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RESEARCH ARTICLE

LIPID PROFILE OF WISTAR RATS SUBMITTED TO DIETS WITH WASTE FLOUR FROM PASSION FRUIT PROCESSING

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ABSTRACT

The waste generated from passion fruit juice processing is very significant and it is important to find solutions for the use thereof, since they exhibit high potential as a functional food. This study had as objective to make the chemical characterization of the waste generated from passion fruit juice processing (peel) of different species, as well as the supplementation effect with passion fruit peel flour on lipid profile plasma levels of the male Wistar rats. The peels after hygienized and lyophilized were finely ground to obtain the passion fruit flour (sour yellow – SYPFF, sweet - SwPFF and purple - PPFF) for carrying out chemical analysis and it was used 18 rats divided into 3 groups (n = 6) which received different treatments for 42 days: T1 - feed (control); T2 - feed + roast chicken fat; T3 - feed + roast chicken fat + passion fruit peel flour, for in vivo tests. By the results we can infer that passion fruit peel flour, provides to be a source of fiber and minerals with great potential to be used in human food as an alternative to reduce the lipid profile, which was proven in vivo test.

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INTRODUCTION

Brazil is a leading food producer in the world and annually wastes more than R$ 12 billion in food, which could be for food of 30 million poor people. Studies conducted by the Brazilian Agricultural Research Corporation (Embrapa) in Agribusiness Food Center have shown that the Brazilians discard more food than consume (Raza, 2011). Improper disposal to the tons of waste generated from passion fruit juice processing is quite significant and therefore it is very important to find solutions for utilization them, as have high nutritional and functional potential. According to several researchers the generated wastes constitute 65-70% of the total weight of the fruit, varying according its species.

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In recent years, the functional properties of passion fruit peel have been studied, especially those related to the content and type fibers, thereby increasing product obtained from passion fruit peel, directed to people who need to increase the intake of fibers are formulated (Cordova *et al*., 2005). Passion fruit (*Passiflora sp.*) have three more commercialized plant species, and the sour yellow passion fruit (*Passiflora edulis f. flavicarpa*), corresponding to 95% of the crop in Brazil, followed by the sweet passion fruit (*Passiflora alata*) and sour purple passion fruit (*Passiflora edulis*) (Meletti, 2000).

According Zeraik, *et al*. (2010), many substances present in the pulp and in passion fruit peel may be beneficial to the human body, such as antioxidant activity, antihypertensive, decreased glucose levels and blood cholesterol. Despite the potential and applicability of passion fruit as a functional food, are still scarce studies. It is noteworthy that, despite the generated volume of sub-products (peels and seeds) to be susceptible to utilization, also have pharmacological actions and nutritional value; since peel is rich in pectin, in addition to containing other substances such as flavonoids and the seeds are rich in polyunsaturated acids such as ω-3 and ω-6. The passion fruit peel flour has been the focus of great speculation regarding to its use in decreasing plasma cholesterol. Numerous reports and interviews have been broadcast by the media showing its power in the combating dyslipidemia (Ramos *et al*., 2007). The passion fruit peel is rich in pectin, niacin (vitamin B3), iron, calcium and phosphorus. In humans, pectin helps the body in the removal of excess and harmful substances out of the body (Gonçalves *et al*., 2007). Niacin acts on the growth and hormones production, so as to prevent gastrointestinal problems. The minerals act to prevent anemia (iron), in growth and bone strengthening (calcium) and cell formation (phosphorus) (Córdova, *et al*., 2005). Due to the high pectin content on its composition, has shown hypocholesterolemic effect, hypoglycemic and inducing the secretion of insulin on plasma, these characteristics mean that it is no longer considered only industrial waste (Queiroz *et al*., 2008). Currently the functional properties of passion fruit peel have been studied, especially those related to the content and fiber type. Thus, more products obtained from the passion fruit peel have been directed to people who need to increase the fiber intake (Córdova *et al*., 2005). Therefore, this study had as objective to make the chemical characterization of waste flour generated from the passion fruit juice processing (peels) of different species, as well as check the supplementation effect with passion fruit peel flour on plasma levels of total cholesterol and fractions of male Wistar rats.

