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Original Article

Evaluation of the effects of passion fruit peel flour (*Passiflora edulis* fo. *flavicarpa*) on metabolic changes in HIV patients with lipodystrophy syndrome secondary to antiretroviral therapy

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ABSTRACT

This study evaluated the effects of using passion fruit peel flour together with diet therapy and counseling in 36 patients with HIV lipodystrophy who were in an ambulatory clinic in a university hospital. The patients were divided into two groups. One received 30 g of passion fruit peel flour daily for 90 days and diet therapy counseling. The other group received only diet therapy counseling. The metabolic changes were analyzed before and after the intervention, with a significance level predetermined at $p \leq 0.05$. The use of passion fruit peel flour was effective in reducing total cholesterol and triacylglycerides after 30 days. The concentrations of LDL-C decreased, while HDL-C increased in the blood of lipodystrophy patients after 90 days passion fruit peel flour treatment. No significant differences in food consumption were seen between groups. The use of 30 g of passion fruit peel flour for 90 days together with diet therapy counseling was effective in improving plasma concentrations of total cholesterol, LDL-C, HDL-C and triacylglycerides.

Introduction

The treatment of acquired immunodeficiency syndrome (AIDS) with reverse transcriptase and protease inhibitors represented a positive impact on the survival rate of individuals carrying the human immunodeficiency virus, or HIV (Smith, 2014). However, when HAART or highly active antiretroviral therapy (Smith, 2015) became a routine treatment, changes in body fat distribution and dyslipidemia were observed (Guimarães et al., 2007). These changes are called HIV Lipodystrophy syndrome (Tien et al., 2006; Guimarães et al., 2007; Araújo et al., 2007), which can increase the risk of cardiovascular diseases (Friis-Møller et al., 2003).

Soluble fiber may increase short-chain fatty acid synthesis (Wong et al., 2006), thereby reducing endogenous cholesterol production and associated with a diet low in cholesterol and saturated fat can reduce blood levels of LDL-C and triacylglycerides (Liu et al., 2000; Schneeman, 2002; Solà et al., 2007). The American Heart Association (2006) recommended the use of 5–10 g/day of soluble fiber for the reduction of dyslipidemia. A fiber-rich diet may be beneficial in preventing the development of fat deposition in people with HIV (Hendricks et al., 2003).

Passion fruit is the common name for various species of plants in the genus *Passiflora* (Zeriak et al., 2010). The two species with the most commercial value are *P. edulis* fo. *edulis* (red passion fruit) and *P. edulis* fo. *flavicarpa* O. Deg. (yellow), with the yellow species being the most widely cultivated (Zeriak et al., 2010). It has several health effects, including lowering the concentrations of LDL-C and cholesterol in the blood (Ramos et al., 2007). The peels can be dried

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and made into a food product called passion fruit peel flour (PFPF) that has several health effects and is sold in Brazil as an adjuvant treatment for diabetes due to its hypoglycemic effect (Smith et al., 2012). Flour prepared from yellow passion fruit peels has also been shown to reduce blood glucose in diabetic people. In a phase I clinical study, passion fruit peel flour was well tolerated in 36 people between ages 20 and 60, of both sexes. They received 10 g of flour three times a day and were told to put it in their choice of juice, soup, or any other food or beverage. There was an average reduction of blood glucose, triacylglycerides, total cholesterol and LDL of 5.2, 15.0, 18.2 and 19.0%, respectively.

In phase II studies, flour prepared from yellow passion fruit peels reduced blood glucose, cholesterol, LDL, blood pressure and body weight in diabetic patients.

The peels, or rinds, of Hawaiian yellow passion fruit have been fed to cattle and found an increase in milk production (Otagaki and Matsumoto, 1958). There are many anecdotal accounts of it increasing milk production and preventing bacterial infection, but these effects have not been quantified.

PFPF contains about 10% moisture, 7.5% ash, 4% protein, 19% soluble fiber, 38% insoluble fiber and 21% soluble carbohydrates (Córdova et al., 2005; Pinheiro et al., 2008). The major compound in PFPF is pectin, a dietary fiber that is rich in polygalacturonic acid and its methyl ester (Smith et al., 2012). However, to the best of our knowledge, the soluble carbohydrates and other soluble compounds have never been identified or analyzed by either HPLC or NMR.

Thus, considering that PFPF is a good source of soluble fiber, this study evaluated the effectiveness of PFPF in improving the blood lipid profile of individuals carrying HIV and receiving HAART who developed lipodystrophy. In addition, the hot, pressurized methanolic extract (100 °C and 10 MPa pressure) was analyzed by HPLC and NMR.

