

The Effect of Diets Enriched with Avocado (*Persea americana* cv Hass) on Cholesterol and Triglyceride Decrease Evaluated in Hamsters

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Abstract Avocados are characterized by their high dietary fiber and unsaturated fatty acids content. This study aimed to evaluate the hypolipidemic and hypocholesterolemic effect of avocado Hass on hamsters fed with diets based on freeze-dried pulp avocado (FAD) and defatted paste avocado (DPAD). Total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), serum triglycerides (TG), and atherogenic index of plasma (AIP) were evaluated along with total lipids in liver and feces. Compared to the control diet, FAD and DPAD reduced the serum TG levels by 76.47 and 46.06%, respectively. FAD also reduced the serum TC levels by 16.39%. FAD and DPAD decreased the lipids in the liver by 33.61% and 10.0% and the amount of lipid excreted in feces by 73.47% and 41.50%, respectively. FAD showed the lowest AIP, 0.60, compared to that of DPAD and the control, 1.83 and 1.94, respectively. Freeze-dried avocado pulp was found to be efficient in reducing dyslipidemia values in hamsters.

Keywords: avocado, freeze-drying, hamsters, triglycerides, cholesterol

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1. Introduction

Avocado (*Persea americana* Mill., Lauraceae) is a fruit native to Central America and Mexico [1]. It grows in tropical and subtropical climates in countries such as Australia, the United States, and Malaysia [2]. *Persea americana* cv Hass is the most important commercial avocado. Mexico is the main producer and exporter of avocado cv. Hass worldwide [3].

Cardiovascular diseases are one of the main causes of mortality across the globe [4]. A close relationship between these diseases and the high concentration of LDL-cholesterol (LDL-C), total cholesterol, and blood triglycerides (TG) have been reported [5]. Low consumption of saturated fatty acids and a higher intake of unsaturated fatty acids, it has been documented, prevents the incidence of cardiovascular disease by reducing the TC and TG serum levels. Avocados have a high amount of lipids, around 60% dry basis, being mainly of the unsaturated type, such as oleic acid, linoleic acid, and linolenic acid [6].

The use of avocado has been mentioned as an alternative to prevent overweight and type 2 diabetes [7];

it also regulates glucose tolerance and insulin resistance [8]. Avocados are an excellent source of different bioactive compounds that act as antioxidant molecules, including polyphenol compounds [9], phytosterols, such as β -sitosterol, campesterol, stigmasterol, and avenasterol [10,11]. Phytosterols are plant sterols that reduce blood cholesterol levels by different metabolic processes, such as the inhibition of intestinal cholesterol absorption and liver synthesis of cholesterol. Another component present in avocados is dietary fiber, of which around 70% is insoluble and 30% soluble [12], which is associated with cardiovascular health [13]. Studies show a beneficial effect of monounsaturated fatty acids (MUFAs), mainly oleic acid, on lipoprotein metabolism. In a study [14], it was documented that MUFAs exert synthetic and rapid catabolic pathways for triglyceride-rich lipoprotein metabolism that are mediated through apolipoproteins E and C-III and suppress the secretion of more slowly metabolized types of VLDLs and IDLs that do not contain these apolipoproteins. This is a possible mechanism that helps control the serum LDL-C levels.

Avocados have a short post-harvest shelf-life; therefore, it is suggested that some conservation process be applied to preserve their therapeutic and nutritional qualities. Freeze-drying is a preservation system based on a

sublimation process. The food is frozen and introduced into a vacuum chamber that generates sublimation by lowering the temperature. The final product is a dehydrated food with an excellent rehydration capacity, and its nutritional properties are preserved [3]. Countries like Mexico are characterized as large exporters of avocado var. Hass worldwide. The commercialization of freeze-dried avocado pulp has been increasing. However, there is little information on the effects of the consumption of freeze-dried avocado on the control of lipid levels. It is documented [15,16] that the Syrian hamster is the species with the greatest similarity to humans regarding lipoprotein metabolism, representing the most appropriated preclinical model for investigating the treatments of dyslipidemia in humans. This study aimed to evaluate the hypolipidemic effect in hamsters' diets containing either lyophilized whole avocado pulp or avocado paste (the oil was previously extracted from freeze-dried avocado pulp).