MATERIALS AND METHODS

This work was carried out in the Federal University of Lavras, at the Central Laboratory of Food Analysis from Food Science Department and parcel in Biotherium of the Multidisciplinary laboratory of Animal Experimentation, University Center of Lavras - UNILAVRAS, located in the city of Lavras - MG.

Obtaining passion fruit flour

The raw materials used in the study were passion fruit peels of the cultivars: yellow passion fruit (*Passiflora edulis Sims f. flavicarpa Deg*), purple passion fruit (*Passiflora edulis Sims*) and sweet passion fruit (*Passiflora alata*), and these wastes generated from juice processing that were provided by industry juices *Frutilavras*, located in Lavras/MG. Transportation was held in coolers, hermetically sealed until to the Central Laboratory of Food Science Department DCA/UFLA, located in Lavras-MG, where the wastes were hygienized, then cut into small cubes and kept under refrigeration until they were lyophilized and analyzed.

Preparation and characterization of passion fruit flour

After lyophilized, the peels (each species) were mixed and then finally ground and processed into passion fruit flour that was characterized as described below:

Yield

The yield of passion fruit peel flour was established in accordance with the production yield, based on the calculation of the raw material initial weight and water weight loss of the final product.

Chemical composition of passion fruit flour

Analyses of flour chemical composition were carried out according to the methodology proposed by the Official Analytical Chemistry Association (AOAC, 2000). For the dietary fiber total determination, soluble and insoluble fiber, it was used Kit-dietary fiber total SIGMA®, following the techniques proposed by the AOAC (2000). For pectin extraction of passion fruit peel, it was used the methodology of McCready & McComb (1952). It was analyzed the crude fiber submitting the peel to acid digestion with sulfuric acid 1.25% solution, followed by alkaline digestion with sodium hydroxide 1.25%, according to the methodology proposed by Institute Adolfo Lutz (1985). It was calculated the total amount of carbohydrates in samples from the difference between initial weight of sample (100 g) and the total mass of proteins, lipids, of fixed mineral waste and crude fiber. Its total caloric value was calculated applying the conversion values for carbohydrates (4.0 kcal), lipids (9.0 kcal) and protein (4.0 kcal) (Instituto Adolfo Lutz, 1985). Minerals readings were held in the Leaf Analysis Laboratory at the Chemistry Department - UFLA in a flame photometer, from samples extracts obtained by digestion nitric perchloric (DNP) and determined by Malavolta, Vitti and Oliveira (1989).

Microbiological analysis

To check the processing conditions, hygiene and handling of different passion fruit peel flour, microbiological analyzes were performed in order to determine the microorganism count, according to the methodology by Silva *et al*. (2007).

Statistical design

The design was completely randomized, with 15 fruits of each passion fruit species (yellow, sweet and purple), providing three flour, which were evaluated in three repetitions each. Data were statistically analyzed using variance analysis of Scott-Knott test at the level of 5% probability using the SISVAR program (Ferreira, 2000).

Experimental design 'in vivo'

The experimental protocol of this study follows the ethical principles for animal experimentation adopted by the Brazilian College of Animal Experimentation (Cobea, 1991) and was approved by the Ethics Committee in Animal Experimentation of the University Center of Lavras - UNILAVRAS. The experiment was conducted with 18 adult Wistar rats (± 170 g), males with 90 days old, coming from the Biotherium of the Multidisciplinary laboratory of Animal Experimentation, University Center of Lavras. For the animals air conditioning, they were kept in individual cages with water *ad libitum* and commercial feed (NUVILAB CR1, NUVITAL®) for 3 days at temperature and light ambient, and the Biotherium is equipped with ventilation and exhaust system, alternating in 12-hour periods of light and dark. After this period they were separated into groups of six animals to compose the three treatments (T), these being: T1 - feed (control); T2 - feed + roast chicken fat; T3 - feed + roast chicken fat + passion fruit peel flour, for 42 days.

