ORIGINAL ARTICLE

Plant physiology and biochemistry

Lipid Profiles are Altered in Rats Fed with Different Garlic Cultivars

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Abstract

Garlic has antioxidant and hypocholesterolemic properties that are attributed to its organosulfur compounds being allicin, which is reported to be the most active of these compounds. We hypothesized that allicin content could reduce plasma concentrations of triglycerides (TG), total cholesterol (TC), HDL (high density lipoproteins), VLDL (very low density lipoproteins), and glucose. Two different cultivars of commercial garlic, Peruano and Jinxiang, were used. Thirty male Wistar rats were distributed into 6 groups and fed for 15 days with standard diet (Control), Control with Peruano garlic treatment (CGP), Control with Jinxiang garlic treatment (CGCH), cholesterol-added control diet (CholC), cholesterol-added diet with Peruano garlic treatment (CholGP), and cholesterol-added diet with Jinxiang garlic treatment (CholGCH). Garlic treatment consisted of a daily oral dose of 1ml of lyophilized garlic. We observed that garlic treatment in Control group significantly reduced plasma TG and VLDL concentrations. The CGCH group presented a significant increase in plasma TC levels (25.5%) and glucose (11%). No significant changes in TC, HDL, TG and VLDL were observed in CholGP and CholGCH, but levels of fasting plasma glucose were increased: CholGP (23%) and CholGCH (27.5%). Results suggested allicin treatments alter lipid profile in rats. Nevertheless, further studies are necessary to address the increase in plasma glucose levels. **(Int J Biomed. 2015;5(3):155-161.)**

Keywords: powder garlic; allicin; rats; cholesterol; fasting plasma glucose.

Abbreviations

TC, total cholesterol; **HDL**, high density lipoprotein; **VLDL**, very low density lipoprotein; **TG**, triglycerides; **Control**, standard diet; **CGP**, Control with Peruano garlic treatment; **CGCH**, Control with Jinxiang garlic treatment; **CholC**, cholesterol added control diet; **CholGP**, cholesterol-added diet with Peruano garlic treatment; **CholGCH**, cholesterol-added diet with Jinxiang garlic treatment; **OSC**, organosulfur compound; **CVD**, cardiovascular disease.

Introduction

Garlic contains 33 organosulfur compounds (OSC), 17 amino acids (including all essential amino acids), minerals such as phosphorus, calcium, iron, potassium, magnesium, selenium, zinc, and vitamins A, B, C, and E [1]. This bulb and its preparations have been widely recognized as an agent capable of preventing and treating cardiovascular diseases (CVD), atherosclerosis, thrombosis, hypertension, and diabetes [2,3]. It has been suggested that garlic's beneficial properties are attributed to specific OSC, including sulphoxides and γ-glutamil peptides that are present in the crude clove [4,5].

Allicin (diallylthiosulfinate), a volatile liquid, is responsible for the pungent odor of garlic, representing approximately 70% of all thiosulfinates present in the crushed clove [6]. The compound is not found in intact plants, but it is formed by action of the enzyme named alliinase (EC4.4.1.4), derived from a non-proteinogenic amino acid *S*-allylcysteine *S*-oxide (aliin) at the time garlic is crushed [7]. In garlic powder the conversion of aliin to allicin begins when water is added to the powder, being quickly degraded into diallyl disulfide (DADS), vinyldithiins and ajoenes [8].

Atherosclerosis is one of the highest risk factor in hypertension development and CVD [9]. CVD are the main causes of death among the Western population. Risk of CVD is higher in men than in women who are in the pre-menopausal

