</sup> vacuum sealer system (V2450 model, Miami, FL) was used for sample storage.

### Chemical composition

Proximate composition was determined following AOAC (1984) methods: moisture (32.083), ash (14.006), and crude fat (7.062). Proteins were calculated using a 6.25 factor (42.014) (AOAC, 1970), and total soluble and insoluble fiber fractions were separated and quantified (985.29) (AOAC, 1997). The fiber content was also analyzed with a commercial kit (Sigma TDF-100A Kit, Saint Louis, MO). Carbohydrates were calculated by difference.

### Micronization

The IFRF was micronized by passing the material three times through a hammer mill (Glen Creston 17-140 model, Retsch, Haan, Germany) and screening through several sieves as it is described below. The micronized fiber was stored in vacuum-sealed polyethylene bags in a desiccator until used.

### Particle size distribution

IFRF samples obtained were screened in a Ro-Tap device (RX-29-E Model, W.S. Tyler Test Sieve Shakers, Milwaukee, WI) to calculate the percentage of retained particle size in each of the sieves used, which were 60 (opening of 250 μm), 80 (opening 177 μm), 100 (aperture of 149 μm), 200 (opening of 74 μm), and 400 (opening of 35 μm) US. The screening was performed using samples of 150 g and stirring for 15 min to effect separation of the particles. Of each mesh, the weight of the retained material was determined and then the percentage retained was calculated in relation to the total sample.

### Preparation of cookies

The recipe used for cookie preparation included corn starch, soy oil, sucrose, baking powder (0.1%), vanillin (3%), and water (8%), the first three ingredients were considered in a half proportion as mentioned in the experimental diet formulation and the other half was added at the moment of preparing the corresponding diet. The dough was allowed to equilibrate for 1 h at 4 °C and divided into 15 g portions made into round shape and baked in a commercial oven (Mabe HM8015 model, Mexico City, Mexico) on a tray at 180 °C for 25 min. Baked cookies were cooled to room temperature. Cookies were stored under vacuum in sealed bags (Food Saver<sup>®</sup>, Miami, FL) at 8 °C until used.

### Diets

A standard diet AIN-93M Purified Rodent Diet (Panlab S.L., Barcelona, Spain) that provides all the nutrients required by adult rats according to the National Research Council guidelines (Ramos et al., 2008) with slight modifications was used for this experiment: the diet contained 10.1% of metabolizable energy from fat, 65.6% from carbohydrates, and 20.4% from proteins. The source of lipids of the four diets was soybean oil (5.4% of metabolizable energy) providing 1.7% of metabolizable energy as palmitate, 3.0% as oleate, and 3.6% as linoleate (only fatty acids with contribution ≥1% are included).

Experimental diets were positive control, IFRF, and FF and were supplemented with 1% (w/w) reagent grade cholesterol (Sigma C8667, grade ≥99%, St. Louis, MO) to induce food hypercholesterolemia in mice (Table 1). A negative control diet was included to which no cholesterol or fiber was added. For four diets, the source of protein was casein, and the sources of carbohydrate were dextrose, maltodextrin, sucrose, and corn starch. The diets had low sodium content ≤0.3% by weight) and were matched for content of mineral elements, trace elements, and vitamins.

Table 1. Experimental diets formulations (d.b).

Ingredients (g kg <sup>-1</sup> )	Negative control	Positive control	IFRF	FF <sup>a</sup>
Casein	140	140	140	140
IFRF <sup>b</sup>	–	–	50	–
Cookie <sup>c</sup>	–	–	–	128 (5% IFRF)
Sucrose	100	100	100	100
Corn starch	673.2	663.2	613.2	535.2
Soybean oil	40	40	40	40
L-Cysteine	1.8	1.8	1.8	1.8
Vit/min premix	45	45	45	45
Cholesterol	–	10	10	10

<sup>a</sup>Functional food.

<sup>b</sup>Insoluble fiber-rich fraction.

<sup>c</sup>Cookie.

