Effects of passion fruit (*Passiflora edulis*) byproduct intake in antioxidant status of Wistar rats tissues

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**Abstract**

Passion fruit (*Passiflora edulis*) is a native species from Brazil explored in fruit culture. Peels are byproducts of juice industrial processing and are usually discarded. They are source of fibers and other bioactive substances, which may have antioxidant role and to protect cells against oxidative stress. This study aimed to evaluate the antioxidant status of *P. edulis* peel flour *in vivo*. The male Wistar rats (77 days old) were divided in Peel and Control groups (*n* = 4). Control group was fed with standard diet (AIN-93M) and Peel group, received a diet in which fifty percent of the cellulose content was replaced by fiber from PPF. After 15 days, the animals were anaesthetized and sacrificed. Peel group consumed greater amount of diet without alterations in body weight of animals. The experimental group showed no differences in serum antioxidant status (FRAP and TBARS assays) (*P* > 0.05), but they presented lower antioxidant potential according to ORAC assay (*P* < 0.05). In the kidneys of Peel group, a reduction in lipid peroxidation by TBARS assay (*P* < 0.05) was observed. In addition, the liver of the animals fed with PPF showed high TBARS levels, increased glutathione reductase activity (GR), decreased glutathione peroxidase activity (GPx) and superoxide dismutase activity (SOD).

1. Introduction

The excess of reactive species may result in loss of function and even cellular death due to damage to cell lipids, proteins and DNA (Habib & Ibrahim, 2011; Kaliora, Dedoussis, & Schmidt, 2006; Libby, 2007), which has been linked to many diseases, such as coronary heart disease, diabetes, obesity, and cancer (Elleuch et al., 2011). The organisms use endogenous and exogenous antioxidant defenses to protect cells against the harms of oxygen and nitrogen reactive species. These defenses include the activity of enzymes, such as catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase activity (SOD), as well as a non-enzyme system, like reduced thiols (GSH) (Rezaie, Parker, & Abdollahi, 2007).

Nevertheless, sometimes, endogenous antioxidants are unable to prevent oxidative damage and require help from exogenous scavengers that are obtained from the diet, such as vitamins, minerals, carotenoids and polyphenols (Cerqueira, Medeiros, & Augusto, 2007; Habib & Ibrahim, 2011). In this way, the interest in fruits has increased due to the presence of antioxidants in these foods, which can prevent cellular oxidative stress (Beecher, 2003; Cerqueira et al., 2007; Habib & Ibrahim, 2011; Holt et al., 2009; Scalbert, Johnson, & Saltmarsh, 2005).

Passion fruit (*Passiflora edulis*) is a source of bioactive compounds, which could alleviate oxidative stress. Some phenolic compounds were identified in *Passiflora* species such as like C-glicosil derivatives apigenin and luteolin, as vitexin, isovitexin, orientin, schaftoside, 2′-O-rhamnoside and luteolin-7-O-(2′-rhamnosylglucoside) (Ferrer& et al., 2007; Muller, Vasconcelos, Coelho, & Biavatti, 2005; Zeraik & Yariwake, 2010) and they have shown therapeutic effects such as immunomodulation, anticarcinogenic and antioxidant activities (Neuza Jorge, 2009; Tommonaro et al., 2007; Zeraik & Yariwake, 2010). Recent study showed that *P. edulis* juice intake during 28 days reduced the lipid peroxidation in Wistar rats (de Souza et al., 2012).

In addition to the edible portion of whole fruits and vegetables, their non-edible portions could also be good sources of bioactive compounds, such as polyphenols, that could be supplemented in the diet (Leite-Legatti et al., 2012). *P. edulis* leaves extract demonstrated antioxidant properties *in vitro* and *in vivo*, according to antioxidants tests, level of lipid peroxidation and antioxidant enzymes’ activities (Da Silva et al., 2013; Ferreres et al., 2007).
P. edulis is a native fruit from Brazil. Nearly 40% of passion fruit production is directed to the juice industry (Meletti, 2011; Meletti & Maia, 1999). Large amounts of byproduct (seeds and peels), that contains functional substances, are carried in passion fruit juice processing (Gorinstein et al., 2011). Powders of industrial residues of passion fruit showed amounts of total phenolic compounds five times higher than the pulp of this fruit (103 ± 10.4 and 20 ± 2.6 mg GAE g⁻¹, respectively) (de Oliveira et al., 2009). Saravanan and Parmelazhagan (2013) related that Passiflora seeds' extract had antioxidant action in vitro, which was evaluated by methods of DPPH®, ABTS®, superoxide radical scavenging and metal chelating activities.