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period. Multiple factors contribute to its development, such as lifestyle (smoking, physical inactivity, etc.) and stress. High levels of cholesterol in the plasma, particularly LDL and TG, are associated with an increase in CVD risk [10]. Oxidative change of LDL by reactive oxidative specie (ROS) is also considered an important mechanism in atherosclerosis and hypertension [10,11]. The cardio protector effect of garlic has been extensively assessed. Several in vitro studies have indicated that garlic and its components inhibit the key enzyme HMG-CoA reductase (*3-hydroxi-3 methylglutaryl Coenzime A*), which is associated with cholesterol and fatty acids synthesis [12-14]. In different assays carried out with animals, garlic extracts showed the ability to reduce cholesterol and lipid levels in the blood plasma of rats [15,16]. In humans, garlic significantly reduced plasma lipids levels, especially TC and LDL [17,18]. However, studies continue to be conducted due to the differences found. Active components of raw garlic may vary according to cultivar, harvest, and storage conditions. Various garlic preparations have been used in different studies. However, there is a lack in the literature concerning which are the most important active ingredients and how they impact lipid metabolism. Given this scenario, we hypothesized that the amount of allicin present in garlic could reduce the plasma concentration of TG, TC, HDL, VLDL, and glucose. To test our hypothesis we used an animal model and two commercial cultivars of garlic, Peruano and Jinxiang, grown in Brazil and China, respectively, which were submitted to a lyophilized process in order to preserve their properties and facilitate handling of the garlic throughout the experiment. This study aimed to: (a) assess the content of allicin in garlic cultivars (Peruano and Jinxiang), commercially available in the local market and (b) study the effect of allicin levels in reducing plasma concentrations of TG, TC, HDL, VLDL and plasma glucose in animals fed with either standard or cholesterol-added diets.

Material and Methods

1. Garlic processing

Bulbs of fresh garlic (*Allium sativum* L.), cultivar Peruano, were obtained from commercial and experimental fields in Brasília, DF, Brazil. Bulbs were harvested 150 days after planting and submitted to curing. Bulbs from Jinxiang cultivar, imported from China, were obtained at the local wholesale market. Fresh and healthy bulbs were selected and graded. Garlic cloves were manually stripped without harming the product, using a kitchen knife. Then they were frozen at a temperature of -70±1°C for approximately 8 hours, using an ultra-freezer (ULT1386-5-D40, Revco, Illinois, USA), sliced in an industrial processor (CL50, Robot Coupe, USA) with 5mm thickness and immediately lyophilized (LS3000, Terroni, São Carlos, Brazil) for approximately 3 days. The product was then crushed in a knife mill (SL31, Solab, Piracicaba, Brazil), and put in sealed polyethylene bags.

2. Allicin content determination

Allicin content was determined according to the Institute for Nutraceutical Advancement 110.001 method INA [19]. Analyses were performed by a reversed phase,

high-performance liquid chromatography (RP-HPLC) system (Shimadzu, Japan). Oven temperature was set at 28±0.5°C. Samples were eluted with methanol and water (50:50 v/v), using a flow of 1.0mL/min for 20 minutes and detected at 240 nm. Injection volume was 25 µL. Allicin determination was performed comparing the area under the peak produced by the aqueous extract of garlic to a standard peak of allicin. Allicin standard solution was obtained by the oxidation of *diallyl disulfide,* according to Lawson & Wang [20]. Allicin concentration (C) in the solution was calculated according to Eq.:

$$E_{1cm}^{1\%} = \frac{Absorbance}{C(\mu g\,/\,mL)}\,x10000$$

, in which:

E=extinction coefficient for allicin in water
(145.4, considering a cell of 1 cm of wavelength of 240 nm).

A standard curve was obtained by subsequent dilutions (5, 10, 15, 20, 30, 40, 50, 60, 70 and 80 µg/mL) of the standard solution. To determine allicin content, lyophilized garlic powder was reconstituted with water. Samples of 0.4 g of garlic powder were placed in 50 mL plastic tubes, 10mL of deionized water was added at room temperature and tubes were sealed using plastic film (Parafilm). Samples were homogenized using a tube agitator (IKA®, model MS1, German) and left at room temperature for approximately 6 minutes in order to produce OSC. Then samples were filtered through a filtrating membrane of 0.45 µm (Millipore, USA) and transferred to an HPLC vial.

3. Animals and diets

The Institute of Biological Sciences/University of Brasilia (Brasilia, DF, Brazil), Ethical Committee for Animal Research approved all the adopted procedures (Protocol CEUA/ICB/UnB no. 18914). Wistar male rats (n=30) approximately 7 weeks old, used in the present study, were obtained from the Institute of Biological Sciences of University of Brasília. For 15 days, the animals were individually kept in polypropylene boxes in the following conditions: 23±2°C, 50%–60% relative humidity, and a photoperiod of 12 hours. All animals had free access to food and water. All experimental diets were distributed in a pellet form, based on the diet for growing rodents of the American Institute of Nutrition (AIN)-93G. After adaptation for one week, animals were randomly distributed in 6 groups of 5 animals each. Group 1 received the standard diet (Control). Groups 2 (CGP) and 3 (CGCH) received the same diet as Group 1, with the addition of Peruano garlic (Group 2) and Jinxiang garlic (Group 3). Group 4 (CholC) received a cholesterol-based diet, containing 0.125% of sodium cholate and 0.5% of cholesterol (Control containing cholesterol), according to the model of Yanagita et al [21]. Groups 5 (CholGP) and 6 (CholGCH) received the same diet as Group 4, with Peruano garlic (Group 5) and Jinxiang garlic (Group 6) added (Table 1).