2. Materials and Methods

The freeze-dried avocado pulp var. Hass was provided by the company SíoSí Alimentos S.A. de C.V. located in Morelia, Mich., México. The avocado oil was extracted with hexane at 70°C from the freeze-dried avocado pulp. The solvent/oil phase was separated. The remaining solid was oven-dried at 70°C to remove the residual solvent and the product obtained was the avocado paste.

2.1. Chemical analyses

Analyses of the freeze-dried avocado, defatted paste avocado, and diets were performed according to the AOAC [17] methods as follows: moisture (934.01), protein (960.52), lipids (920.85), and ash (942.05). Soluble fiber (SF), insoluble fiber (IF), and total dietary fiber (TDF) were measured according to Prosky et al. [18]. To measure the fatty acid profile of avocado oil, derivatization of the fatty acids was first performed in order to convert them into non-polar low molecular weight substances, so that both volatilization of compounds and their detection could be carried out. The methodology used for the derivation and quantification of the fatty acids in the oil is briefly described below. 3 ml of ethyl ether was added to 0.1 g of the oil sample. The mixture was stirred, and then 1 ml trimethyl ammonium hydroxyl was added. It was stirred again, and the upper fraction that formed during the process was separated. The fatty acid profile of the oil was evaluated with a gas chromatograph coupled to an Agilent Technologies® model 6890N mass spectrometer. The equipment used a capillary column of silica. The temperature conditions for the injector were 300 and °C and for the detector 325°C.

2.2. Animals and Diets

Thirty 10-week-old male golden Syrian hamsters were submitted to a four-week adaptation period ($22 \pm 2^\circ\text{C}$, 12 h light/12 h dark cycles) and fed with commercial feed. Subsequently, the animals were weighed and randomly distributed to individual cages, forming five experimental

groups ($n = 6$ animals per group). Initial hamster weights were similar for all groups (115-119 g). The hamsters received food and water *ad libitum* for 21 days. The diets elaborated (Table 1) were as follows: Control diet, based on the formulation of Reeves et al. [19] for adult animals in maintenance (CD); diet based on defatted paste avocado (DPAD); diet based on freeze-dried avocado (FAD). For all the diets, 2% coconut oil and 10% cholesterol were added to induce hypertriglyceridemia and hypercholesterolemia in hamsters. An amount of 300g of defatted avocado paste and 270 g of freeze-dried avocado were added to each diet (DPAD and FAD, respectively) to complete 1000 g of diet. 300 g of paste avocado provided DPAD with 29.55 g of protein, 12.20 g of lipids, 147.10 g of fiber, 53.82 g of minerals, and 57.33 g of carbohydrates. 270 g of freeze-dried avocado provided FAD with 10.53 g of protein, 163 g of lipids, 52.0 g of fiber, 19.17 g of minerals, and 18.94 g of carbohydrates. Cellulose was not added to DPAD and FAD because they already contain a considerable content of TDF, and additionally, it allowed us to evaluate the effect of the dietary fiber provided by the avocado. The energy content in the diets was CD (4420 kcal), DPAD (3995), and FAD (5030), considering the following factors: protein 4 kcal/g, carbohydrates 4 kcal/g, and fat 9 kcal/g. At the end of the experiment, the hamsters were fasted for 12 h, anesthetized, and sacrificed by cervical dislocation. Blood was drawn by cardiac puncture in all animals and centrifuged at 5,000 rpm for 10 min. The serum was separated and stored at -20°C . The livers were removed, cleaned with distilled water, and stored at -20°C . The feces of the animals were collected during the last week of the study. The liver lipids and moisture and lipids of feces were determined by the previously mentioned methods. Total cholesterol (TC), high-density lipoproteins (HDL-C), and triglycerides (TG) levels in serum were determined using commercial kits (Total Cholesterol, Reference 1001090; Triglycerides, Reference 41032; HDL Cholesterol colorimetric, Reference 1001095. SPINREACT, Girona, Spain).