In the literature, data relative to effects of the Passiflora peel in vitro and in vivo are not available, but as indicate studies with other fruits in this part could be concentrated more part of bioactive compounds. Then, this work intended to investigate the antioxidant potential of P. edulis peel intake in Wistar rats’ tissues.

2. Material and methods

2.1. Chemicals

Reduced l-glutathione, glutathione reductase, oxidized glutathione disodium salt, β-nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt hydrate (NADPH), hypoxanthine, xanthine oxidase, nitroblue tetrazolium blue chloride (NBT), 5,6-dithio-bis-2-nitrobenzoic acid (DTNB), bovine serum albumin (BSA), (±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX), 2,2'-azobis (2-methylpropionamidine) dihydrochloride (AAPH), 2,4,6-tri (2-piridil)-1,3,5-triazina (TPTZ), were obtained from Sigma—Aldrich (São Paulo, Brazil). Fluorescein sodium (Fl) was purchased from Chayman Chemical Company (Michigan, USA).

2.2. Passiflora edulis peel flour (PPF)

Organic P. edulis harvested in June 2010 in Torre de Pedra, São Paulo, Brazil was used to produce the peel flour. The fruits were cleaned and separated into pulp and peel (epicarp + mesocarp). The peels were cut into small pieces and dried in an oven with air circulation at 50 °C (Marconi, Piracicaba/SP, Brazil) until approximately 10% moisture. Dried samples were grounded into a fine powder using a hammer mill (20 mesh). P. edulis peel flour (PPF) was stored in amber flask at room temperature (24 °C).

2.3. Total phenolic and flavonoid contents

One gram of PPF was extracted with 50 mL of boiled water. After 35 min the extract was filtered under vacuum and stored under refrigeration (2–8 °C) in amber glass bottles. The total phenolic content was determined according to Folin-Ciocalteu’s method (Swain & Hillis, 1959) with adaptation. The adaptation was only a scale reduction (to do the analysis in microplate) in order to save reagents and solvents. The absorbance was read at 725 nm, gallic acid was used in a standard curve and the results were expressed in terms of gallic acid equivalent (mg GAE g⁻¹). The flavonoids contents were determined by colorimetric reaction with aluminum chloride as described by (Zhishen, Mengcheng, & Jianmin, 1999) with adaptations. Catechin was used as standard and the absorbance was read at 510 nm and the results were expressed in mg catechin g⁻¹. These analyses were done in a microplate reader (Synergy HT, Biotek, Winooski, USA) spectrophotometer with Gen5™ 2.0 data analysis software.

The presence of some antioxidant phenolic compounds (vitexin, isovitexin, orientin and apigenin), previously described in P. edulis (Simirgiotis, Schmeda-Hirschmann, Borquez, & Kellenny, 2013; Zeraik & Yariwake, 2010), were investigated in this extract, in a previously work (Cazarin, Da Silva, Colomeu, Zollner, & Maróstica Jr., in press).

2.4. In vivo experimental design

The study was approved by the Institutional Animal Care and Use Committee (CEUA- UNICAMP 110#2385-1, Campinas, Brazil). All the procedures were in accordance with the institutional ethical guidelines. Male Wistar rats were maintained under controlled conditions of temperature (22±2 °C), humidity (60–70%), and a light–dark cycle (12/12 h). They were fed with a commercial diet (Nuvilab®) for rodents until they reached adult age. Seventy-seven-day-old rats were randomly distributed into 2 groups (n = 4), namely Peel and Control. Both groups were fed with standard diet (AIN-93M) (Reeves, Nielsen, & Fahey, 1993); however, 50% cellulose were replaced with fiber from PPF in the Peel group diet. The composition of the diets is presented in Table 1. The body weight of the animals was determined weekly, and food intake was determined three times per week. After 15 days of consuming the experimental diets, the animals were anesthetized with ketamine and xylazine (40 and 5 mg kg⁻¹, respectively) and euthanized by exsanguination by cardiac puncture.

2.5. Samples

Blood samples were collected in appropriate tubes and centrifuged at 2,000 x g for 20 min. Serum was separated and stored at −80 °C until further analyses. The whole liver and kidneys were removed, weighted and frozen in liquid nitrogen and stored at −80 °C. The carcasses were weighted and stored at 4 °C until the analysis.

