

Beneficial effects of consumption of acerola, cashew or guava processing by-products on intestinal health and lipid metabolism in dyslipidaemic female Wistar rats

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Abstract

This study assessed the effects of diet supplementation with industrial processing by-products of acerola (*Malpighia emarginata* D.C.), cashew (*Anacardium occidentale* L.) and guava (*Psidium guajava* L.) fruit on the intestinal health and lipid metabolism of female Wistar rats with diet-induced dyslipidaemia. Female rats were randomly divided into five groups: healthy control, dyslipidaemic control and dyslipidaemic experimental receiving acerola, cashew or guava processing by-products. Fruit processing by-products were administered (400 mg/kg body weight) via orogastric administration for 28 consecutive days. Acerola, cashew and guava by-products caused body weight reduction (3.42, 3.08 and 5.20%, respectively) in dyslipidaemic female rats. Dyslipidaemic female rats receiving fruit by-products, especially from acerola, presented decreased faecal pH, visceral fat, liver fat and serum lipid levels, as well as increased faecal moisture, faecal fat excretion, faecal *Bifidobacterium* spp. and *Lactobacillus* spp. counts and amounts of organic acids in faeces. Administration of the tested fruit processing by-products protected colon and liver from tissue damage (e.g. destruction of liver and colon cells and increased fat deposition in hepatocytes) induced by dyslipidaemic diet. Dietary fibres and phenolic compounds in tested fruit by-products may be associated with these positive effects. The industrial fruit processing by-products studied, mainly from acerola, exert functional properties that could enable their use to protect the harmful effects on intestinal health and lipid metabolism caused by dyslipidaemic diet.

Key words: By-products: Dyslipidaemia: Fibres: Intestinal microbiota

Excessive intake of dietary *trans*-fat, saturated fat and cholesterol induces changes in lipid metabolism and leads to dyslipidaemia, which is one of the most prevalent chronic non-communicable diseases worldwide. Dyslipidaemia results from changes in lipid metabolism, being characterised by increased plasma levels of total cholesterol (TC), LDL and TAG and reduced levels of HDL. These may or may not occur simultaneously⁽¹⁾. Furthermore, excessive dietary fat may lead to the accumulation of fat in the liver (liver steatosis)⁽²⁾ that

predisposes to the development of non-alcoholic fatty liver disease (NAFLD)⁽³⁾ and atherosclerotic CVD (ACD)^(4,5).

Regular ingestion of fruit and dietary fibre (DF) has been recommended as primary prevention or treatment strategy for dyslipidaemia^(6,7), NAFLD and ACD^(8,9). Acerola (*Malpighia emarginata* D.C.), cashew (*Anacardium occidentale* L.) and guava (*Psidium guajava* L.) are tropical fruits consumed 'in natura' or processed to produce mostly juices and frozen pulp^(10–12). Agro-industrial processing of these fruits generates a

Abbreviations: DC, non-treated dyslipidaemic control; DEA, dyslipidaemic group supplemented with acerola by-product; DEC, dyslipidaemic group supplemented with cashew by-product; DEG, dyslipidaemic group supplemented with guava by-product; DF, dietary fibre; HC, healthy control; IDF, insoluble dietary fibre; SDF, soluble dietary fibre; TC, total cholesterol.

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large amount of by-products (such as, peel, seeds and flesh) that if inappropriately discarded cause damage to the environment, requiring high costs to reduce their negative impacts⁽¹³⁾.

Use of fruit industrial processing by-products in the human diet should be an interesting alternative destination because some of these materials contain high amounts of DF, as observed in by-products generated from acerola (37.32–80.42 g/100 g dry basis)⁽¹⁴⁾, cashew (76.20 g/100 g dry basis)⁽¹⁵⁾ and guava (88.7 g/100 g dry basis) processing⁽¹⁶⁾. In addition, high amounts of total phenolics have been observed in processing industrial by-products of acerola (7265.29 mg gallic acid equivalents (GAE)/100 g dry basis), cashew (6588.41 mg GAE/100 g dry basis) and guava (1987.19 mg GAE/100 g dry basis)⁽¹⁷⁾. Consumption of DF may modulate the composition of intestinal microbiota and fermentation activity of beneficial bacteria (e.g. *Bifidobacterium* and *Lactobacillus*) found there^(18,19), increasing the production of SCFA that contribute to the maintenance of intestinal health⁽²⁰⁾. Phenolic compounds have been also associated with positive effects on intestinal microbiota composition and SCFA production^(21–23). SCFA are saturated aliphatic organic acids exerting positive effects on intestinal and liver health and function, as well as on the regulation of lipid metabolism^(24–26).