Materials and methods

Chemicals

Chloroform ($CHCl_3$), deuterated chloroform ($CDCl_3$), methanol (CH_3OH) and deuterated methanol (CD_3OD) were from Sigma–Aldrich, St. Louis, MO.

Preparation and characterization of the PFPF

The PFPF was produced from sanitized ripe yellow passion fruits (*Passiflora edulis* fo. *flavicarpa* O. Deg), that were identified by AUOS-S and stored at the Federal University of Rio de Janeiro for further reference. The peel was separated from the pulp, dehydrated, and transformed into flour using a knife mill. In order to characterize the PFPF, 10 g of it was mixed with enough HydroMatrix™ (Sigma–Aldrich, St. Louis, MO) to fill the 100 ml stainless steel sample cell used in an Accelerated Solvent Extractor (ASE, ThermoFisher Scientific, Sunnyvale, CA). Then, CH_3OH was added while the temperature and pressure were increased to 100 °C and 10.3 MPa (1500 psi, 100 atm) over a 3 min time (static time). Next, the solvent was purged into a collection vessel. A total of four cycles were run to statically extract the sample, resulting in a total volume of about 160 ml. The solvent was evaporated off and the oily residues remaining were weighed. A portion of the residue remaining after evaporating the CH_3OH from the methanolic extract of each sample was redissolved in CD_3OD for NMR analysis. Another portion was dissolved in methanol for HPLC analysis.

NMR analyses were done using an Agilent DD2 600 MHz NMR (Santa Clara, CA). A 30° pulse width and 1 s pulse delay were used for the ^1H NMR, while a 30° pulse width and 2 s pulse delay were used for the ^1H-coupled ^{13}C-NMR spectra, also known as $^{13}C\{^1H\}$-NMR. Chemical shifts were referenced to the CD_3OD signals at 3.35 and 4.78 ppm (for ^1H) and 49.30 ppm (for ^{13}C) for the spectra of the methanolic extracts and to the $CDCl_3$ signals at 7.27 and 77.23 ppm, for ^1H and $^{13}C\{^1H\}$-NMR, respectively.

The FTIR spectrum of the residue remaining from the methanolic extract, after evaporating off the methanol was acquired using ATR attachment on Shimadzu IRAffinity-1S. The UV–Vis spectrum of a 5 μg/ml solution of the extract in methanol was obtained using Shimadzu UV-2600. Plain methanol was used as blank. The source of light was shifted to deuterium bulb at 300 nm and the spectrum window was opened between 900 nm and 185 nm.

Experimental design

A clinical therapeutic randomized trial was performed with individuals carrying HIV, presenting Lipodystrophy Syndrome, receiving HAART, and showing dyslipidemia (hypercholesterolemia, hypertriglyceridemia, or both) as defined by the National Cholesterol Education Program (2002). These individuals were monitored at the Lipodystrophy Ambulatory Center in the João de Barros Barreto University Hospital (HUJBB), during the period of January to December 2009, and fulfilled the criteria for participation in the study. The sample was calculated through the calculation of Proportion: two samples. For the first sample (from the group using PFPF), the estimated proportion of plasma cholesterol improvement was 50%; for the second sample, this estimate was of 10%. The ratio between these samples was stipulated as 1:1. The power of the test was fixed as 0.8, and the unilateral alpha level in 0.05. The sample was calculated from sixteen individuals in each group.

The 36 individuals participating in the study were systematically divided into two groups. Group 1 ($n = 18$) received diet therapy counseling and 30 g/day of PFPF, consumed as a dilute solution in water, juices, and/or fruit smoothies. Group 2 ($n = 18$) received diet therapy counseling only. There were 12 men and 6 women in each group.

Information about pathology, weight, height, and food intake was assessed in the initial evaluation by the 24 h recording method (R24) at the time of the initial (beginning of the intervention) and last assessments (end of the study).

Individuals from the two groups were instructed to have biochemical tests performed monthly (total cholesterol – TC, LDL-C, HDL-C, and triacylglycerides) at the HUJBB's laboratory. These tests were performed in the morning, after 12 h fasting and at 4 time points in the study: before the intervention (T0), at 30 (T30), 60 (T60), and 90 days (T90) after the intervention.

The following parameters were followed as inclusion criteria in the study: be an adult patient (any gender) receiving HAART, attending treatment at the Lipodystrophy ambulatory center at the HUJBB, presenting changes in the TC and/or LDL-C and/or triacylglycerides, presenting undetectable HIV viral load and LTCD4+ above 300 cells/mm^3 and agreeing to participate in the study by voluntarily signing an informed consent form.

The following criteria were followed for study exclusion: individuals presenting triacylglycerides above 700 mg/dl, or using lipid-lowering drugs, who could not complete the 90 days of monitoring, did not tolerate the use of PFPF, missed appointments, displayed mental illness, were under age 18 (children, and adolescents), and did not accept to participate in the study.