2.6. Proximate composition of the carcasses

The carcasses were weighted without some organs (liver, kidneys, brain, intestine, stomach, spleen and pancreas) and freeze-dried. The protein, moisture and ash contents were then determined using standard methods (AOAC International, 1995). The lipid content was determined by the Soxhlet method using petroleum ether as the extractor (AOAC International, 1995).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Formulations of experimental diets.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AIN-93M (AIN)</td>
</tr>
<tr>
<td></td>
<td>g kg⁻¹ diet</td>
</tr>
<tr>
<td>Corn starch</td>
<td>465.69</td>
</tr>
<tr>
<td>Casein (≥85% protein)</td>
<td>140.00</td>
</tr>
<tr>
<td>Dextrinized corn starch (90–94%)</td>
<td>155.00</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100.00</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>40.00</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50.00</td>
</tr>
<tr>
<td>Passiflora peel flour</td>
<td>_</td>
</tr>
<tr>
<td>Mineral Mix (AIN-93M-MV)</td>
<td>35.00</td>
</tr>
<tr>
<td>Vitamin mix (AIN-93-Vx)</td>
<td>10.00</td>
</tr>
<tr>
<td>l-cysteine</td>
<td>1.80</td>
</tr>
<tr>
<td>Choline bitartrate (41.1% choline)</td>
<td>2.50</td>
</tr>
<tr>
<td>Butylhydroquinone</td>
<td>0.01</td>
</tr>
</tbody>
</table>

AIN-93M refers to the maintenance phase of growth (Reeves et al., 1993).

b Amount needed to obtain 25 g of Passiflora fiber.

b Values were corrected according to the content of these nutrients in the PPF.
2.7. Antioxidant potential in serum

Serum was treated with ethanol: ultrapure water (2:1, v/v) and 0.75 mol L\(^{-1}\) metaphosphoric acid (Leite et al., 2011). These extracts were used in ORAC (Hydrophilic Oxygen Radical Absorbance Capacity) (Davalos, Gomez-Cordoves, & Bartolome, 2004; Prior et al., 2003) and FRAP (Ferric Reducing Antioxidant Power) (Benzie & Strain, 1996) assays, carried out as described below.

2.7.1. ORAC assay

Twenty microliters of each sample extract or standard solution, 120 \(\mu L\) of fluorescein diluted in phosphate buffer (pH 7.4) and 60 \(\mu L\) of AAPH were added to black microplates in the dark. Trolox was used as a standard, and the readings were performed in a Synergy HT microplate reader with the following fluorescent filters: excitation wavelength, 485 nm; and emission wavelength, 520 nm. The ORAC values were expressed in \(\mu mol\) trolox equivalents (\(\mu mol\) TE) per liter of sample using the standard curves (2.5–80.0 \(\mu mol\) TE \(L^{-1}\)) for each assay. The fluorescence readings were used for the appropriate calculations (Davalos et al., 2004; Prior et al., 2003).

2.7.2. FRAP assay

The ferric reducing ability of the serum was determined by the FRAP method (Benzie & Strain, 1996) with modifications. The FRAP reagent was prepared in the dark with 300 mmol L\(^{-1}\) acetate buffer (pH 3.6), 10 mmol TPTZ in a 40 mmol L\(^{-1}\) HCl solution and 20 mmol L\(^{-1}\) FeCl\(_3\). The sample or standard solutions, ultrapure water and FRAP reagent were mixed and incubated in a water bath for 30 min at 37 °C. After cooling to room temperature, the absorbance of the samples and standard were read at 595 nm. The trolox standard curve was prepared using concentrations ranging from 10 to 800 \(\mu mol\) TE \(L^{-1}\). The results were expressed as \(\mu mol\) TE \(L^{-1}\).

2.7.3. Lipid peroxidation by thiobarbituric acid reactive substances (TBARS) assay

The TBARS determinations were performed in the liver, kidneys and serum according to the method described by Ohkawa, Ohishi, and Yagi (1979) with modifications. The organ samples were macerated in liquid nitrogen. The samples (organs or serum) were mixed with 8.1% sodium dodecyl sulfate (SDS) and working reagent (TBA 5% acetic acid and 20% sodium hydroxide). After heating at 95 °C for 60 min, the samples remained in the ice bath for 10 min and then were centrifuged at 10,000 \(g\) for 10 min. The supernatant was read at 532 nm in a clear 96-well microplate. The standard curve (0.625–50 \(\mu mol\) MDA mL\(^{-1}\)) was prepared using a malondialdehyde (MDA) standard. The results were expressed as \(nmol\) MDA mg\(^{-1}\) tissue (or \(nmol\) MDA mL\(^{-1}\) serum).