3.1. Samples preparation for garlic treatment

Lyophilized garlic powder was daily reconstituted with water and fed to the animals at the same time, in the morning, before the regular diet. The quantity was equivalent to 500

mg of lyophilized garlic/kg of the animals' body mass [22]. Garlic powder was added with 1 mL of filtered water at room temperature, homogenized and left at the counter top for 6 minutes to yield OSC. Then rehydrated garlic was fed to animals by gavage. Each animal of every group (CGP, CGCH, CholGP and CholGCH) received 1 mL of reconstituted garlic and the other groups (Control and CholC) received 1mL of physiologic saline solution, once a day. During the experiment, garlic doses were adjusted according to the mass increase of the experimental animals.

Table 1.
Composition of experimental diets (g/Kg)

Ingredients	Control	CGP/ CGCH	CholC	CholGP/ CholCH
Cornstarch†	529.49	524.49	523.24	518.24
Casein (≥ 85% of protein) †	200	200	200	200
Sucrose‡	100	100	100	100
L-cystine†	3	3	3	3
Soybean oil§	70	70	70	70
Microcrystalline cellulose †	50	50	50	50
Mineral mixture - AIN93G†	35	35	35	35
Vitamin mixture - AIN93G†	10	10	10	10
Choline bitartrate †	2.5	2.5	2.5	2.5
Tert-butylhydroquinone£	0.014	0.014	0.04	0.014
Cholesterol£	-	-	5	5
Sodium cholate£	-	-	1.25	1.25
Lyophilized garlic	-	5	-	5

Tested diets were formulated based on the diet AIN-93G.
† Rhoster Indústria e Comércio Ltda (Vargem Grande Paulista, SP, Brazil).
‡ Vetec Química Fina (SP, Brazil).
§ Refinações de Milho Brasil
£ Sigma Chemical Co.(St Louis, MO, USA).

4. Data collection

Rats were daily monitored and individually weighed every 2 days and before blood collection at the end of the experiment. Their daily food ingestion and weight increments were registered during the experimental period.

5. Blood collection

Blood samples of each individual rat were collected at the end of the experiment with animals having fasted for 12 hours. Rats were anesthetized by the intraperitoneal route with quetamine association (100 mg/Kg) and xilazine (10 mg/Kg). Blood collection was performed by cardiac punction. The animals' euthanasia was performed with an overdose of barbiturics. Blood was collected using sterilized syringes and needles and immediately transferred to dry hemolysis tubes with cap, stored in an ice bath, and taken for biochemical processing a maximum of 1 hour after collection. Plasma was separated using a centrifuge (SIGMA, 2-5, Osterode am Harz, Germany) at 1200g, for 10 minutes, at room temperature.

6. Enzymatic analysis

TC, HDL, TG and glucose were assessed using an enzymatic analytical kit from Abbott Laboratories (Illinois, USA) in automated equipment (ARCHITECT C8000, USA). VLDL fraction was calculated according to the following

equation: VLDL=(Tryglicerides/5) used for triglyceride values < 400 mg/dL [23].

Statistical analysis: Results were expressed by mean±SD. Data were subjected to the chi-square test with 5% of probability, with the purpose of checking population adherence to the normal distribution curve. As there was no population adjustment to normal distribution, the non-parametric method of analysis using Kruskal Wallis test (*P<.05*) was chosen.

Results

1. Allicin content

No significant differences in allicin content for fresh Peruano and Jinxiang garlics were observed. However, significant differences were verified when these varieties were lyophilized. The lyophilization process caused a significant reduction in allicin content for the Jinxiang cultivar (91%) (Table 2).

Table 2.