The IFRF was included as an ingredient in cookies to produce a FF. Test diets were prepared by incorporating the IFRF, cookie, or cholesterol by substituting the equivalent amount of corn starch and were balanced by making theoretical calculations per serving. Four kinds of pellets were prepared for feeding. Diets formulated in dry powder form were mixed with a gelatin solution (7 g/100 mL purified water) to form pellets.

Experimental animals were 28 male mice (strain C57BL/6, Harlan, Mexico City, Mexico), 6–7 weeks of age with an average weight of  $18.69 \pm 1.53$  g. The animals were housed in individual cages with a 12/12 h light/dark cycle at 25 °C. At the end of 7-d adaptation period, animals were randomly assigned to one of the four diet groups. Each diet group comprised seven mice.

The corresponding feed treatment and purified water were offered *ad libitum* for 30 d. Feed was weighed, and feeders were cleaned and refilled daily. Uneaten food was removed daily and replaced with fresh food. Every third day, the cages were disinfected and fresh, and sterile wood shavings were placed on the cage floor. Animal weights were recorded weekly.

### Sample collection and laboratory analysis

At the end of the 30-d experimental diet period, food was removed at 7:30 h, 1 h after the beginning of the light cycle. Water was provided *ad libitum* after food removal. Animals were anesthetized with ether and samples were taken by cardiac puncture and blood and liver samples were collected for serum cholesterol and triacylglycerides determination. Mice were killed between 11:30 and 14:00 h. The project was approved by the Animal Care and Use Committee CCUAL-FM-UAEM (Autonomous University of Morelos State). A bioethical approval was obtained from the committee for animal use and procedures performed on them.

### Serum cholesterol and triglycerides

Blood samples were taken from the submandibular vein in anesthetized mice after 4 h of food deprivation. After centrifugation of blood samples (10 min at  $600 \times g$ ; 4 °C), serum was divided into 100  $\mu$ L aliquots and stored in Ependorff tubes at –70 °C. Serum samples were analyzed by colorimetric methods using enzymatic diagnostic kits for cholesterol (CHOLESTEROL-LQ, Spinreact, Barcelona, Spain), triacylglycerides (TRIGLYCERIDES-LQ, Spinreact, Barcelona, Spain), and high-density lipoproteins (HDLc-P, Spinreact, Spain). Low-density lipoprotein (LDL) cholesterol was quantified according to Friedewald et al. (1972).

### Liver histological analysis

Four animals from each group were used for histopathological analyses. The livers were first fixed in a 10% formaldehyde solution and then in paraffin. Sections (4–6  $\mu$ m) were prepared with a Minot microtome (American Optical) and stained by the hematoxylin–eosine technique. Histological changes were observed with a microscope (Zeiss Axiostar plus, Göttingen, Germany) equipped with a digital camera (Canon Power Shot A640, Canon, Tokyo, Japan).

### Statistical analysis

Experimental data were subjected to a one-way analysis of variance (ANOVA) followed by Tukey's multiple-range test using the Statistica v. 8.0 software (StatSoft Inc., Tulsa, OK). Differences were considered to be significant at  $p < 0.05$ . All experiments were made at least in triplicate.