The data obtained were tabulated and analyzed using the Biostat 5.0 program (2007) with a significance level of $p \leq 0.05$. The dietpro program (Esteves and Monteiro, 2002) was used for the evaluation of R24 h. The bilateral t-test was used for the verification of homogeneity in the observed values between the groups.

The paired *t*-test was used to assess the variation in the results from laboratory tests.

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and Nuremberg Code respecting the standards of research involving human subjects (resolution 196/96) of the National Health Council. This study was authorized by the Ethics Committee for Research with Human Beings of the HUJBB from the Federal University of Pará under Protocol No. 1945/08. Written informed consent was obtained from all patients.

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Results

Dry methanol at 100 °C and 10 MPa (100 atm) pressure in a sealed container solubilized 31% of the PFPF. The FTIR spectrum of the residue remaining after evaporating off the methanol is shown in Fig. 1. The peaks in the region from 1400 to 1800 cm^{-1} are listed in Table 1. The UV–Vis spectrum of a 5 μg/ml solution of the extract in methanol is shown in Fig. 2. The ^{1}H and $^{13}C\{^{1}H\}$-NMR spectra of the extract are shown in Figs. 3 and 4. The ^{13}C chemical shifts are tabulated in the Supplementary Material. Signals due to the CHO and CH_2O of carbohydrates accounted for 75.9% of the total peak area (Fig. 1). There were also signals due to $CH_3(CH_2)_n$ groups in fatty acyls that are covalently bound to carbohydrates as fatty acid glycosides in both the ^{1}H and $^{13}C\{^{1}H\}$-NMR spectra (Figs. 1 and 2). On the other hand, several of the signals that are produced by D-fructose were not seen, including the ones at 105.93, 99.30, 84.24, 83.31, 77.60, 76.84, 71.93, 66.00, 65.19 and 64.28 ppm. So, even though D-fructose is soluble in methanol,

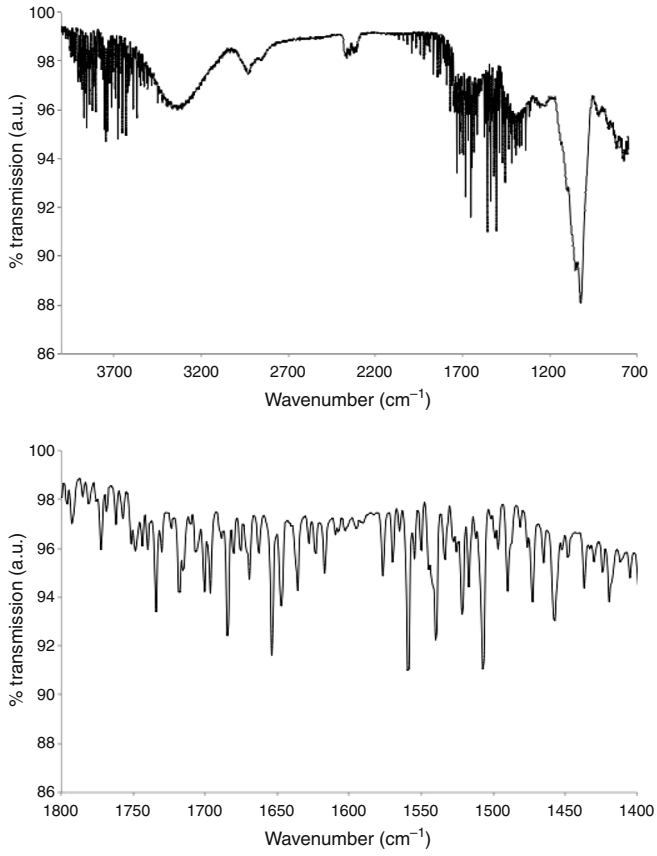

Fig. 1. FTIR spectrum of the hot, pressurized methanolic extract (100 °C and 10 MPa pressure) of passion fruit peel flour, after evaporating off the methanol. The peaks can be used as a type of fingerprint that can be used to identify it.