Allicin content in the samples of aqueous extract of crude garlic and after lyophilization processing

Cultivars	Crude Garlic*		Lyophilized garlic *
	mg/g FM product	mg/g DM product	mg/g DM product
Peruano	7.90 ± 0.09 a	21.95 ± 2.65 a	27.04 ± 0.32 a
Jinxiang	6.73 ± 0.03 a	20.04 ± 0.49 a	1.88 ± 0.26 b

* a,b *Means in the same column, followed by different letters, are statistically different among them at the level of 5 % (P< 0.05). FM = fresh matter and DM = dry matter.*

Jinxiang garlic was included in the present study once it was imported from China, and it was not possible to define all postharvest procedures until it reached the final market. On the other hand, Peruano garlic is grown locally and all the postharvest steps are known. These differences are probably associated with the observed distinct levels of allicin. Our study also showed that garlic variety influenced allicin during the lyophilization process.

2. In vivo experiment

Garlic treatment in the standard diets and cholesterol-based diets influenced the body weight of experimental animals (Table 3).

Table 3.

Effect of lyophilized powder garlic in standard diets and cholesterol added diets in relation to body weight, (g) weight gain (%) and consumption of feed (g/day) in the rats groups

| Study Group	Body weight 1st day*	Body weight 15th day*	Body gain	Feed consumption
Control	184.17 ± 12.36 a	267.43 ± 18.30 a	45.34 ba	32.25 ± 2.66 ca
CGP	172.17 ± 12.09 a	222.56 ± 7.57 b	29.58 b	26.65 ± 1.56 dc
CGCH	179.05 ± 8.61 a	264.14 ± 15.09 a	47.93 ba	30.98 ± 3.72 ca
CholC	177.66 ± 3.17 a	267.41 ± 5.13 a	50.53 ba	33.22 ± 2.15 a
CholGP	165.88 ± 11.39 a	218.46 ± 6.19 b	32.24 b	24.32 ± 0.51 db
CholGCH	173.34 ± 8.06 a	284.84 ± 10.34 a	64.42 a	32.16 ± 1.31 a

Results expressed as mean ± SD.
ba Different letters, in a same column, and in different assays, indicate a significant difference (P <0.05).

Rats that were treated with Peruano garlic (CGP and CholGP) showed lower body weight, lower increment in body weight after 15 days of study, and consumed lower amounts of feed compared to other groups. However, there were no statistical differences in body weight, weight increment, and feed consumption for the CholC group in relation to the Control group.

We verified similar trends in the CGP group, fed with 13.52 mg of allicin/kg of animal mass, to the study conducted by Elkayam et al [24]. Other studies conducted by Lee et al.[25] and Sohn et al. [9], both with diets enriched with garlic powder, did not show any change in food intake and in weight gain.

Our results showed that garlic can help in weight loss by reducing the appetite (Table 3). The possible mechanism of appetite reduction is probably associated with the strong odor of garlic, which stimulates the brain satiety center, reducing the desire to eat. It is still believed that garlic can stimulate the nervous system to release hormones such as adrenalin, which can accelerate the metabolic rate, helping in losing weight [26]. As Peruano garlic has higher amounts of allicin, it consequently has a more pungent odor, generating a stronger stimulus in the brain and, thus, accelerating metabolic rate.

Changes in lipid concentration in blood plasma after 15 days are shown in Figure 1. Group CGP kept cholesterol levels similar to the Control group, whereas the CholGCH group showed a significant increase (25.5%). Rats fed with the cholesterol-based diet presented a significant increase in cholesterol levels in blood plasma when compared to the group fed with a standard diet. Groups CholGP and CholGCH did not present significant differences in relation to the CholC group.
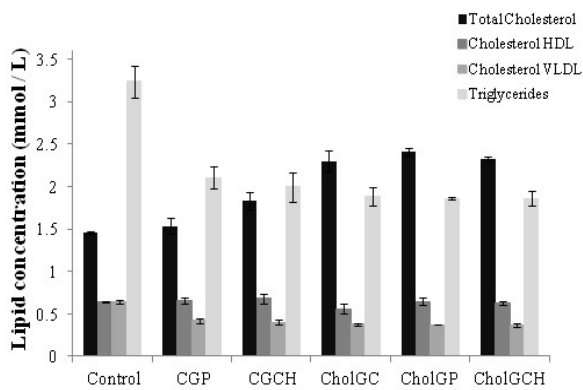
Fig. 1. *Changes in lipid levels in blood plasma of rats fed with a diet either with or without cholesterol. Values are means. Vertical bars represent standard deviation.*