This study evaluated whether the consumption of by-products generated from industrial processing of acerola, cashew and guava can affect selected parameters that indicate the intestinal health and lipid metabolism of female Wistar rats with induced dyslipidaemia.

Methods

Acquisition and preparation of fruit processing by-products

By-products, mostly composed of mashed peels and seeds and small amount of flesh, generated from acerola, cashew and guava industrial processing were obtained from a fruit pulp processing industry (Polpa Ideal Indústria Ltda). The by-products were homogenised after freezing in liquid N₂ and freeze-dried (−40°C, vacuum pressure <150 μmHg) for approximately 12 h in a bench lyophilizer model L-101 (LIO-TOP). The freeze-dried material obtained was ground in a domestic blender, sieved through a 1.0-mm mesh and stored (−10°C) in glass containers under light protection.

Moisture, ash, protein and lipid content and the total energy value⁽²⁷⁾ of these freeze-dried by-products were analysed in triplicate; total, soluble (SDF) and insoluble DF (IDF), respectively, were measured using an enzymatic-gravimetric method⁽²⁸⁾, and fructan content was measured by enzymatic hydrolysis⁽²⁷⁾. Organic acids, sugars and phenolic contents were determined by HPLC under analytical conditions described elsewhere⁽²⁹⁾. Nutritional composition and amounts of different phenolic compounds in freeze-dried acerola, cashew and guava processing by-products used in this study are shown in Table 1.

Animals

In all, forty female adult Wistar rats, approximately 90 d old, were maintained individually in metabolic cages with water and food *ad libitum* at 21 ± 1°C, relative humidity in the range of 50–55% and alternating 12 h light–12 h dark cycles. The

experimental protocol used in this study was approved by a Committee on Ethics in Animal Experimentation (Center of Biotechnology, Federal University of Paraíba, João Pessoa, Brazil) under the no. 0505/14. All experiments were performed in agreement with the guidelines of Brazilian College for Animal Experimentation (COBEA, Brazil).

Experimental design

After an acclimatisation period of 1 week, the female Wistar rats were randomly put into five groups: a healthy control group (HC, *n* 8), which received saline solution via gavage and was fed on an AIN 93M diet⁽³⁰⁾ (Rhostrer) throughout the experiment; a dyslipidaemic control group (DC, *n* 8), which received saline solution via gavage and was fed on a dyslipidaemic diet (Rhostrer), containing 6% lard, 5% non-hydrolysed vegetable fat, 1% cholesterol and 0.5% cholic acid (online Supplementary Table S1), throughout the experiment; and three dyslipidaemic experimental groups that were fed a dyslipidaemic diet throughout the experiment and received supplementation with processing by-products of acerola (DEA, *n* 8), cashew (DEC, *n* 8) or guava (DEG, *n* 8).

Initially, the female rats forming the dyslipidaemic groups (DC, DEA, DEC and DEG) had undergone previous treatment by consuming a dyslipidaemic diet for 2 weeks⁽³¹⁾. After this period, the detection of dyslipidaemia was performed through biochemical examination of the lipid profile in all groups (online Supplementary Table S2). Afterwards, the female rats forming HC and DC groups received a saline solution via gavage for 28 consecutive days, and those forming the DEA, DEC and DEG groups received acerola, cashew or guava processing by-product at a dose of 400 mg/kg body weight (fruit by-products were diluted (1.6%, w/v) in saline solution), respectively, via gavage⁽³²⁾ for the same time period. Gavage was performed twice a day at 4-h intervals in the morning and afternoon periods. Body weight and food consumption were assessed weekly.

On day 29 of treatment, the animals were fasted for 12 h and then anaesthetised by intraperitoneal injection of 1 ml of ketamine hydrochloride (75 mg) and 1 ml of xylazine hydrochloride (5 mg) per kg body weight. Following the euthanasia of animals via aortic transection, visceral fat samples were collected and weighed. Colon and liver were removed for histological analysis and faeces were collected from caecum for microbiological counts and quantification of organic acids and total fat. Serum was collected and maintained at room temperature (25 ± 1°C) for determination of lipid profile.