Feed preparation

Three diets were prepared, constituted the treatments in which the first group (T1) the diet was standard, group (T2) the standard diet was enriched with 30% roast chicken fat as a cholesterol source, which is reminiscent of the rotating machine to roast chickens *Brasgrill* brand, for the purpose to hypercholesterolaemia animals. As for the diet of the group T3 was composed of passion fruit peel flour at concentration 10% plus 30% roast chicken fat. Based on dietary fiber content, pectin and better yield, and analyzing the large volume of waste generated in the industry, the sour yellow passion fruit was the qualified species as the most suitable to be used *in vivo* tests, since it corresponds to 95% of the crop in Brazil. The ingredients of the diets were weighed, mixed in a container with the standard feed ground and add water, which are made weekly and stored under refrigeration.

Euthanasia and Biochemical Analysis

To carry out the clinical examination, the animals remained in fasting for a period 12 hours. Subsequently, to euthanasia, animals were anesthetized with chloral hydrate (dosage 1g/kg; volume 0.5 mL/100g animal) by intraperitoneally via, using a syringe 5ml and needle 25x7; after certification of anesthesia occurred skin incision, dilatation, muscle incision and aortic artery rupture with surgical scissors, to provide the realization of blood collection. The blood was collected with syringe and deposited in the pipe for collecting and forwarded to the *Santa Cecília Clinical Analysis Laboratory* - Lavras/MG, where was determined triglycerides, total cholesterol and its fractions (HDL, LDL, VLDL). The measurement of total cholesterol and triacylglycerols were analyzed by Trinder Enzyme method, using the *kit* Sera-Pak ® Plus (Bayer, 2003). Cholesterol HDL was analyzed by direct Homogeneous method using *kit* HDL LE®. As for, LDL cholesterol was calculated using the equation: LDL = Total Cholesterol - HDL-C - Triglycerides/5 and VLDL cholesterol was calculated by the formula: VLDL-cholesterol = triacylglycerols/5 (Friedewald, 1972). For biochemical studies it is considered as normal parameters values presented by the control group, UNILAVRAS biotherium own, since there is no scientific consensus on reference values for rats.

Statistical analysis

After data collection, they were submitted to statistical analysis by Scott-Knott test at 5% probability using the program SISVAR (Ferreira, 2000).

RESULTS AND DISCUSSION

Chemical composition

According to average values found for the yield calculation (Table 1) it is observed that flour average value of the different species of passion fruit peel were around 5.5; 8.0 and 10.8%, significantly differing from each other (P<0.05). The passion fruit flour that stands out in relation to the largest yield percentage was the SwPFF, followed by the SYPFF, which showed intermediate value and PPFF, which had lower income to the others. One alternative that has shown technological and economic feasibility is the enrichment of high acceptance product and consumption within the population, with fruit and vegetables industrial waste, thus reducing nutritional deficiencies resulting from the change in the standard food, consuming ever more processed foods instead of *in natura*, naturally rich in fiber, vitamins and minerals (Santana, *et al*., 2011).

Table 1. Analysis average values of yield, moisture, ash, crude protein, ether extract, carbohydrates and calories of yellow passion fruit peel flour (SYPFF), sweet passion fruit (SwPFF) and purple passion fruit (PPFF) in g/100g

Variable	SYPFF	SwPFF	PPFF	CV
Yield	8.00 b	10.83a	5.50 c	3.24
Moisture	18.74 a	8.28 c	12.44 b	11.06
Ashes	5.64 c	8.54 b	10.02 a	6.38
Crude protein	8.21 c	9.73 b	10.26 a	2.71
Ethereal extract	0.59 a	0.55 a	0.68 a	10.95
Carbohydrates	40.48 a	41.66 a	34.61 b	4.06
Calories (KCAL)	200.04 a	210.52 a	185.55 b	3.26

Average followed by the same letter on the line, does not differ by the Scott-Knott test at 5% probability.