Glucose levels in the plasma were altered due to different diets (Fig. 2). There was a significant increase in the CGCH group when compared to Control, as well as for CholGP and CholGCH when compared to CholC and Control. Therefore, in a standard diet, CGCH garlic consumption can increase the glucose level in the blood, as well as in CholGP and CholGCH.
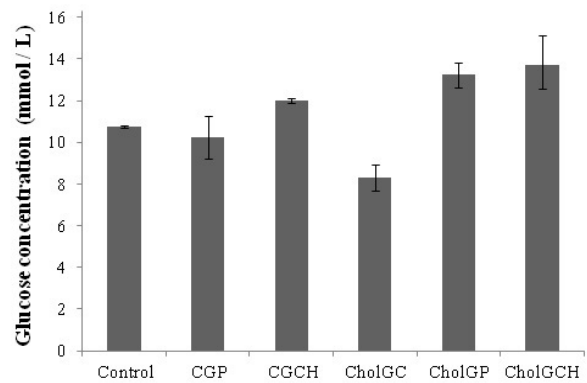
Fig. 2. *Changes in glucose levels in blood plasma of rats fed with a diet either with or without cholesterol. Values are means. Vertical bars represent standard deviation.*

Discussion

The observed difference is probably associated with allicin instability, which can vary during the process and with different cultivars [27,28]. Lawson and Hughes found 7.52 mg/g of allicin in garlic powder, dried in slices with 3 mm thickness at 60°C for approximately 57 hours, followed by spraying using a mill. Garlic powder extract was obtained by adding water (20 mL/g) and incubating at 23°C for 8 minutes, followed by filtration and evaluation with HPLC (240 nm and mobile phase of 50% of methanol in water). In the same study carried out by Lawson and Hughes [29], authors assessed the production of thiosulfinates during the garlic drying process. The amount of allicin formed in the fresh product was around 12.1 mg/g (dry weight) and the garlic powder was 11.6 mg/g(dry weight). Loss caused by the drying procedure was nearly 4%. In our study, we observed higher values either in fresh product or in garlic powder. According to Calín-Sánchez et al. [30], different drying methods can alter the final results, as one could expect. Garlic homogenization by spray-drier results in aliinase loss of activity, the same occurring with drying methods that use very high temperatures. Drying in low temperatures (<60°C) has minor effects on the production of main thiosulfinates (allicin and alil methyl thiosulfinates). We also noticed that garlic cultivars influenced the activity loss of aliinase.

In another study, Lawson et al. [31] assessed different thiosulfinates, including allicin, present in tablets of garlic powder marketed in Australia, Germany, the USA and Japan. The amount found corresponded to 3.60mg/g product (Australia); 3.10 mg/g product (USA); 0.26 to 2.55 mg/g product (Germany) and not detected (Japan). According to the authors, this wide range was due to different procedures used to prepare garlic powder, which has a potential to preserve the releasing capacity of allicin in garlic. However, while some garlic powders release a significant amount of allicin when in contact with water, many do not indicate any variation. Therefore, it is possible to observe variations in allicin content depending on garlic powder processing [32-34].

Future studies should address different processes to

minimize allicin reduction during the drying process, searching for the best drying process for each cultivar, besides assessing possible formation of other organosulfur compounds and their effects in experimental animals.

Results of the present study associated with the CholGP group were similar to the ones observed in the investigation conducted by Chi at al. [35], in which rats fed with 1% of cholesterol, 15% of pig fat, and supplemented with 2% (320 mg/day) and 4% (640 mg/day) of garlic powder for 4 weeks, presented a significant reduction in feed ingestion and weight gain in the group with the cholesterol-based diet supplemented with 2% garlic.

Findings of the present investigation regarding cholesterol levels for the CGP group are in line with the results verified by Gorinstein et al. [21]. These researchers observed that rats fed with a standard diet supplemented with 25 mg of lyophilized garlic powder, equivalent to 500 mg of fresh garlic/kg of body weight, for 4 weeks did not present significant differences in relation to control. For the groups CholGP and CholGCH, our results were similar to the ones observed by Asdaq [36].