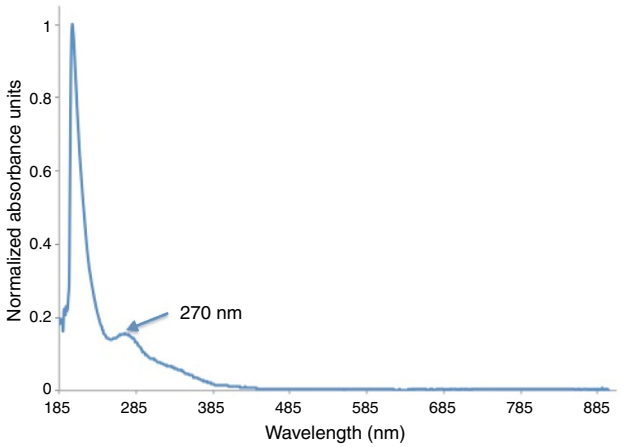

Fig. 2. UV–Vis spectrum of the hot, pressurized methanolic extract (100 °C and 10 MPa pressure) of passion fruit peel flour, in methanol. The maximum at 270 nm can be used to help identify the passion fruit peel flour.

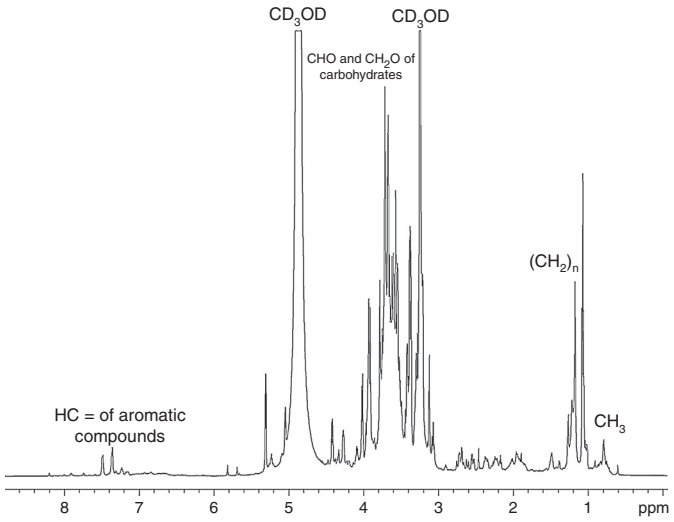

Fig. 3. ^{1}H NMR of the hot, pressurized methanolic extract (100 °C and 10 MPa pressure) of passion fruit peel flour. The signals can be used as a type of fingerprint that can be used to identify it.

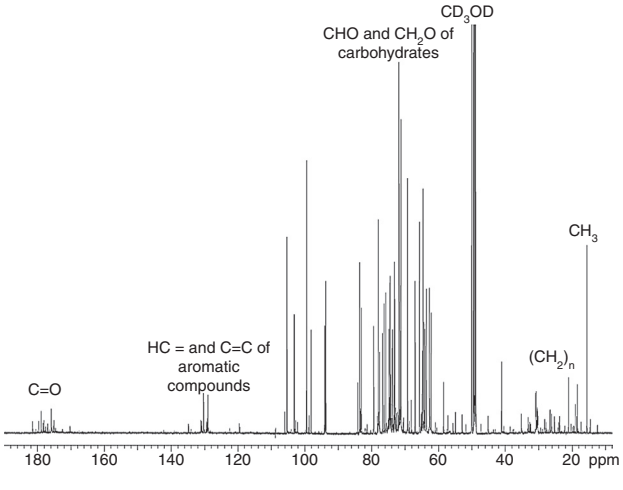

Fig. 4. $^{13}C\{^{1}H\}$-NMR of the hot, pressurized methanolic extract (100 °C and 10 MPa pressure) of passion fruit peel flour. The signals can be used as a type of fingerprint that can be used to identify it.

Table 1
Peaks in the FTIR spectrum of the hot, pressurized methanolic extract (100 °C and 10 MPa pressure) of passion fruit peel flour.

cm^{-1}	cm^{-1}
1796	1610
1792	1609
1785	1603
1781	1595
1723	1591
1767	1577
1762	1570
1757	1565
1751	1559
1749	1555
1744	1549
1740	1540
1734	1533
1730	1528
1723	1521
1719	1517
1716	1507
1707	1499
1700	1497
1696	1490
1684	1481
1681	1477
1676	1473
1669	1465
1663	1458
1654	1448
1647	1437
1636	1430
1628	1424
1624	1420
1617	1412

it is not present at a sufficient concentration to be detected by $^{13}C\{^1H\}$-NMR. Glucose and sucrose are not soluble in methanol, but are not known to be in PFPF, which does not have a sweet taste. Still, there is a significant concentration of fatty acid glycosides in the methanolic extract of PFPF. They produced not only signals due to $CH_3(CH_2)_n$, but also the carbonyl carbon due to esters at 170.397 and 172.635 ppm. There is also at least one free fatty acid, as indicated by the carbonyl carbon at 181.573 ppm. Finally, there are also signals due to HC=CR carbons and protons due to aromatic compounds in the ^1H and $^{13}C\{^1H\}$-NMR spectra.