2.7.4. Enzyme and non-enzyme endogenous antioxidant system

2.7.4.1. Thiol group content (GSH). The GSH levels were determined in the phosphate buffer (PB) homogenates of the liver and kidneys using Ellman’s reagent (DTNB) (Ellman, 1959) with modifications. The intensity of the yellow color was read at 412 nm, and GSH was used as the external standard. Reduced thiol contents were expressed as nmol GSH mg\(^{-1}\) protein.

2.7.4.2. Glutathione peroxidase activity (GPx). The GPx activity was quantified in PB homogenates from the liver as described by Flohe and Gunzler (1984). The decrease in absorbance was monitored at 365 nm after induction with 0.25 mmol L\(^{-1}\) \(H_2O_2\) in the presence of 10 mmol L\(^{-1}\) reduced glutathione, 4 mmol L\(^{-1}\) NADPH and 1 U GR enzyme activity. The results were expressed as nmol NADPH consumed min\(^{-1}\) mg\(^{-1}\) protein.

2.7.4.3. Glutathione reductase activity (GR). GR activity in the liver was measured in PB homogenates by determining the decrease in absorbance at 340 nm induced by 1 mmol L\(^{-1}\) oxidized glutathione in the presence of 0.1 mmol L\(^{-1}\) NADPH in phosphate buffer (Carlberg & Mannervik, 1985). The results are expressed as nmol NADPH consumed min\(^{-1}\) mg\(^{-1}\) protein.

2.7.4.4. Superoxide dismutase activity (SOD). The SOD activity was analyzed in liver and kidney tissues. One hundred microliters of appropriately diluted PB homogenates were added to a 96-well microplate and 50 \(\mu L\) of a previously prepared solution (0.1 mmol L\(^{-1}\) hypoxanthine, 0.07 U xanthine oxidase and 0.6 mmol L\(^{-1}\) NBT in phosphate buffer in 1:1:1 proportions) were added just before the readings. The reading was done at 560 nm, and the reaction was monitored for 10 min. The SOD activity is expressed as \(U\) mg\(^{-1}\) protein (Winterbourn, Hawkins, Brian, & Carrell, 1975).

The protein concentration of the tissues homogenates was determined using the Bradford method (Bradford, 1976).

2.8. Statistical analyses

Data were expressed as the means ± standard error (SEM). The statistical analyses were carried out using GraphPad Prism 5.0 (GraphPad Software, Inc. La Jolla, CA, USA) software. The significance of the data was determined using Student’s t-test, and the limit of significance was set at \(P < 0.05\).

3. Results

The total phenolic content in PPF extract was 4.67 ± 0.38 mg GAE g\(^{-1}\); this result compared with our previous works suggest that the high amounts of dietary fiber present in PPF can have strong influence in the phenolic compounds bioavailability (Cazarin et al., in press; Da Silva, Cazarin, Bogusz Jr., Augusto, & Maróstica Jr., 2014). This result was similar some fruits’ peel (mandarin, lemon, calamondin, pamplemousses and bergamot) (Ramful, Bahourun, Bourdon, Tarnus, & Aruoma, 2010). The flavonoid content in PPF extract was 1.17 ± 0.05 mg catechin g\(^{-1}\) which was higher the content in orange and grapefruit peels (aqueous extract) (Oboh & Ademosun, 2012), it was similar to that found in methanolic extract of calamansi (citrus fruit) (Samonte & Trinidad, 2013), pineapple, and mango peels (Kalpna, Mital, & Sumitra, 2011). Flavonoids are hydroxylated phenolic substances responsible for some pharmacological activities, especially, through their antioxidant activity (Kumar & Pandey, 2013). However, the phenolic compounds vitexin, isovitexin, isoorientina and apigenin have been identified in P. edulis, in this extract was not possible to identify and quantify them by chromatographic analysis, probably because their lower concentration in the extract (Cazarin et al., 2014).