Determination of lipid profile

Serum concentrations of TC and HDL were measured using the Trinder enzymatic method and the accelerator selective detergent method using Liquiform Cholesterol and HDL LE kits, respectively (Labtest). TAG levels were determined using the Trinder method with a TAG Liquiform kit (Labtest). All analyses followed the manufacturer's recommendations, and absorbance was determined using a LabMax 240 Premium automatic analyser (Labtest) at 505 nm (TAG), 500 nm (TC) or 600 nm (HDL). LDL and VLDL values were calculated using previously described equations⁽³³⁾, as follows: LDL = TC − HDL − TAG/5; and VLDL = TAG/5.



Fat in liver and moisture, pH, fat and microbial counts in faeces

For three consecutive days, that is, on days 26, 27 and 28 of treatment, faecal samples were collected and stored at -20°C . For analysis, the samples were diluted in deionised water (1 mg/ml) and faecal pH was measured using a digital potentiometer (Q400AS; Quimis)⁽³⁴⁾. A separate portion of faecal sample was dried in an oven (320-SE; Fanem) at 105°C for 24 h to determine faecal moisture^(27,35).

Another portion of faecal samples collected from caecum was diluted (1:9) in sterile peptone water and inoculated (20 μl), using the microdrop technique⁽³⁶⁾, on selective agar for counting *Lactobacillus* spp. (de Man, Rogosa and Sharpe (MRS); HiMedia), *Bifidobacterium* spp. (*Bifidobacterium* agar; HiMedia) and Enterobacteriaceae (MacConkey agar; HiMedia). Agar plates for counting *Lactobacillus* spp. and *Bifidobacterium* spp. were incubated under anaerobic conditions (Anaerobic System Anaerogen; Oxoid Ltd) and agar plates for counting Enterobacteriaceae were incubated under aerobic conditions, all at 37°C for 24–48 h. At the end of the incubation period, characteristic colonies on the selective media were counted, and the results were expressed as log of colony-forming units per g of faeces (\log_{10} CFU/g)⁽³⁷⁾. Total lipid in faeces and liver was determined by cold extraction⁽³⁸⁾.

Quantification of organic acids in faeces

Contents of acetic, butyric, propionic, formic, citric, lactic and malic acids in faeces were quantified by HPLC using a 1260 Infinity LC system (Agilent Technologies) coupled to a PDA detector (G1315D; Agilent Technologies). For analysis, an Agilent Hi-Plax H (300 \times 7.7 mm) column with 8.0 μm particle size was used; the column was protected with a PL Hi-Plax H (5 \times 3 mm) guard column (Agilent Technologies). Column temperatures were maintained at 50°C . Each sample was diluted in ultrapure water filtered through a 0.45- μm -pore membrane, with an injection volume of 10 μl , at a flow rate of 0.5 ml/min and a runtime of 20 min. The phase was 4.0 mm H_2SO_4 in ultrapure water. Obtained data were processed using Open LAB CDS Cessation Edition (Agilent Technologies). HPLC sample peaks were identified by comparing their retention times with those of organic acid standards⁽³⁹⁾. Duplicate injections were performed, and average peak areas were used for quantification. Standard of formic acid was obtained from Sigma-Aldrich, and standards of acetic, butyric, propionic, citric, lactic and malic acids were obtained from Vetec, all with a purity of $\geq 99\%$. Ultrapure water was obtained from a Mille[®] system (EMD Millipore), and sulphuric acid was obtained from Merck.

Histopathological evaluation of the intestine and liver

Colon and liver fragments were excised, washed in saline solution (0.9% NaCl), fixed in 10% buffered formalin for 48 h and subjected to histological processing. Samples were dehydrated in an increasing ethanol series, clarified in xylene, embedded in paraffin and sectioned in a microtome to obtain 5 μm -thick slices. Sections were stained with haematoxylin–eosin (H&E) and periodic acid–Schiff (PAS) and analysed under a light microscope (Motif BA 200) at 4 \times , 10 \times , 20 \times and 40 \times magnifications. Morphological analysis

of the intestine included evaluation of inflammatory processes presence, such as stasis, leucocyte migration, haemorrhage, vasodilation and necrosis, as well as evaluation of epithelial preservation, hypertrophy and hyperplasia of the outer muscular layer. The morphological analysis of liver included evaluation of the occurrence of degenerative processes by fatty degeneration and inflammatory parameters, including leucocyte migration, oedema, hyperaemia, haemorrhage, necrosis, preservation of liver parenchyma and presence of microthrombi.