As shown in the Supplementary Material, analysis by HPLC supports the hypothesis that there are aromatic compounds.

Out of the 41 individuals selected to participate in the study, three were eliminated from Group 1 (two reported unpleasant taste when using PFPF, and one initiated the use of lipid-lowering drugs during the study). In Group 2, two patients were eliminated (one initiated the use of lipid-lowering drugs, and one missed subsequent appointments). Thirty-six patients remained in the study and were evaluated. Table 2 describes the characteristics of the patients. The statistical analysis demonstrated no difference between the groups according to the evaluated variables. The average values for TC, LDL-C, HDL-C, and triacylglycerides are presented in Tables 3 and 4 for patients in Group 1 and 2, respectively.

The increase in HDL-C, in the group using PFPF during 90 days averaged 5.9 ± 10.7 mg/dl, which corresponded to 13.4% of the initial value. On the other hand in Group 2, the increase averaged 1 ± 10.4 mg/dl and corresponded to 2.3% of the initial value.

The average value (Table 3) for basal total cholesterol detected in Group 1 (227.3 ± 42.9 mg/dl) is considered a borderline value according to the NCEP III (2003), however, after 90 days of intervention with PFPF, this value decreased to 192.1 ± 45.2 mg/dl, which is classified as optimal. This reduction was highly significant ($p = 0.001$) and already noticed as a significant decrease 30 days after the intervention ($p = 0.007$). The LDL concentrations in individuals who used PFPF during 30 and 60 days showed discrete and non-significant reduction. A significant LDL-C reduction was only observed after 90 days of intervention ($p = 0.0082$).

A discrete and not significant elevation in the HDL-C concentrations were observed in individuals who used PFPF during 30 days; however, a significant increase was observed after 90 days of its use ($p = 0.0294$). There were no significant changes in the HDL-C concentrations in Group 2 throughout the intervention period.

The plasma triacylglycerides concentrations decreased significantly ($p = 0.034$) after 30 days of PFPF consumption together with diet therapy counseling. However, there was no further significant reduction afterwards, showing that PFPF assisted in reducing the levels of triacylglycerides in just the first 30 days and maintaining this lower level throughout the study.

Table 2
Effects of PFPF on the plasma concentrations of cholesterol, LDL-C, HDL-C and TG in HIV patients with lipodystrophy syndrome secondary.

Variable	Group 1		Group 2		*p* value
	Average	SD	Average	SD	
Age (years)	46.0	8.4	46.6	7.0	0.83
Time since diagnosis (years)	9.1	4.9	11.0	5.4	0.28
Duration of HAART (years)	8.2	4.5	10.0	5.0	0.25
Weight (kg)	59.7	10.1	57.5	10.4	0.52
BMI (kg/m^2)	23.3	2.8	22.1	3.5	0.28

There were 12 men and 6 women in each group. The data obtained were tabulated and analyzed using the Biostat 5.0 program (2007) with a significance level of $p \leq 0.05$. The dietpro program (Esteves and Monteiro, 2002) was used for the evaluation of R24 h. The bilateral *t*-test was used for the verification of homogeneity in the observed values between the groups. The paired *t*-test was used to assess the variation in the results from laboratory tests.

Table 3
Averages and standard deviations and differences between the average values of the evaluated parameters during the intervention in Group 1 (which received diet therapy counseling and 30 g/day of PFPF). Comparison between the basal values and values observed in other periods throughout the study.

	Evaluation		Difference between averages (SD)		
	Average	SD	T0/T30	T0/T60	T0/T90
TC					
T0	227.3	42.9	26.8 (40.8)	34.5 (27.4)	35.2 (39.5)
T30	200.6	51.2	$p = 0.007$[a]	$p < 0.0001$[a]	$p = 0.001$[a]
T60	192.8	45.5			
T90	192.1	45.2			
LDL-C					
T0	125.8	33.1	13.2 (36.4)	14.5 (32.6)	32.3 (34.72)
T30	112.6	47.7	$p = 0.14$	$p = 0.1$	$p = 0.0082$[a]
T60	111.3	42.6			
T90	93.5	45.8			
HDL-C					
T0	43.9	12.2	−1.1 (9.2)	0.6 (9.7)	−5.9 (10.7)
T30	44.8	16.9	$p = 0.33$	$p = 0.4$	$p = 0.0294$[a]
T60	43.1	13.9			
T90	49.7	19.1			
TG					
T0	323.9	114.4	68.6 (140.3)	97.7 (149.1)	96.47 (164.6)
T30	255.2	115.2	$p = 0.034$[a]	$p = 0.0078$[a]	$p = 0.0140$[a]
T60	226.1	118.4			
T90	227.4	139.3			

TC, total cholesterol; TG, triacylglycerides.