## Results and discussion

### Chemical composition of bagasse and IFRF

Total DF (TDF) in the starfruit bagasse ( $631 \text{ g kg}^{-1}$ ) (Table 2) was higher than the  $573 \text{ g kg}^{-1}$  TDF reported for starfruit native to China (Chau et al., 2004a), but lower than the  $723 \text{ g kg}^{-1}$  TDF in starfruit native to Bangladesh (Friedewald et al., 1972). Insoluble fiber constituted the majority ( $507.8 \text{ g kg}^{-1}$ ) of the bagasse TDF, followed by carbohydrates ( $203.8 \text{ g kg}^{-1}$ ); soluble fiber ( $123.1 \text{ g kg}^{-1}$ ), and other minor components: proteins ( $71 \text{ g kg}^{-1}$ ), lipids ( $69.6 \text{ g kg}^{-1}$ ), and ash ( $24.4 \text{ g kg}^{-1}$ ). This coincides with previous reports that the insoluble fraction is the principal constituent in starfruit TDF (Chau et al., 2004a; Nahar et al., 1990). Compared with other fruit bagasses, starfruit TDF content ( $631 \text{ g kg}^{-1}$ ) was greater than mango ( $370 \text{ g kg}^{-1}$ ) or pineapple ( $289 \text{ g kg}^{-1}$ ), but smaller than latkan ( $790 \text{ g kg}^{-1}$ ), lukluki ( $712 \text{ g kg}^{-1}$ ), and guava ( $744 \text{ g kg}^{-1}$ ) from Bangladesh. Its soluble fiber proportion ( $123.1 \text{ g kg}^{-1}$ ) was higher than lukluki ( $630 \text{ g kg}^{-1}$ ) and guava ( $70 \text{ g kg}^{-1}$ ), which has a relatively a low soluble fiber proportion despite their high TDF content (Nordby & Hall, 1979). Starfruit bagasse is clearly an overall good DF source, including insoluble and soluble fibers, which makes it a promising raw material in food industry applications.

Separation of the IFRF from the starfruit bagasse reduced its ether extract content by 79.17% due to pigments removal (probably flavonoids) (Table 2). The remaining 1.45% could correspond to water- and alcohol-insoluble polymeric lipid compounds such as waxes and sterols on the fruit epidermis (Nordby & Hall, 1979), and other pigments such as tannins (Narain et al., 2001). Due to their water and alcohol solubilities, ash and carbohydrate contents decreased considerably (62.70% and 73.25% reduction, respectively). This ash content is higher than the values reported for apple fiber concentrate, but lower than those reported for date, pea, grapefruit, lemon, and orange concentrates (Besbes et al., 2010; Figuerola et al., 2004). The TDF fraction was concentrated to  $921.8 \text{ g kg}^{-1}$ , with 76.11% insoluble DF (IDF) and  $160.7 \text{ g kg}^{-1}$  soluble DF (SDF). The starfruit TDF content observed here exceeds the 51.7–89.8% levels reported previously, although under different fiber extraction conditions (Besbes et al., 2010; Figuerola et al., 2004). The IDF: SDF ratio in the IFRF was 4:1, similar to that in the bagasse. The fiber separation method employed resulted in higher insoluble fiber-rich concentrate recovery from this fiber-rich raw material.

### Particle size

Particle size in the IFRF showed that the highest recovery was in the 100 (25.34%), 200 (30.93%), and 400 (39.06%) mesh, meaning the 149–37.5  $\mu$ m particle size interval

Table 2. Chemical composition of starfruit bagasse, starfruit insoluble fiber-rich fraction (IFRF).

Composition ( $\text{g kg}^{-1}$ ) (d.b)	Starfruit bagasse	IFRF
Proteins	$71.0 \pm 5.0^a$	$26.8 \pm 0.8^b$
Ether extract	$69.6 \pm 0.0^a$	$14.5 \pm 0.7^b$
Total dietary fiber	$631.0 \pm 6.8^a$	$921.8 \pm 14.1^b$
Insoluble dietary fiber	$507.8 \pm 1.8^a$	$761.1 \pm 14.1^b$
Soluble dietary fiber	$123.1 \pm 4.9^a$	$160.7 \pm 3.5^b$
Ash	$24.4 \pm 0.9^a$	$9.1 \pm 0.3^b$
Carbohydrates <sup>b</sup>	$203.8^a$	$27.8^b$

<sup>a</sup>Data are expressed as mean  $\pm$  standard deviation ( $n = 3$ ).