Table 1. Formulation of the experimental diets

Ingredients (g)	CD	DPAD	FAD
Starch	545	370	394.5
Sucrose	100	100	100
Casein	100	70	90.5
Cellulose	50	-	-
Lipids	40	30	-
Minerals	35	-	15
Vitamins	10	10	10
Cholesterol	20	20	20
Coconut oil	100	100	100
Avocado oil	-	-	-
Defatted paste avocado	-	300	-
Freeze-dried-avocado	-	-	270

CD, control diet; DPAD, defatted avocado paste diet; FAD, freeze-dried avocado diet.

According to Martinez-Flores et al. [20], the low-density lipoproteins (LDL-C) + very low-density lipoproteins (VLDL-C) value is estimated using the following formula: $\text{LDL-C} + \text{VLDL-C} = (\text{TC} - \text{HDL})$. The study was carried out according to the Mexican Official Guidelines for the production, care, and use of laboratory

animals (NOM-062-ZOO-199) and approved by the Universidad Michoacana de San Nicolas de Hidalgo Bioethics Committee.

2.3. Statistical Analysis

Data obtained was represented as mean values \pm standard deviation. One-way ANOVA and Tukey tests were employed to determine the difference among treatments, considering a significance level of $P < 0.05$. The statistical analyses were performed using the JMP Software ver. 6.0 [21].

3. Results

3.1. Chemical Composition

The chemical composition of freeze-dried whole pulp avocado and defatted avocado paste is shown in Table 2. The lipid content was the highest in the freeze-dried sample (60.4%). This value is higher than that in the study by Yanty et al. [22], where 54.90% lipid content was found in Hass avocado from Australia, and similar to that indicated by Tan et al. [2], which found 61.27% lipid content in a Hass variety of avocado from Mexico. The TDF found in the freeze-dried sample was 19.4%, of which 13.87% corresponds to IF and 5.53% corresponds to SF. This value is within the ranges reported by other authors [23,24] who report TDF values in Hass avocado of 16.53% and 24.55%, respectively. The main component found in our avocado paste was the TDF, which was 49.04% (34.87% IF and 14.00% SF).

Table 2. Chemical composition of freeze-dried pulp avocado and defatted paste avocado (dry basis)

Component	Freeze-dried avocado g/100 g	Defatted avocado (g/100 g)
Protein	3.90	9.85
Lipids	60.42	4.01
Total dietary fiber	19.41	49.04
Insoluble fiber	13.80	34.87
Soluble fiber	5.53	14.00
Ashes	7.10	17.94
Nitrogen-free extract*	9.11	19.16

* Nitrogen-free extract = 100 - (moisture + protein + lipids + total dietary fiber + ashes).

The composition of fatty acids in avocado oil extracted from freeze-dried avocado is shown in Table 3. The sample presented 15.8% of saturated fatty acids, 71.5% of monounsaturated fatty acids, and 12.7% of polyunsaturated fatty acids. A high amount of unsaturated fatty acids (84.2%) was observed, which is consistent with that found by Yanty et al. [22] and Dreher and Davenport [24], who report a total of 84.94% and 84.0%, respectively, of unsaturated fatty acids in avocado oil of the Hass variety. In our study, the main saturated fatty acid was palmitic acid, corresponding to 15.23% of total lipids, and 96.5% of total saturated lipids. The highest content of monounsaturated fatty acids was oleic acid, which was 65.66% of the total lipids, representing 91.8% of the total monounsaturated fatty acids. Palmitoleic acid amounted to 5.69%, representing 7.96% of the entire monounsaturated fatty acids. Among the polyunsaturated fatty acids,

linoleic and linolenic acids were found in greater presence, at 11.25% and 0.84% of the total lipids, and representing 91.4% and 6.8% of the total polyunsaturated fatty acids. These values were similar to those obtained by Yanty et al. [22], who found values for palmitic, oleic, and linoleic acids at 14.80%, 63.73%, and 15.27%, respectively.