[a] *p*-value < 0.05. The data obtained were tabulated and analyzed using the Biostat 5.0 program (2007) with a significance level of $p \leq 0.05$. The dietpro program (Esteves and Monteiro, 2002) was used for the evaluation of R24 h. The bilateral *t*-test was used for the verification of homogeneity in the observed values between the groups. The paired *t*-test was used to assess the variation in the results from laboratory tests.

Table 4
Averages and standard deviation and differences between the average values of the evaluated parameters during the intervention in Group 2 (which received diet therapy counseling only). Comparison between the basal values and values observed in other periods throughout the study.

	Evaluation		Difference between averages (SD)		
	Avg	SD	T0/T30	T0/T60	T0/T90
TC					
T0	234	59.7	8.8 (36.2)	7.0 (26.3)	4.83 (35.4)
T30	226	56.7	$p = 0.16$	$p = 0.14$	$p = 0.28$
T60	228	55.3			
T90	230	50.1			
LDL-C					
T0	143	52.6	16.9 (49.5)	18.5 (51.8)	12.7 (34.0)
T30	126	51.6	$p = 0.11$	$p = 0.10$	$p = 0.09$
T60	125	45.8			
T90	131	42.5			
HDL-C					
T0	43.6	16.5	−0.6 (10.6)	2.77 (7.8)	−1 (10.4)
T30	44.2	17.3	$p = 0.40$	$p = 0.09$	$p = 0.35$
T60	40.9	14.9			
T90	44.6	17.7			
TG					
T0	294	120.4	−16.2 (131.4)	5.33 (72.3)	−7.94 (118.9)
T30	310	137.4	$p = 0.30$	$p = 0.38$	$p = 0.39$
T60	288	115.9			
T90	302	153.6			

TC, total cholesterol; TG, triacylglycerides.
The data obtained were tabulated and analyzed using the Biostat 5.0 program (2007) with a significance level of $p \leq 0.05$. The dietpro program (Esteves and Monteiro, 2002) was used for the evaluation of R24 h. The bilateral *t*-test was used for the verification of homogeneity in the observed values between the groups. The paired *t*-test was used to assess the variation in the results from laboratory tests.

Table 5 shows the evaluation of effects from the consumption of macronutrients and dietary fibers, constituents of the diet used in the study, before and after intervention. There was no difference in the constituents of the diet between the groups, this confirming the effects of the PFPF in the obtained results.

Table 5
Average and standard deviation of the consumption levels of the main food components, before and after intervention, in Groups 1 and 2.

Nutrients	Group 1		Group 2		*p*-value
	Average	SD	Average	SD	
Calories (kcal)					
Before	2190	688	2265	830	0.77
After	1883	499	1804	670	0.69
Saturated fat (g)					
Before	16.9	7.9	16.0	6.7	0.70
After	13.3	7.0	14.5	13.7	0.74
Monounsaturated fat (g)					
Before	16.5	7.4	18.5	9.2	0.47
After	12.4	8.0	14.3	10.3	0.56
Polyunsaturated fat (g)					
Before	11.8	9.1	16.7	15.5	0.25
After	5.9	4.7	5.7	4.4	0.87
Cholesterol (mg)					
Before	354.6	304	383	327	0.79
After	184.7	120	221	191	0.50
Fibers (g)					
Before	32.7	55.5	29.6	20.7	0.48
After	36.6	56.4	32.7	7.1	0.62

The data obtained were tabulated and analyzed using the Biostat 5.0 program (2007) with a significance level of $p \leq 0.05$. The dietpro program (Esteves and Monteiro, 2002) was used for the evaluation of R24 h. The bilateral *t*-test was used for the verification of homogeneity in the observed values between the groups. The paired *t*-test was used to assess the variation in the results from laboratory tests.

Discussion

To the best of our knowledge, this is the first report that describes the FTIR, UV–Vis and NMR analysis of PFPF. Hot, pressurized, dry methanol was chosen as the solvent to extract the PFPF, because previous studies have shown that it is better than ultrasound, Soxhlet extraction or other methods for solubilizing solids in fruits (Richter et al., 1996; Richards et al., 2014). Dry methanol at 100 °C and 10 MPa (100 atm) pressure in an accelerated solvent extractor was able to solubilize D-fructose, fatty acid glycosides and other compounds from a variety of fruits (Richards et al., 2014). D-fructose produced more than six signals, because it exists as a mixture of three isomers in methanol: β-fructopyranose, α-fructofuranose and β-fructofuranose (Richards et al., 2014). Fatty acid glycosides have been shown to have important neuromodulatory effects (Akihisa et al., 2007; Li et al., 2008; Smith, 2015).