<sup>b</sup>Calculated by difference. Values in the same row with different letters are significantly different (Tukey,  $p < 0.05$ ).

represented 70% of the recovered fiber (Figure 1). Smaller particle sizes could be collected by using a larger number of powder recirculation steps, high pressure micronizers, or jet grinding (Chau et al., 2006). Some works have demonstrated that micronization treatment could effectively reduce the particle size of DF to microsized (Chau et al., 2006; Chou et al., 2008; Huang et al., 2008). Feeding the micronized insoluble fibers, particularly the micronized IFRF, significantly ( $p < 0.05$ ) improved their abilities in lowering ( $p < 0.05$ ) serum triglyceride concentrations, serum total cholesterol (TC), and liver lipids to different extents by significantly enhancing ( $p < 0.05$ ) the excretion of lipids, cholesterol, and bile acids via feces. Chou et al. (2008) found that the particle size was a crucial factor affecting the characteristics and physiological functions of insoluble fibers.

### Hypolipidemic activity

Average feed intake ( $4.51\text{--}5.78\text{ g d}^{-1}$ ) did not vary ( $p > 0.05$ ) between the four experimental groups. At the end of the experimental period, average animal weight was  $20.22 \pm 0.95\text{ g}$  in the negative control,  $19.83 \pm 0.60\text{ g}$  in the positive control,  $19.33 \pm 1.50\text{ g}$  in the IFRF group, and  $21.68 \pm 1.89\text{ g}$  in the FF group. The FF group exhibited the highest relative weight gain ( $0.05/\text{d}$ ), although it was not statistically different ( $p > 0.05$ ) from the other groups. Serum TG in the positive control group (no fiber +1% cholesterol) was  $99.76\text{ mg dL}^{-1}$ , that is 14.2% and 30.18% higher than in the IFRF and FF groups, respectively (Table 3). This coincides with reports that fibers from some fruit and vegetable bagasses reduced serum triacylglycerols levels in rodents (Chou et al., 2008). Serum TG levels in the IFRF and FF groups were within desirable levels ( $10\text{--}160\text{ mg dL}^{-1}$ )

(Jehle, 2002). Triacylglycerides levels did not surpass these desirable levels in any of the four groups, since the diet was a maintenance formula, with no excess of carbohydrates, proteins, or fats to be converted into triacylglycerides (Jehle, 2002; Millán et al., 2009).

Serum TC was reduced ( $p < 0.05$ ) by 25.40% in the IFRF group and 39.47% in the FF group, levels similar to those reported for carrot fiber (Chou et al., 2008). This reduction may be due to the formation of a protective membrane by the DF around the cholesterol-containing lipid drops, which prevents absorption in the small intestine (Mun et al., 2006). DF also increases viscosity, possibly altering lipid coalescence in the stomach and small intestine (Yangilar, 2013). Chou et al. (2008) observed increased bile salt excretion, which is related to cholesterol metabolism. This increased excretion could be in response to entrapment of bile salts by DF, thus avoiding lipid emulsification in the small intestine or transport of lipid digestion products from lipid drops to the intestinal mucosa, a mechanism proposed by Thongngam & McClements (2005). A similar succession of events may have occurred in the FF group since TC levels in this group were near those of the negative control group. In the IFRF group, serum TC levels fell within desirable levels.