Table 3. Avocado oil fatty acid profile (dry basis)

Fatty acids	Content (%)
Saturated	15.79
Lauric acid	0.04
Myristic acid	0.05
Palmitic acid	15.23
Stearic acid	0.47
Monounsaturated	71.49
Palmitoleic acid	5.69
Oleic acid	65.66
Gadoleic acid	0.15
Polyunsaturated	12.72
Linoleic acid	11.25
Linolenic acid	0.84
Others	0.63

3.2. Feed Consumption and Rodent Weight Loss

Feed intake for each animal was evaluated throughout the experiment and an average per group was obtained. The hamsters fed with CD consumed the greatest ($P < 0.05$) amount of feed (149.1^a g). There was a significant difference ($P < 0.05$) among the rodent groups fed with DPAD and FAD, which consumed 102.5^b g and 74.1^c g of feed, respectively. The initial and final weights of the animals are shown in Figure 1. For all the hamsters that were fed diets containing avocado, the weight decreased (FAD = 26.9% weight loss; DPAD = 26.9% weight loss), with a significant difference ($P < 0.05$) with respect to CD, and with no significant difference ($P < 0.05$) among them, which clearly indicates that avocado consumption positively influenced the decrease in body weight. The differences could be due to the DPAD having a high amount of TDF (DPAD). The presence of SF and IF in DPAD and FAD was important, as both components generate satiety at the gastrointestinal level. Puhua-Ramos et al. [25], observed weight loss in Wistar rats when consuming an avocado paste. There are reports that palmitoleic acid, which is also present in the freeze-dried avocado pulp used in our study, could help decrease body weight.

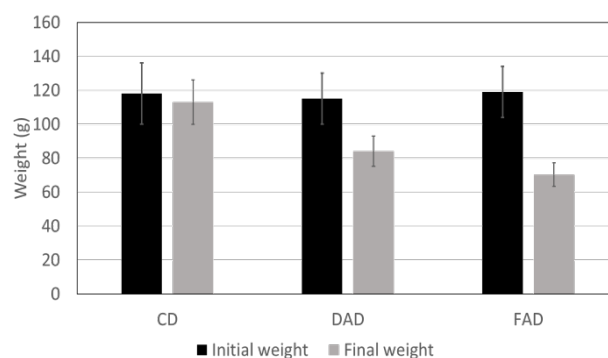


Figure 1. Animal weight in animals fed with experimental diets. CD = Control diet; DPAD = Diet based on defatted paste avocado; FAD = Diet based on freeze-dried avocado. N = 6.

3.3. Lipid Determinations in Serum

TG, CT, HDL-C, LDL-C, and VLDL-C levels in the serum of hamsters are presented in Table 4. A decrease in serum TG levels was observed in the hamsters that were fed diets based on avocado. The hamsters fed the FAD and DPAD decreased TG content by 76.47% and 46.06%, respectively, compared to the hamsters fed CD. TG results suggest that the decrease in serum TG values was possibly due to the combined presence of TDF and unsaturated fatty acids in FAD. DC and FAD were formulated with similar TDF, 5.0% and 5.2%. DC was formulated with cellulose, which is an IF. In FAD, TDF was supplied from freeze-dried pulp avocado, of which 1.49% corresponds to SF and 3.71% to IF. On the other hand, the DPAD group had a high amount of TDF in their formula - 14.20%. High dietary fiber has been shown to trap cholesterol in the gastrointestinal tract, preventing its absorption, and therefore, there is a decrease in serum cholesterol [20]. Other authors [26,27] also observed the hypolipidemic effect in Wistar rats dosed with avocado pulp. They induced hypercholesterolemia using 1% cholesterol and reported a significant decrease in TG and TC. Pahu-Ramos et al. [25] also fed Wistar rats with a lipid-free avocado paste and observed a reduction in TG compared to hypercholesterolemic animals. Avocados are rich in β -sitosterol, polyphenols, and vitamin E, which together help to prevent atherogenic problems [25].

Table 4. Effect of diets on lipids measured in serum, liver, and feces of hamsters

Analysis	CD	DPAD	FAD
TG	219.7±102.1 ^a	118.5±57.8 ^b	51.7±28.5 ^c
TC	263.5±18.1 ^a	282.0±14.1 ^a	220.3±32.6 ^b
HDL-C	120.10±37.3 ^a	120.1±14.8 ^a	86.2±20.7 ^b
VLDL-C + LDL-C	143.4±12.4 ^{ab}	161.9±12.1 ^a	134.1±11.4 ^c
AIP (TC/HDL-C)*	1.94	1.8	0.60
Lipids in liver (%)	36.01±0.01 ^a	32.4±2.8 ^b	23.9±0.1 ^c

* AIP = Atherogenic index of plasma. TC/HDL-C = Total cholesterol/High-density lipoproteins cholesterol. Equal letters in the same row show no significant difference between groups ($P < 0.05$). CD, control diet; DPAD, diet based on defatted paste avocado diet; FAD, a diet based on freeze-dried avocado diet.