Statistical analysis

Statistical power of 0.80 (80%) was obtained by estimating forty adult female Wistar rats (eight females per group) when the minimally detectable effect size was 1.0 and the significance level was 0.05. Food intake, body weight and lipid intake data were compared by a two-way ANOVA for independent measures with a factor for group (five levels) and a factor for time (four or five levels). Other data with single factor for group were analysed via one-way ANOVA. When there was a difference between the obtained data, Tukey's *post hoc* test was performed at a significance level of 5% ($P \leq 0.05$). Results are expressed as the means and standard deviations. The software SigmaPlot 12.5 for Windows (Systat Software Inc.) was used to perform the statistical analysis and graphic design.

Results and discussion

Food intake and body weight

Food intake and body weight of the HC, DC, DEA, DEC and DEG groups were monitored during the 28-d experimental period (online Supplementary Fig. S1(a) and S1(b)). The HC group presented the highest food consumption during the monitored period ($P \leq 0.05$) when compared with the other groups. DEA, DEC, DEG and DC groups presented similar food consumption over time ($P > 0.05$), with exception of the DEA group that presented lower food consumption than the HC group during the 1st week of the experimental period ($P \leq 0.05$). These results indicate that the dyslipidaemic diet consumption promoted greater satiety^(40,41) in the DEA, DEC, DEG and DC groups. The fruit by-products tested had little influence on the appetite of the animals, probably owing to the lower energy value provided by DF, which was detected in high amounts in these materials. These findings are similar to those found in a previous study that administered different amounts of prebiotic fibres in diet, and observed no difference in diet intake among the groups of animals receiving these fibres⁽⁴²⁾.

Although consuming a high-lipid and high-energy diet (online Supplementary Table S1 and Supplementary Fig. S1(c)), the DC group showed body weight gain similar to HC group ($P > 0.05$), which could be related to a lower dyslipidaemic diet intake by the DC group during the experimental period. Short-term dietary fat consumption is associated with satiety-inducing intestinal peptides expression, such as cholecystokinin, glucagon-like peptide-1, pancreatic polypeptide, peptide YY and oxymomodulin. Interestingly, these effects are attenuated with long-term dietary fat consumption^(40,41).

DEA, DEC and DEG groups showed body weight loss during the monitored experimental period ($P \leq 0.05$). Body weight loss among these groups followed the order $DEG > DEA > DEC$. Although the DEA, DEC and DEG groups consumed an amount of dyslipidaemic diet similar to the DC group, the DF of the fruit by-products contributed to the weight loss of the animals, as the consumption of the tested fruit by-products may have facilitated greater faecal excretion of dietary fat, implying i) lower energy absorbed and metabolised; ii) increased postprandial energy expenditure by favouring greater gastrointestinal motility; and iii) increased excretion of bile acids, inducing the mobilisation of body fat reserves for the bile acids hepatic synthesis⁽⁴³⁾. A previous study found that high-fat-diet consumption resulted in increased body weight in rats over time, whereas the same high-fat diet to which had been added 10% dehydrated guava reduced rats' body weight gain⁽⁴⁴⁾. Consumption of acerola juice has shown to be effective in preventing body weight gain in mice fed a cafeteria diet⁽⁴⁵⁾.

Effects on lipid profile

The DC group exhibited higher TC, TAG and LDL levels compared with the HC, DEA, DEC and DEG groups ($P \leq 0.05$) (Fig. 1(a), (b) and (d)). The DEA and DEG groups presented lower VLDL levels compared with the DC group ($P \leq 0.05$) (Fig. 1(e)). The DEA and DEC groups presented increases in HDL levels (Fig. 1(c)) by 104.96 and 40.74%, respectively, compared with the DC group ($P \leq 0.05$).

An increase in HDL levels is associated with increased protection against atherosclerosis, whereas an increase in LDL levels is associated with increased atherosclerosis risks. Similarly, high cholesterol and VLDL serum levels indicate a higher atherogenic risk^(7,46). Considering the results of this study, consumption of acerola, cashew and guava by-products, particularly the acerola by-product, was effective in preventing changes in lipid metabolism induced by the dyslipidaemic diet.

Improvement in lipid profile of dyslipidaemic animals receiving fruit processing by-products could be related to the fibre content and phenolic compounds (Table 1) found in these materials. SDF increases intraluminal viscosity, affects the enterohepatic circulation of bile acids and reduces fat absorption⁽⁴⁷⁾. Phenolic compounds increase bile acids excretion and inhibit fat and cholesterol intestinal absorption by modulating expression and interacting with the intestinal cholesterol transporter Niemann-Pick C1-Like 1, which prevent the absorption of dietary cholesterol^(48,49). Excretion of bile acids in faeces induces the production of bile via cholesterol in the liver, lowering cholesterol plasma levels and cholesterol-transporting lipoproteins⁽⁵⁰⁾.