The group that received the experimental diet showed no difference in antioxidant potential in their serum than the Control group according to the FRAP assay (\(P > 0.05\)) (Fig. 1a). In addition, the Peel group showed lower lipid peroxidation levels in their serum compared to the Control group (Fig. 2a). However, according to ORAC assay, Peel group had lower antioxidant potential (Fig. 1b) (\(P < 0.05\)).

PPF intake reduced lipid peroxidation in the kidneys. The animals that received PPF had a 30% lower kidney TBARS than the Control group (Fig. 2b) (\(P < 0.05\)). Nevertheless, the opposite was observed in the liver, where there was higher peroxidation in experimental group (Fig. 2c) (\(P < 0.001\)). Regarding antioxidant defenses, there were no differences in the thiol group content (GSH) between the groups either in the liver or kidneys analyzed (\(P > 0.05\)), 38.99 ± 5.79 and 37.69 ± 2.01 nmol mg\(^{-1}\) protein in the
liver and kidneys, respectively. The liver SOD activity of the Peel group was 50% lower than the Control group, and there were no differences in the SOD activity in the kidneys between the groups (Fig. 3a and b). The Peel group had 3 times lower GPx activity than the control group; however, the GR activity in the Peel group was 2 times higher than the Control group (Fig. 4a and b).

Food intake from Peel group was nearly 20% higher than the animals in the Control group (25.93 ± 0.62 and 20.25 ± 0.61 g day⁻¹ in the Peel and Control groups, respectively) during the experimental period. However, there were no significant differences in the body weight (Table 2), of the organs (liver, kidneys, whole brain) or of the carcasses weights between the groups (P > 0.05) (Table 2). In addition, the proximate composition of the freeze-dried carcasses was similar between the two groups (Table 3).

4. Discussion

The intake of fruits has been considered important for health maintenance and the prevention of chronic diseases, since they are great sources of micronutrients, fiber and other bioactive compounds (Jaime & Monteiro, 2005; Ramalho, Dalamaria, & de Souza, 2012; Record, Dreosti, & McInerney, 2001). The processing of fruit leads to large amounts of byproducts that may have functional properties. In the production of passion fruit juice, the residues’ amount originated are above 50% of total raw material (Habib & Ibrahim, 2011; Kulkarni & Vijayanand, 2010; Meletti & Maia, 1999; Oliveira, Nascimento, Borges, Ribeiro, & Ruback, 2002; Tommonaro et al., 2007).

Non-edible fruit portions have shown high levels of polyphenols (Babbar, Oberoi, Uppal, & Patil, 2011; Barros, Ferreira, & Genovese, 2012; Ignat, Volf, & Popa, 2011). Ho, Su, and Lin (2014) found great amount of total phenolic compounds in peels of nine citrus fruits, ranging from 450 to 1160 mg GAE 100 g⁻¹ dried peel. These authors related antioxidant actions of non-edible portions of these fruits by ORAC, DPPH and peroxynitrite-scavenging tests.

The extract made with Passiflora endocarp plus seeds significantly retarded lipid peroxidation in a peroxyl radical-mediated peroxidation in a membrane model (de Oliveira et al., 2009). Similar effect was also observed in the serum of rats treated with P. edulis juice in according to de Souza et al. (2012). In our experiments, there was improving in the oxidative status in kidneys, although PPF intake have not been increased the antioxidant power in the serum.

Nevertheless, toxicological tests in the liver have shown that Passiflora peel has hepatoprotective effects (Zibadi et al., 2006), and negative effects of PPF intake (30 g day⁻¹) were not observed in a...
clinical trial (Medeiros et al., 2009). In this report, the consumption of PPF showed higher lipid peroxidation in liver than the Control group. However, more detailed investigations should be made to explain the observed results in the liver because they could be attributed to several factors such as reduced antioxidant effects or decreased ability to neutralize reactive species. The literature has demonstrated that polyphenols are able to inhibit the activity of hepatic cytochrome P450 enzymes, which are involved in detoxification and could explain the aforementioned results (Si et al., 2009).

In this same tissue, higher GR and lower GPx and SOD activities were observed in PPF group. Some hypotheses have risen to explain these results. High lipid peroxidation in the liver could yield reactive species, which may have overloaded the SOD and GPx enzymes, resulting in a reduction of their activities as noted. These enzymes are responsible for controlling the prevalence of hydrogen peroxide and lipid hydroperoxides. The increase in GR activity may occur to recover reduced glutathione involved in the glutathione cycle (Rover Jr., Höehr, Vellasco, & Kubota, 2001). However, it is difficult to draw conclusions regarding the variations in the enzymes or substrates of the glutathione cycle because there are many interactions among these species (Rover Jr. et al., 2001).