The average values for moisture were significantly different, where SwPFF had the lowest value 8.28%, followed by PPFF presenting 12.44% and SYPFF had the highest value 18.74%. For moisture content, the results do not corroborate with Córdova *et al*. (2005) and Oliveira *et al*. (2002), which in their studies found similar content to the yellow passion fruit peel flour (88.37 and 89.08%, respectively) (Table 1). For the ashes content and crude protein, it is verified that they showed values in ascending order, where SYPFF was significantly lower (P<0.05) content (5.64% and 8.21%), followed by SwPFF (8.54% and 9.73%) and PPFF (10.02% and 10.26%). The results found for the ashes content (Table 1) do not corroborate with the observers by Oliveira, *et al*. (2002) and Souza, *et al*. (2008), who found ashes content for SYPFF 9.20% and 8.66%, respectively. In relation to crude protein content, different results were obtained by Souza *et al*., (2008), who found higher content (12.52%). This difference can be explained by the presence of proteins in various components suffering modifications (biological oxidation) during respiratory metabolism which occurs in the passion fruit maturation process (Chitarra and Chitarra, 2005). It is observed that there were no significant differences (P>0.05) for the lipids, where average content ranged from 0.55% to 0.68% (Table 1). These results are similar to Reolon, Braga and Saliba (2009) and Martins Guimarães and Pontes (1985), that in their studies found average content 0.74% and 0.51%, respectively. However, do not corroborate with Oliveira *et al*., (2002) who report having obtained higher values than the present study (1.07%). The carbohydrates content and calories were significantly equal to SYPFF (40.48% and 200.04Kcal) and SwPFF (41.66% and 210.52Kcal), differing from PPFF (34.61% and 185.55Kcal). The carbohydrate contents results in this study (Table 1) do not corroborate with Córdova *et al*. (2005) and Souza *et al*. (2008), that in their studies had higher content (55.96% and 77.07%).

Table 2. Percentage average values of crude fiber, total dietary fiber, soluble dietary fiber and insoluble dietary fiber and total pectin flour from peels of yellow passion fruit (SYPFF), sweet passion fruit (SwPFF) and purple passion fruit (PPFF)

Cultivar	Crude fiber	Total dietary fiber	Soluble dietary fiber	Insoluble dietary fiber	Total pectin
SYPFF	26.34 b	55.01 a	7.94 b	47.09 a	15.62 a
SwPFF	31.28 a	45.01 b	5.75 c	39.27 b	12.63 c
PPFF	31.03 a	56.62 a	8.38 a	48.24 a	14.10 b
CV	3.20	4.12	2.39	4.69	3.53

Average followed by the same letter on the column, does not differ by the Scott-Knott test at 5% probability.

Table 3. Average values of mineral composition (g/100g) of dry matter of the yellow passion fruit peel flour (SYPFF), sweet passion fruit (SwPFF) and purple passion fruit (PPFF)

CULTIVAR	P	K	Ca	Mg	Cu	Zn	Mn	Fe
SYPFF	1.96 a	22.03 a	0.55 a	0.24 b	0.22 b	0.12 b	0.12 b	0.58 a
SwPFF	1.90 a	23.01 a	0.61 a	0.14 c	0.20 b	0.12 b	0.13 b	0.61 a
PPFF	2.52 a	21.01 a	0.50 a	0.56 a	0.66 a	0.32 a	0.45 a	0.67 a
CV	15.22	5.24	9.37	6.75	8.87	9.33	11.29	8.77

Average followed by the same letter in the column does not differ by the Scott-Knott test at 5% probability.