The FTIR spectrum had a large, broad peak from about 3000 to 3500 cm^{-1}. This indicates the presence of hydroxyl groups (Smith, 2014). There were also peaks from 1650 to 1780, and 1600 to 1650 cm^{-1} due to C=O and C=O bonds, respectively (Smith, 2014). The UV spectrum had maximum absorbance (λ_{max}) at 205 and 270 nm. The λ_{max} at 270 nm indicates the presence of one or more aromatic compounds. The ^{1}H and $^{13}C\{^{1}H\}$-NMR spectra contained signals from about 3.0 to 5.4 ppm and 64 to 110 ppm, respectively, due to CHO and CH_2O groups in carbohydrates. Fructose is the only simple sugar that dissolves in methanol. However, PFPF does not taste sweet and there were several signals that fructose is known to produce in ^{13}C-NMR spectra (Richards et al., 2014) that were not seen in the spectrum. Also, there were signals from 0.6 to 1.0 ppm and 14.5 to 34 ppm in the ^{1}H and $^{13}C\{^{1}H\}$-NMR spectra due to the CH_3 and $(CH_2)_n$ of fatty acyl groups. This supports the hypothesis that PFPF contains fatty acid glycosides. There were signals from about 6.8 to 8.2 ppm and 128 to 136 ppm in the ^{1}H and $^{13}C\{^{1}H\}$-NMR spectra due to C=C and HC=C bonds. This is consistent with the UV spectrum that showed the presence of aromatic compounds. Finally, there were signals in the $^{13}C\{^{1}H\}$-NMR spectrum from about 171 to 182 ppm due to C=O bonds in esters and carboxylic acids. The FTIR spectrum is a good fingerprint of the PFPF, while the UV and NMR spectra were also quite informative.

A clinical trial was conducted in women presenting dyslipidemia (Ramos et al., 2007) using the same 30 g/day dose of PFPF that was used in the present study. Similar reductions in the concentrations of cholesterol in the blood were seen (Ramos et al., 2007). A reduction of 31.7 ± 28.0 mg/dl in this value was observed after 30 days of intervention and 47.0 ± 29.5 mg/dl after 60 days (Ramos et al., 2007). Both were statistically significant, with $p = 0.0001$ and $p = 0.0000$, respectively (Ramos et al., 2007).

In another open clinical trial was conducted on 36 healthy adults of both genders, who were instructed to eat 10 g of PFPF three times a day, daily for during eight weeks (Medeiros et al., 2009). A significant reduction in total cholesterol concentrations in the blood (17.0% for women and 19.5% for men) was observed (Medeiros et al., 2009).

Elevated total cholesterol is a risk factor for developing cardiovascular disease (Smith, 2015). This has become more frequent among HIV+ individuals who receive HAART drug therapy. Cardiovascular diseases are the leading cause of death in the US, but their incidence can be lowered by taking drugs like statins that reduce blood cholesterol levels (Smith, 2015), and by consuming more dietary fiber (Brown et al., 1999). The National Cholesterol Education Program in the USA (2002) estimated that for every 1% reduction in cholesterol concentration in the blood, a decrease of 2% in the risk of cardiovascular diseases could be observed.

PFPF may improve the concentrations of cholesterol, LDL-C, HDL-C and triacylglycerides in several ways. First, it can make one feel satiated and eat less. Moreover, dietary fiber can improve the

composition of healthy bacteria in the gut, with concomitant health effects. Finally, the fatty acid glycosides may be able to boost the immune system, reduce smoldering inflammation, which can help prevent cardiovascular and neurodegenerative diseases as well as cancer (Smith, 2015).

The reduction of LDL-C observed in this study is similar to the results observed by others (Ramos et al., 2007) who saw a decrease of 33.6 ± 33.3 mg/dl in the LDL-C ($p = 0.0003$) when introducing 6 g of soluble fiber in the usual diet of 52 women. One study evaluated the introduction of 14 g of fiber per day in women living in a community in Fort Collins and there was an 8% reduction in the amounts of LDL-C after 90 days of follow-up, lower than the present study that the decrease was on average 32.3 ± 34.7 mg/dl in group 1 which equals 25.6% (Davy et al., 2002). A meta-analysis was performed on the effects of fiber intake on cholesterol levels and concluded, that the increase in soluble fiber in the diet, produce significant results on the LDL-C values (Brown et al., 1999).