The positive control, IFRF, and FF groups had lipoprotein contents within normal levels ( $60\text{--}160\text{ mg dL}^{-1}$ ) (Jehle, 2002). Reductions in serum TC affected lipoprotein levels in the FF and IFRF groups. LDL levels decreased ( $p < 0.05$ ) appreciably (43.18%) in the FF group, but no difference was observed between the IFRF and the positive control groups. According to results from other studies (Chen et al., 2008; Erkkilä & Lichtenstein, 2006; Lairon, 2001), it was proposed that the enhancement in lipid- and cholesterol-lowering activities by the consumption of micronized fibers could be a combination of the improved physiological performances, including decreased transit time, increased binding of lipids and bile acids in the intestinal lumen, reduced absorption of lipids and bile acids, increased bile acid excretion, elevated cholesterol catabolism to bile acids, delayed cholesterol biosynthesis, and subsequently, up-regulation of the LDL receptor to compensate for the loss of cholesterol (Chou et al., 2008). HDL levels were reduced by 35.11% in the FF group and 55.06% in the IFRF group compared to the positive control group, probably due to the reduction in TC considering that DF increases the enzymatic activity of cholesterol-7- $\alpha$ -hydroxylase, the major regulatory enzyme in the hepatic conversion of cholesterol to bile acids, thus contributing to a higher depletion of hepatic cholesterol (Roy et al., 2002). The FF treatment controlled both LDL and HDL levels, keeping them within desirable levels. In contrast, the IFRF treatment had a  $28.06\text{ mg dL}^{-1}$  HDL concentration and a  $108.14\text{ mg dL}^{-1}$  LDL concentration, suggesting that the HDL were inactive or beginning activation to control serum cholesterol levels (Millán et al., 2009), which were within normal levels ( $35\text{--}65\text{ mg dL}^{-1}$ ). TC and lipoproteins levels in the FF treatment resulted in a TC/HDL ratio of 3.13 and a LDL/HDL ratio of 1.72, both better than the 3.28 (TC/HDL) and 1.97 (LDL/HDL) of the positive control; lower

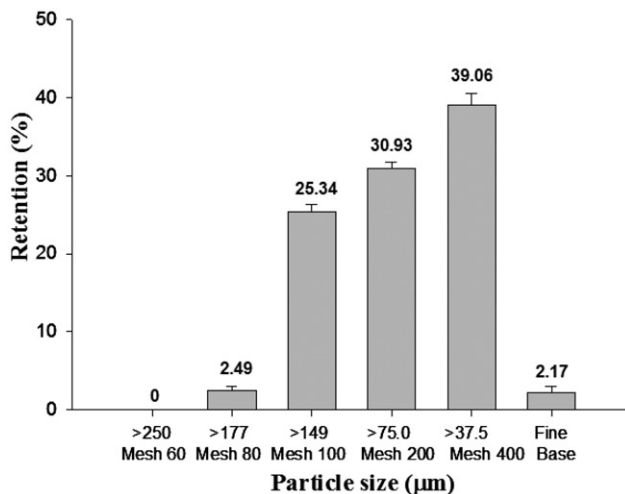


Figure 1. Particle size distribution (% weight) of micronized starfruit insoluble fiber-rich fraction. Values represent the average of three replicates  $\pm$  standard deviation.

Table 3. Serum triacylglycerols (TG), total cholesterol (TC), HDL, and LDL concentrations in mice fed the experimental diets for 30d.

Diet	TG ( $\text{mg dL}^{-1}$ )	TC ( $\text{mg dL}^{-1}$ )	HDL ( $\text{mg dL}^{-1}$ )	LDL ( $\text{mg dL}^{-1}$ )
Neg. control	$60.67 \pm 19.99^{\text{ac}}$	$123.46 \pm 27.13^{\text{a}}$	$72.43 \pm 14.55^{\text{a}}$	$38.90 \pm 23.28^{\text{a}}$
Pos. control	$99.76 \pm 7.82^{\text{b}}$	$205.53 \pm 21.03^{\text{b}}$	$62.43 \pm 6.25^{\text{a}}$	$123.14 \pm 22.78^{\text{b}}$
IFRF	$85.59 \pm 5.48^{\text{a}}$	$153.32 \pm 23.27^{\text{a}}$	$28.06 \pm 13.02^{\text{b}}$	$108.14 \pm 24.79^{\text{b}}$
FF	$69.65 \pm 7.21^{\text{c}}$	$124.41 \pm 9.23^{\text{a}}$	$40.51 \pm 5.62^{\text{b}}$	$69.97 \pm 6.78^{\text{c}}$

<sup>a</sup>Data are expressed as mean  $\pm$  standard deviation ( $n = 7$ ). Values in the same column with different letters are significantly different (Tukey,  $p < 0.05$ ). IFRF, insoluble fiber-rich fraction; FF, functional food.