Table 4. Clinical analysis (total cholesterol and fractions and triglycerides) of male Wistar rats fed with standard diet, hypercholesterolemic diet and hypercholesterolemic diet enriched with passion fruit peel flour

Treatment	Total cholesterol (mg/dL)	LDLc (mg/dL)	HDLc (mg/dL)	VLDLc (mg/dL)	Triglycerides (mg/dL)
T1	58.5b	18.55b	28.25b	11.65b	58.25b
T2	66.67a	22.07a	32.25a	12.35a	61.75a
T3	54.07c	18.10b	28.62b	7.35c	36.75c
CV	1.58	5.45	1.88	0.96	0.96

Average followed by the same letter in the column does not differ by the Scott-Knott test at 5% probability.
T1 - feed (control);
T2 - feed + roast chicken fat;
T3 - feed + roast chicken fat + passion fruit peel flour

The differences among the results found in the works can be attributed to the genetic variations, edaphoclimatic, seasonal and other factors that can interfere in the fruit composition (LIMA *et al*., 2012). It is observed in Table 2, the SYPFF (55.01% and 47.09%) and PPFF (56.62% and 48.24%) did not differ significantly and had total dietary fiber content and higher insoluble dietary fiber when compared to SwPFF (45.01% and 39.27%). As for the soluble dietary fiber percentage, all flour showed statistical difference. The PPFF showed higher value (8.38%), followed SYPFF (7.94%) and SwPFF (5.75%). For crude fiber contents, the SwPFF and PPFF were statistically equal (31.28% and 31.03%) differing from the SYPFF (26.34%) (Table 2). Lima, *et al*. (2012), in their research obtained lower crude fiber content for passion fruit flour of the bush (14.6%). Also reports that the crude fiber content is relatively high when compared to the crude fiber content found in other fruits. According Raupp, *et al*. (2000), the soluble fiber fraction is important to provide the mass required for the peristaltic intestine action, whereas the insoluble fraction remains almost totally intact. It is also observed (Table 2) that in the total pectin content, SwPFF was significantly ($P<0.05$) lower content (12.63%), followed PPFF (14.10%) and SYPFF had the highest content (15.62%). These results were lower to those of Reolon, Braga and Salibeb (2009), who found values from 20.7% to 28.5%, observing a reduction in pectin content during the yellow passion fruit maturation process. According Rique *et al*. (2002), soluble fibers represented by pectin have the property to bind to the water forming a gel that reduces sugars and lipids absorption, making it a substrate for the formation of rich bacterial flora. Regarding dietary fiber the values found in this study corroborate with that observed by Cazarin *et al*. (2014), the results demonstrated that passion fruit peel flour showed high fiber content (65.22±0.27%), which 74% are insoluble fibers, significant fiber sources to be included in the diet. These fibers can have various beneficial physiological functions, as peristalsis increase, dyslipidemias prevention, cardiovascular disease, certain types of cancer, among others (Mahan & Escott-Stump, 2005). According to Fernandes (2006), the mechanism of soluble fiber action is based on its action to sequester bile acids at intestine duodenal portion, leading to fecal excretion of bile acids in feces, decreasing the amount that reaches to the liver via enterohepatic. This increased excretion causes higher conversion of hepatic cholesterol into bile acids, reducing intrahepatic cholesterol concentration, which explains the reduction of serum lipids concentrations of the present study.

In Table 3 are presents the average values of the mineral composition (g/100g). It is observed that the mineral content to the average values of elements P (1.90g to 2.52g), K (21.01g to 23.01g), Ca (0.50g to 0.61g) and Fe (0.58g to 0.67g) of the three passion fruit peel flour did not differ. For Cu, Zn and Mn elements, purple passion fruit peel flour showed the highest value (0.66g, 0.32g, 0.45g, respectively) when compared to yellow (0.22g, 0.12g, 0.12g, respectively) and sweet (0.20g, 0.12g, 0.13g, respectively), that presented values equal to each other. As for the Mg content, purple passion fruit peel flour (0.56g) had the highest value, followed by yellow (0.24g) and lower values were observed for sweet passion fruit peel flour (0.14g). Studies by Reolon, Braga and Salibi (2009) showed that minerals from the yellow passion fruit peel at different maturity stages had results lower than this work for P and K elements, as well as similar results for Ca, Mg, Cu, Zn, Mn and Fe (Table 3). However, Martins, Guimarães and Pontes (1985) found 10.98 mg/100g Ca, 3.20mg/100g Fe and 36.36mg/100g P. According to Oliveira *et al*. (2002), certain difference found among the mineral content is related to the fruit maturation stage, because the fruit maturation leads to moisture loss, which leads to the concentration of other constituents in addition to other factors such as the planting site and plant genetic conditions. Felipe, Costa and Maia (2006) observed that the passion fruit peel powders have higher minerals amounts in relation to fruit juice, being good minerals sources, when comparing the values obtained in the study with the values required for the intake daily recommended for adults.