Effects on pH, moisture, organic acids and microbial counts in faeces

The DC group showed a higher faecal pH than the HC group ($P \leq 0.05$) (Table 2). Faecal pH was lower in the DEA, DEC and DEG groups compared with the DC group; this suggests

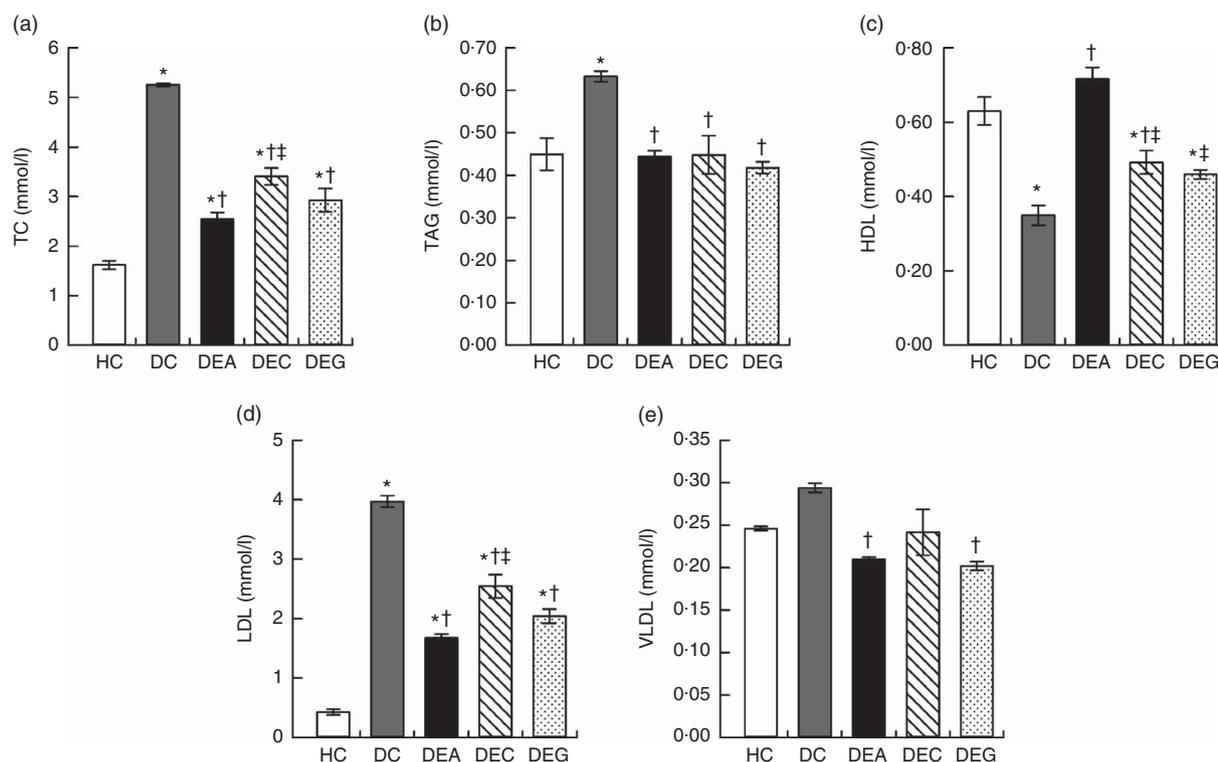


Fig. 1. Total cholesterol (a), TAG (b), HDL (c), LDL (d) and VLDL (e) of the healthy control animals (HC), non-treated dyslipidaemic control animals (DC) and dyslipidaemic animals that received acerola (DEA), cashew (DEC) or guava (DEG) industrial processing by-products. Values are means and standard deviations represented by vertical bars (one-way ANOVA, $P \leq 0.05$, Tukey's *post hoc* test). * Significant difference compared with the HC group, † significant difference compared with the DC group, ‡ significant difference compared with the DEA group.