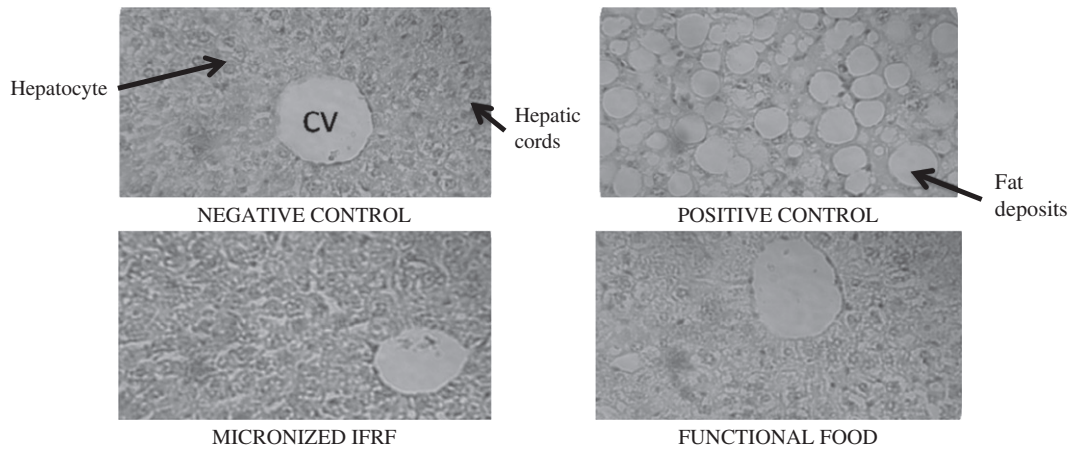


Figure 2. Micrographs (magnification 400 $\times$ ) of mouse liver tissue after the 30-d experimental period. CV, central vein.

ratio values indicate a lower risk of contributing to cardiovascular disease development (Millán et al., 2009).

### Liver histological analysis

Histopathological analysis showed the animals fed the negative control diet to have normal structures in the hepatic cords around the central vein and in the hepatocytes (Figure 2). The mice from the positive control stand in stark contrast, exhibiting steatohepatitis characterized by inflammation of the hepatocytes, with intracytoplasmic lipid deposits and neutrophilic infiltration, all characteristic of non-alcoholic fatty liver (NAFL) (Calderín-Bouza et al., 2009). Livers from mice in the IFRF group did exhibit separated hepatic cords but no hepatocyte damage. Livers from the FF group were similar to those from the negative control, highlighting the ability of this treatment to regulate both serum and liver cholesterol levels. Intake of diets high in soluble fiber (e.g. guar gum and oligofructose) decreased liver damage and can even aid in liver regeneration and decreased hepatocyte apoptosis in fatty liver (Lai et al., 2005). In the present study, the use of insoluble DF as a functional ingredient prevented liver damage and lipids accumulation in hepatocytes, avoiding development of NAFL. In addition to this, starfruit is inexpensive and very accessible in tropical areas with high rates of obesity.

### Conclusions

This work demonstrated that micronization treatment could effectively reduce the particle size of the starfruit bagasse to microsized. Feeding of the micronized insoluble fibers, particularly the IFRF and FF, could significantly ( $p < 0.05$ ) improve their abilities in lowering ( $p < 0.05$ ) the concentrations of serum triacylglycerides, serum TC, and liver lipids to different extents by means of enhancing ( $p < 0.05$ ) the excretion of lipids and cholesterol. Serum lipids levels in the treatments coincided with the presence or absence of fatty liver indicators in the histological analysis. Inclusion of the micronized insoluble fiber-rich fraction in 1% cholesterol supplemented diets for mice lowered serum TC, HDL and triacylglycerides compared with a positive control, but it did not significantly lower LDL. The results suggested that particle size was a key factor in affecting the characteristics and physiological functions of insoluble fibers. It also shed light on the potential applications of micronization technology in the food industry and may offer an opportunity to improve the physiological functions of insoluble fibers, particularly the IFRF from starfruit, in health food applications.

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### Declaration of interest

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