LDL-C is the primary lipoprotein in the plasma, acts as a cholesterol transporter and seems to be the most atherogenic of all (Goldstein and Brown, 1977). The deposition of cholesterol is a consequence of oxidation of circulating cholesterol-rich lipoproteins, in particular LDL-C (Steinber, 1997; Cobbold et al., 2002). Thus, the NCEP has identified this lipoprotein as the main target in the treatment of dyslipidemia (NCEP, 2002). The increase in LDL-C levels promotes atherogenesis however, the restoration of the ideal serum levels reduces the risk of atherosclerotic cardiovascular disease significantly even in patients who already present advanced pathology (Grundy et al., 2004; Tabas et al., 2007). The reduction of 39 mg/dl in this lipoprotein level reduces in 30% the relative risk for developing coronary artery disease (Grundy et al., 2004).

It was found that adults of both sexes who received 30 g of PFPF daily for eight weeks had significantly lower average HDL-C concentrations at the end of the study compared to the beginning (Janebro et al., 2008). This pattern was also observed in the present study, in both fourth and eighth week. The values reached the normal range for both women and men in this latest evaluation, and the respective averages were 53.50 ± 10.8 mg/dl and 43.2 ± 8.13 mg/dl (Janebro et al., 2008).

The results from the *Veterans Affairs High-Density Lipoprotein Intervention Trial* reinforced the idea that elevated HDL-C can reduce the risk of coronary disease (Rubins et al., 2000). In this study, the 22% reduction in deaths from coronary events or non-fatal myocardial infarctions was correlated to the reduction in cholesterol and triacylglycerides levels, by 4% and 31%, respectively, and in the increase of HDL-C by 6%, without significant changes in LDL-C (Rubins et al., 2000). It should be emphasized that in the present study, the HDL-C in the group using PFPF increased from 43.9 ± 12.2 mg/dl at day zero to 49.7 ± 19.1 after 90 days (Table 3). On the other hand HDL-C changed from 43.6 ± 16.5 to 44.6 ± 17.7 mg/dl after 30 days.

Despite the fibers not interfering directly in the HDL-C levels, this lipoprotein varies in a reversed way with the triacylglycerides (Vega et al., 2014). The reduction in lipolysis of the lipoproteins rich in triacylglycerides lowers the levels of available substrate for the maturation of HDL-C (de Man et al., 2003). In addition, the enrichment of HDL-C with triacylglycerides increases the catabolic rate and therefore, reduces its plasma concentration. Also, the lipid exchange between HDL-C and the lipoproteins rich in triacylglycerides are reduced leading to a faster disappearance of the plasma HDL-C (Genest et al., 2006). For each 1% increase in HDL-C there was a 1% reduction in coronary events regardless of the variation of LDL-C, because HDL-C promotes the esterfication of cholesterol and protects the arteries due to the reverse transport of cholesterol. This promotes the withdrawal of cholesterol from the tissues peripheral to the liver where it is excreted in the bile (Abbott et al., 1988; Barter, 2004; Daminelli et al., 2008).

In agreement with the present study, 43 volunteers who received 30 g/day PFPF experienced a significant reduction in triacylglycerides after 8 weeks using this flour. The average values fell from 212.0 ± 119.3 to 161.2 ± 91.1 mg/dl (Janebro et al., 2008).

Hypertriglyceridemia is an independent risk factor for atherogenesis, stroke, and acute myocardial infarction. It was observed in a meta-analysis with seventeen prospective epidemiological studies that every increase of 89 mg/dl in the triglyceridemia led to an increase in the risk of cardiovascular disease of 32% and 76% in men and women, respectively (Abbott et al., 1988). The higher the triglyceridemia, the greater the formation of LDL-C type B, which are smaller and denser. This is important because small and dense LDL-C particles may be more susceptible to oxidative modification. Also, an increased number of atherogenic particles may adversely influence cardiovascular risk (Passareli et al., 2007; Miller et al., 2011).

Conclusion

It was concluded that the use of 30 g of passion fruit peel flour during 30 days together with diet therapy counseling was effective in reducing cholesterol and triacylglycerides levels. PFPF treatment showed good effects against lipodystrophy in HIV patients treated for 90 days. The use of this product for 90 days was effective in reducing the LDL-C, and increasing the HDL-C values in individuals presenting HIV Lipodystrophy syndrome and dyslipidemia. PFPF was found to contain methanol-soluble fatty acid glycosides and aromatic compounds when analyzed by NMR. FTIR and UV–Vis spectra are also useful in establishing a "fingerprint" of PFPF.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors' contributions

SSFM, RMFL and AUOSS designed the experiments and carried them out. SSFM wrote the first draft. RMFL is her advisor. She conceived the project, and did the statistical analyses. RES added more background information, rewrote the paper and submitted it and revised it based on reviewers' comments. TD did the methanol extractions. PS and SH acquired the FTIR and UV–Vis spectra and analyzed them. RL did the NMR analyses.

Conflicts of interest

The authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bjp.2016.03.002.

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