Table 1. Nutritional composition and phenolic compounds determined in freeze-dried acerola, cashew and guava industrial processing by-products (Mean values and standard deviations)

Parameters	By-product					
	Acerola		Cashew		Guava	
	Mean	SD	Mean	SD	Mean	SD
Moisture (g/100 g)	3.30 ^c	0.10	6.28 ^b	0.28	9.78 ^a	0.17
Ash (g/100 g)	3.08 ^b	0.20	2.22 ^c	0.10	3.56 ^a	0.21
Protein (g/100 g)	7.18 ^c	0.10	9.90 ^b	0.01	13.78 ^a	0.19
Lipid (g/100 g)	2.45 ^b	0.09	2.15 ^c	0.00	9.27 ^a	0.13
Simple sugars (g/100 g)						
Fructose	9.64	0.02	18.96	0.02	8.66	0.00
Glucose	9.78	0.02	17.74	0.01	15.96	0.01
Maltose	ND	–	1.46	0.00	ND	–
Dietary fibre (g/100 g)						
Fructan	1.40 ^a	0.01	1.06 ^b	0.02	ND	–
IDF	34.35 ^b	0.29	27.01 ^c	0.05	40.62 ^a	0.14
SDF	14.22 ^a	0.10	7.98 ^b	0.20	3.74 ^c	0.09
TDF	48.57 ^a	0.39	34.99 ^c	0.25	44.36 ^b	0.23
IDF:SDF ratio	2.42	–	3.39	–	10.86	–
Total energy value (kJ)	497.27	–	568.48	–	703.04	–
Total energy value (kcal)	118.85	–	135.87	–	168.03	–
Organic acids (µmol/g)						
Acetic	43.63	0.00	12.99	0.00	9.99	0.00
Citric	ND	–	4.48	0.00	5.10	0.02
Malic	37.29	0.00	19.39	0.00	9.99	0.03
Tartaric	ND	–	4.53	0.00	ND	–
Phenolic compounds (mg/100 g of dry weight)						
Flavanol						
Catechin	125.20 ^a	0.09	67.00 ^b	0.03	4.40 ^c	0.08
Flavanone						
Hesperetin	9.00 ^c	0.01	12.40 ^a	0.01	9.60 ^b	0.01
Naringenin	23.60 ^b	0.20	40.60 ^a	0.11	18.40 ^c	0.05
Flavone						
Chrysin	8.80 ^a	0.04	2.00 ^c	0.02	2.20 ^b	0.01
Flavonol						
Kampferol	42.20 ^a	0.01	ND	–	ND	–
Myricetin	94.20 ^a	0.01	91.80 ^b	0.04	44.60 ^c	0.01
Quercetin	50.80 ^a	0.41	19.80 ^c	0.02	33.80 ^b	0.22
Rutin	68.60 ^a	0.05	67.40 ^b	0.02	14.40 ^c	0.06
Total flavonoids	422.40	–	301.00	–	127.40	–
Hydroxybenzoic acids						
3,4-Dihydroxybenzoic acid	97.80 ^c	0.11	104.80 ^b	0.27	143.40 ^a	0.30
4-Hydroxybenzoic acid	49.60 ^a	0.07	ND	–	21.40 ^b	0.08
2,5-Dihydroxybenzoic acid	737.20 ^a	0.41	ND	–	89.40 ^b	0.78
Ellagic acid	49.00 ^a	0.23	10.40 ^b	0.20	1.00 ^c	0.01
Gallic acid	36.40 ^a	0.07	3.80 ^b	0.08	ND	–
Syringic acid	50.60 ^a	0.07	49.80 ^b	0.02	5.00 ^c	0.02
Salicylic acid	348.64 ^a	0.51	251.60 ^c	0.81	310.60 ^b	0.89
Vanillic acid	19.40 ^a	0.09	4.40 ^b	0.04	2.20 ^c	0.06
Hydroxycinnamic acids						
<i>Trans</i> -cinnamic acid	2.20 ^b	0.01	2.20 ^b	0.01	2.60 ^a	0.02
Caffeic acid	49.40 ^a	0.05	20.60 ^b	0.08	16.20 ^c	0.07
<i>p</i> -Coumaric acid	8.20 ^a	0.06	0.60 ^c	0.01	2.00 ^b	0.03
Felluric acid	28.00 ^a	0.02	10.00 ^b	0.04	ND	–
Synapic acid	ND	–	43.00	–	18.80	–
Total non-flavonoids	1476.44	–	501.20	–	612.60	–
Total phenolic compounds	1898.84	–	802.20	–	740.00	–

IDF, insoluble dietary fibre; SDF, soluble dietary fibre; TDF, total dietary fibre; GAE, gallic acid equivalent; ND, not detected.
^{a,b,c} Mean values in the same row with unlike superscript letters were significantly different between the mean of each group (one-way ANOVA, $P \leq 0.05$, Tukey's *post hoc* test).

increased DF fermentation and organic acid production by colonic microbiota, causing faecal acidification in rats fed on the tested fruit by-products^(51,52). Consumption of a diet rich in resistant starch or fructo-oligosaccharides has also shown an ability to decrease faecal pH in rats^(53,54).