Microbiological quality

The microbiological analyzes results of passion fruit peel flour and its microbiological standards comparison allow its characterization as microbiologically suitable, since the value obtained for Cologne Forming Units (CFU) ($3x10^2$) is below to the standards required by Brazilian law, being permitted its use (Brazil, 2001).

Biochemical composition

In the Table 4 are shown the average values of lipid profile of the male Wistar rats fed with standard diet, hypercholesterolemic diet and hypercholesterolemic diet enriched with passion fruit peel flour. It is observed for total cholesterol levels, VLDL and triglycerides, respectively, that animals when submitted to diet with feed increased fat (T2) had a significant change compared to the other treatments, obtaining an average value 66.67mg/dL; 12.35mg/ dL; and 61.75mg/dL, since the treatment (T1) showed an intermediate value for these variables (58.5mg/dL; 11.65 mg/dL; and 58.25mg/dL) and is higher than (T3), where it can be seen that the inclusion of passion fruit peel flour influenced in total cholesterol content, VLDL and triglycerides, with the lowest value (54.07mg/dL; 7.35mg/dL, and 36.75mg/dL). For variables LDL and HDL, respectively, the animals were submitted to diet with feed increased fat also had higher values (22.07mg/dL; and 32.25mg/dL) when compared to other animals, that showed similar, where the (T1) showed values (18.55mg/dL, and 28.25mg/dL) and (T3) showed (18.10mg/dL, and 28.26mg/dL).

In relation to clinical analysis, Queiroz *et al*. (2008) in their studies on the supplementation effect with passion fruit peel flour on plasma levels of glucose, triglycerides, total cholesterol and fractions, and hepatic glycogen content and cardiac of diabetic rats, found that the use of passion fruit peel flour was effective for glycemic control and increased hepatic glycogen and cardiac, not being effective in reducing plasma lipids during the study period. It also found that passion fruit peel flour fibers intake in the action of lipid and glycemic profile of Wistar rats, showed no reduction of plasma VLDLc that diverges from the present study. Chau and Huang (2005) in their studies with hamsters fed with seed fiber *Passiflora edulis*, whose constitution is like the passion fruit peel, observed a decrease in the triglycerides levels, a similar result to this study. According to Ramos *et al*. (2007) in their study to check the *Passiflora edulis f. flavicarpa* action on LDLc levels in rodents after eight weeks of treatment, there was a reduction in serum values of this lipoprotein which resembles to the present study. Martins *et al*. (2004) in their studies describe that the strategies report for reducing blood cholesterol of National Cholestrol Education Program (NCEP - National Cholestrol Education Program, 1993) estimates that for every 1% reduction in blood cholesterol concentration the risk of cardiovascular disease tends to reduce until 2%, thus, the use of passion fruit peel flour as an alternative dietary therapy to decrease blood cholesterol, thus favoring a decrease of LDL - cholesterol.

Conclusion

By the results obtained in the chemical composition, we can infer that passion fruit peel flour have compatible content with the literature, confirming be a good fiber source and minerals, with great potential to be used in human food as an alternative to reduce lipid profile, since in the *in vivo* test can prove the action of sour yellow passion fruit peel flour in reducing serum lipids of Wistar rats that have undergone diet.

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