On the other hand, other studies verified results that are the opposite of ours. Aouadi et al. [37] observed that supplementing the standard diet with 10% fresh garlic (equivalent to 2%–3% of garlic powder) reduced plasma cholesterol in 12.2% of subjects. The study conducted by Ali et al.[38] showed a reduction of 35% in plasma cholesterol in a high cholesterol diet (2%) when rats were supplemented with 50 mg of garlic powder/kg animal weight, containing 0.6% of allicin. Chi et al. [35] verified a reduction in plasma cholesterol of 45.5% and 44% in rats fed with a diet containing 1% of cholesterol and supplemented with 2% and 4% of lyophilized garlic powder, respectively.

Although our data suggest an increase in HDL levels in the standard diets with garlic treatment, differences observed among treatments were not significant. Other studies verified similar results [13,39].

There was a significant reduction in VLDL and TG serum concentrations for rats of groups CGP, CGCH, CholC, CholGP, and CholGCH when compared to Control. Reduction for the studied groups in both analyzed concentrations was: 35% for CGP, 38% for CGCH, 42% for CholC, CholGP and CholGCH. No significant differences were verified for VLDL and TG levels among the cholesterol-based diets. The present study showed that garlic treatments in a standard diet significantly reduced TG and VLDL concentrations in rats' plasma. Other studies showed a reduction in TG concentrations [40]. However, in general, many studies showed changes in TG and VLDL levels when standard diets, with moderate to high levels of cholesterol, were supplemented with garlic [41,29].

High levels of TG in the serum are associated with pathogenic conditions that accelerate atherosclerosis, such as insulin resistance and low levels of HDL [42]. Consequently, garlic consumption by groups CGP and CGCH can reduce the risk of cardiovascular diseases due to the reduction of TG and VLDL levels.

The effect of garlic in glucose concentrations was not addressed significantly in the searched literature, and the studies carried out had inconsistent results. Chi et al. [35] and Seo et al. [43] showed a significant reduction in glucose levels in rats fed with a high content cholesterol diet supplemented with garlic powder.

Thomson et al. [40] showed in a study that aqueous extract of fresh garlic ingested in small quantities (50mg/kg) reduces the concentration of cholesterol and triglyceride and does not alter the glucose level in the plasma. However, the study found that high doses of garlic (500mg/kg) would reduce plasma glucose levels, which is different from the results verified in this study.

The effect of garlic, and especially allicin, on the lipid profile has been the object of many controversies in animal models, since there is no standardization for the garlic concentration used. There are papers that have mentioned the protective effect to health of fresh garlic but not allicin, since they use garlic as the study base. There are several methods of preparing garlic and, specifically for garlic powder, there are several ways to process it, which implies variation in allicin content. The duration of the experiment duration is also questionable; many studies already published have shown different assessment times. Garlic cultivars present variable results *in vitro* which can generate differences in the *in vivo* results. All these facts, among others not mentioned, can explain the differences presented in our study regarding the literature data on TC, HDL, VLDL, TG and glucose levels in the plasma of experimented animals.

Concerning garlic ingestion, a significant part of the worldwide population consumes garlic as a condiment in their usual meal, without a defined quantity. The effective dose of garlic has not been determined; however, clinical studies in humans have shown that the ingestion of 4 to 6g of garlic powder/day is considered safe when done in a meal [44]. Our results are within the range of these values, after extrapolation of the animal dose of 500mg of garlic powder/kg of the animal weight. This amount corresponds to 4.86g of garlic powder for one person with 60kg, according to the Food and Drug Administration [45].

Conclusions

The present study showed differences in allicin content between two garlic cultivars assessed after a lyophilization process. We also observed differences in the *in vivo* results, probably due to the differences verified for allicin content. The ingestion of Peruano garlic reduced TG and VLDL levels in blood plasma of animals fed with a standard diet. These results indicate that this cultivar might have an important role in the prevention of atherosclerosis. On the other hand, although Jinxiang garlic consumption reduced TG and VLDL levels, it increased TC levels in 25.5% of the animals kept on a standard diet and increased glucose levels 12% and 27.5% in those on standard diets and cholesterol-based diets, respectively. Thus, this cultivar can present a risk factor for diabetes and it is not recommended to be used as a lyophilized product, in the conditions under which the present study was carried out. Our work is, to the best of our knowledge, the first to show

that garlic powder can significantly affect the levels of plasma glucose. Thus, it should be monitored periodically to identify, in future studies, possible mechanisms associated with this increment. For consumption of fresh garlic, additional studies should focus on how these cultivars can contribute to the reduction of the parameters (CT, LDL, VLDL and TG) associated with CVD.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

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