Faecal moisture was higher in the DC group than in the HC group ($P \leq 0.05$). The DEA group presented increased faecal moisture compared with the HC group ($P \leq 0.05$) (Table 2), which could be related to better water retention in the colon caused by a balanced DF intake. Increased faecal moisture was

Table 2. Faecal moisture (g/100 g), pH and organic acids ($\mu\text{mol/g}$) in healthy control animals, non-treated dyslipidaemic control animals and dyslipidaemic animals that received acerola, cashew or guava industrial processing fruit by-products (Mean values and standard deviations)

Parameters	Groups									
	HC		DC		DEA		DEC		DEG	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Moisture	40.44 ^c	0.40	53.20 ^a	0.43	42.64 ^b	0.23	41.55 ^{b,c}	0.42	35.98 ^d	0.33
pH	7.02 ^e	0.01	8.62 ^a	0.01	7.89 ^b	0.02	7.39 ^d	0.05	7.61 ^c	0.06
Acetic acid	59.67 ^a	2.39	27.87 ^c	0.85	45.44 ^b	0.80	27.17 ^c	0.45	29.01 ^c	0.80
Formic acid	ND	–	ND	–	20.92	0.09	ND	–	ND	–
Total SCFA	59.67 ^b	1.19	27.87 ^{c,d}	0.43	66.36 ^a	0.45	27.17 ^d	0.22	29.01 ^c	0.40
Citric acid	1.14 ^c	0.16	8.24 ^a	1.46	3.80 ^b	0.20	7.64 ^a	0.02	2.47 ^{b,c}	0.42
Lactic acid	55.92 ^a	1.88	27.72 ^b	1.60	8.16 ^c	0.67	47.59 ^a	8.77	11.17 ^c	0.86
Malic acid	0.51 ^c	0.02	1.66 ^c	1.20	47.72 ^a	0.94	7.71 ^b	0.28	0.56 ^c	0.08
Total organic acids	117.24 ^b	2.31	65.49 ^d	2.56	126.04 ^a	1.36	90.11 ^c	4.76	43.21 ^e	1.08

HC, healthy control; DC, non-treated dyslipidaemic control; DEA, dyslipidaemic group supplemented with acerola by-product; DEC, dyslipidaemic group supplemented with cashew by-product; DEG, dyslipidaemic group supplemented with guava by-product; ND, not detected.

^{a,b,c,d,e} Mean values in the same row with unlike superscript letters were significantly different between the mean of the each group (one-way ANOVA, $P \leq 0.05$, Tukey's *post hoc* test).

not observed in the DEG group. The guava by-products showed the highest IDF content and IDF/SDF ratio. In addition, the guava and cashew by-products presented high amounts of tannins^(16,55), which have been shown to have anti-secretory activity, inducing decreased intraluminal fluid, increased water reabsorption, reduced intestinal motility and, consequently, reduced moisture in faeces⁽⁵⁶⁾.

Diets presenting IDF/SDF ratio in the range of 1.0–2.3 typically present better capability of modulating physiological processes⁽¹⁰⁾. Supplementation with acerola by-products (IDF: SDF ratio: 2.42) should produce the best DF fractions balance, compared with cashew and guava by-products. Consumption of a diet containing an adequate DF balance might benefit intestinal functions by increasing faecal volume, as a result of increased water absorption⁽⁵⁷⁾, facilitating faecal excretion⁽⁵⁴⁾.

Production of organic acids, particularly SCFA, is directly related to selective fermentation of potentially prebiotic components by colonic bacteria⁽⁵⁸⁾. Although SCFA can be used as energy sources by the host, they can also act as energy consumption and metabolism regulators⁽⁵⁹⁾. SCFA can improve blood lipid levels by suppressing hepatic lipogenesis and increasing oxidative metabolism⁽²⁶⁾. The highest contents of acetic acid were found in faeces of the HC group, followed by the DEA group ($P \leq 0.05$). Formic acid was found only in the faeces of the DEA group. Highest total SCFA contents were found in the faeces of the DEA group ($P \leq 0.05$). Lactic acid was the organic acid found in highest amounts in the faeces of the HC, DC, DEC and DEG groups; malic acid was the organic acid found in the highest amount in the faeces of the DEA group ($P \leq 0.05$) (Table 2).