On the other hand, the animals fed PPF had a higher food intake but no difference in final body weight. These findings associated with greater hepatic lipid peroxidation and reduced SOD and GPx activities and higher GR activity in the liver could suggest that the *P. edulis* peel contains thermogenic/ergogenic compounds. These compounds stimulate energy expenditure but contain no energy themselves. Energetic metabolism produces reactive species, which could explain the greater activity of antioxidant enzymes and lipid

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Peel</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final weight (g)</td>
<td>395.6 ± 7.81a</td>
<td>386.6 ± 13.61a</td>
</tr>
<tr>
<td>Diet intake (g day⁻¹)</td>
<td>25.93 ± 0.62a</td>
<td>20.25 ± 0.61b</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>15.80 ± 0.64a</td>
<td>13.70 ± 0.62b</td>
</tr>
<tr>
<td>Kidneys (g)</td>
<td>2.72 ± 0.15a</td>
<td>2.61 ± 0.18a</td>
</tr>
<tr>
<td>Brain (g)</td>
<td>1.98 ± 0.01a</td>
<td>2.07 ± 0.07a</td>
</tr>
<tr>
<td>Carcass (g)</td>
<td>342.32 ± 9.72a</td>
<td>328.73 ± 12.34a</td>
</tr>
</tbody>
</table>

Peel group – animals fed the AIN-93M with 50% of the fiber content replaced with fiber from PPF; Control group – fed the AIN-93M. Data are expressed as the mean ± SEM (n = 4). The superscript letters indicate significant difference (P < 0.05) according to Student’s t-test.

### Table 3

Proximate composition of freeze-dried carcasses.

<table>
<thead>
<tr>
<th>Group</th>
<th>Moisture (g/100 g)</th>
<th>Ash (g/100 g)</th>
<th>Proteins (g/100 g)</th>
<th>Lipids (g/100 g)</th>
<th>Carbohydrates (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peel</td>
<td>2.41 ± 0.18a</td>
<td>8.22 ± 0.43a</td>
<td>49.13 ± 1.51a</td>
<td>38.11 ± 1.36a</td>
<td>2.13 ± 2.41a</td>
</tr>
<tr>
<td>Control</td>
<td>1.49 ± 0.14a</td>
<td>8.50 ± 0.15a</td>
<td>49.45 ± 1.62a</td>
<td>39.34 ± 2.11a</td>
<td>1.22 ± 3.05a</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SEM (n = 4). Different superscript letter in the same column indicates significant difference (P < 0.05) according to Student’s t-test. a Calculation per difference and standard deviation determined by means standard deviation of moisture, ash, proteins, and lipids.
peroxidation, and, despite of higher diet intake, the weight maintenance occurred maybe due the action of thermogenics. Natural herbal ingredients are already considered scientifically confirmed thermogenics. For example, flavonoids, catechins and caffeine in tea have such effect (Hursel & Westerterp-Plantenga, 2010). Catechins up-regulate lipid-metabolizing enzymes and thereby stimulate fat oxidation, since it avoid re-uptake of norepinephrine, and the caffeine inactive hormone-sensitive lipase, promoting upper thermogenesis (Hursel & Westerterp-Plantenga, 2010).

Preservation of lean body mass and lower adiposity in rats after viscous fiber intake, hydroxypropyl methylcellulose (HPMC) was reported, and these effects could be involved in increasing mitochondrial biogenesis and fatty acid oxidation in skeletal muscle (Islam, Civitarese, Hesslink, & Gallaher, 2012). In a type of grapefruit, nootkatone (4,4a,5,6,7,8-hexahydro-6-isopropenyl-4,4a-dimethyl-2(3H)-naphthalenone), a sesquiterpenoid present mainly in its peel, was identified as ergogenic compound. Report evaluating nootkatone in experimental and clinical studies showed body weight reduction and improvement in insulin resistance (Jones et al., 2011). The literature also reports the use of extract from the whole or bitter orange’s peel (Citrus aurantium, Rutaceae) in the management of weight (Stohs, Preuss, & Shara, 2011). In addition, whole bitter orange extract has been used in sports performance products because the improvement in the thermogenesis and lipolysis. These effects can be attributed to the proto-flavonoids: nomenclature, occurrence and intake. Journal of Nutrition, 133(10), 32485–32545.