A significant decrease ($P < 0.05$) in TC was observed in animals fed with DPAD (220.3 mg/dL) - a decrease of 16.39% was found in relation to those fed with CD (263.5 mg/dL). Additionally, LDL-C + VLDL-C serum values were reduced in hamsters fed with FAD, with a value of 134.1 mg/dL compared to the value obtained from rodents fed with CD (143.4 mg/dL). This is important because high LDL-cholesterol (LDL-C) values are associated with the risk of CVD [28]. This indicates that FAD showed positive effects in controlling LDL-C + VLDL-C, possibly due to the constituents present in avocado oil. Another important parameter evaluated was Atherogenic Index of Plasma (AIP), where the ratio was calculated by dividing TG/HDL-c, which is a strong predictor of myocardial infarction [29]. The ratio TG/HDL-C ≥ 3.5 provides a means of identifying dyslipidemic patients who are likely to be at increased risk of CVD [30]. In our results, the best AIP was found in rodents fed with FAD (0.60), followed by DPAD (1.83) and DC (1.94).

The lipids measured in the liver and the moisture and lipids measured in the feces of hamsters fed with the experimental diets are shown in Table 4 and Figure 2, respectively. FAD and DPAD groups showed a significant decrease in the content of lipids in the liver, 33.61% and 10.0%, respectively, compared to CD. Fatty livers can be mitigated by high TDF intake [31]. Freeze-dried avocado used in this study had a TDF value of 19.4%. Hamsters fed with FAD and DPAD had 23.19% and 17.39% more moisture content compared to the CD group. TDF present in DPAD could have better physiological effects than the source of dietary fiber used in CD, which was cellulose. It is worth mentioning that the DPAD presented 10.10% IF and 4.05% SF and FAD presented 1.49% corresponding to SF and 3.71% to IF. The IF can retain water in its structural matrix, forming low-viscosity mixtures that produce an increase in fecal mass that accelerates intestinal transit and stimulates evacuations more quickly. In addition, IF decreases the concentration and contact time of carcinogenic potentials with the mucosa of the colon. SF captures a higher water content and increases the volume of feces in the colon, promoting the evacuation of softer feces. The diets containing avocado dietary fiber, FAD, and DPAD, excreted higher concentrations of lipids in feces, 73.46% and 41.49%, respectively. The presence and type of dietary fibers present in diets containing avocado trapped fat in the intestinal lumen, which was excreted in the feces, so that the content of absorbed fat in the intestine was lower, and therefore the TG and TC contents in plasma and lipids in the liver were also lower, particularly in FAD-fed hamsters.

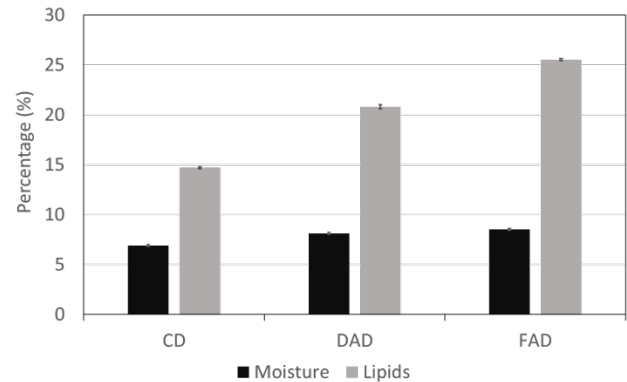


Figure 2. Moisture and lipids in the feces of hamsters fed with experimental diets. CD = Control diet; DPAD = Diet based on defatted paste avocado; FAD = Diet based on freeze-dried avocado.

4. Conclusions

In this study, it was shown that avocado is an excellent option to mitigate hyperlipidemia. The freeze-dried avocado allowed to decrease TC, TG, and LDL-C + VLDL-C by 16.4%, 76.5%, and 6.5%, respectively.

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Statement of Competing Interests

The authors have not competing interests.

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