Considering that the acerola by-product exhibited high SDF content, the increased acetic acid content observed in the faeces of the DEA group could be related to increased colonic SDF fermentation⁽¹⁸⁾. Moderate amounts of acetic acid detected in the acerola by-product (Table 1) could be related to the detection of increased amounts of formic acid in faeces of the DEA group. Formic acid is used as substrate for acetic acid production by acetogenic bacteria via the Wood–Ljungdahl

pathway⁽⁶⁰⁾. In the present study, butyric and propionic acids were not detected in the animals' faeces regardless of the consumption of acerola, cashew or guava by-products. One possible reason for this is that 95 % of SCFA produced in large intestine is quickly absorbed by colonocytes, whereas only the remaining 5 % is expelled in faeces⁽²⁴⁾.

The DC group exhibited higher faecal counts of Enterobacteriaceae (Fig. 2(b)) and lower counts of *Bifidobacterium* spp. and *Lactobacillus* spp. (Fig. 2(a) and (c)) than the HC, DEA, DEC and DEG groups ($P \leq 0.05$). The DEA group showed higher faecal counts of *Bifidobacterium* spp. and *Lactobacillus* spp. than the DC group ($P \leq 0.05$) and similar to HC group ($P > 0.05$), suggesting that the DF and fructans present in acerola by-products (Table 1) may stimulate the increase in populations of these beneficial microorganisms in the intestine^(35,61). Phenolic compounds present in acerola processing by-products (Table 1) might also contribute to these stimulatory effects, as up to 90 % of plant phenolics may reach the colon where they are used as substrates for production of small phenolic acids by intestinal microbiota⁽²¹⁾. Small phenolic acids affect intestinal microbial composition and are used as substrates for organic acid production by colonic bacteria^(21,37). *Bifidobacteria* and *lactobacilli* can normalise fat metabolism through TAG synthesis and mineral absorption, which is also associated with a reduced incidence of colon cancer⁽⁶²⁾. Furthermore, DF can bind to phenolic compounds and, consequently, reduce their absorption in the small intestine. This permits a high proportion of ingested phenolic compounds to reach the large intestine to be fermented, contributing to the growth of beneficial bacterial species (e.g. *Bifidobacterium* and *Lactobacillus*) and inhibiting the growth of pathogenic bacteria (e.g. *Clostridium perfringens* and Enterobacteriaceae)⁽⁶³⁾.

The DEA and DEG groups presented lower faecal counts of Enterobacteriaceae compared with the DC group ($P \leq 0.05$). Increased counts of *Bifidobacterium* spp. and *Lactobacillus* spp. and reduced counts of *C. perfringens* was previously verified in the caecum contents of hamsters fed a diet supplemented with pineapple peel (rich in IDF). These results were

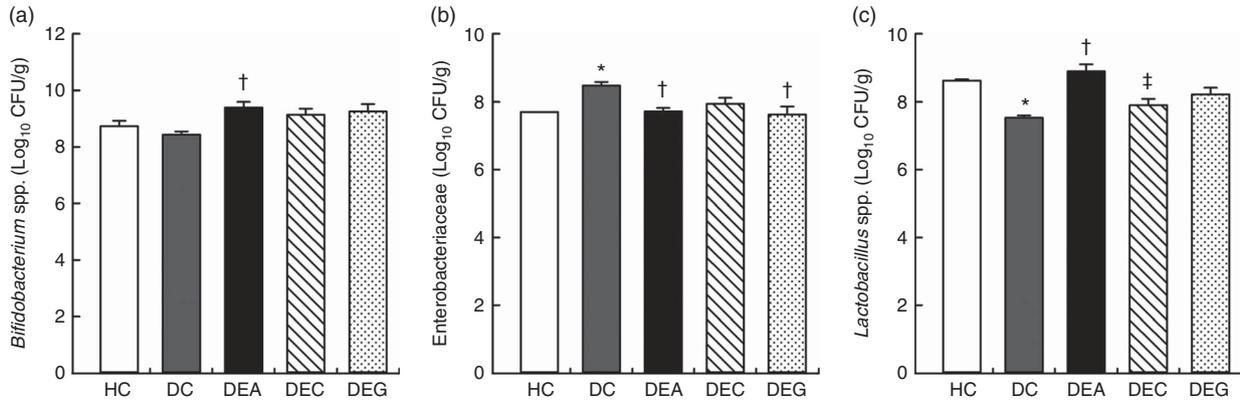


Fig. 2. Viable cell count of *Bifidobacterium* spp. (a), Enterobacteriaceae (b) and *Lactobacillus* spp. (c), in faeces of the healthy control animals (HC), non-treated dyslipidaemic control animals (DC) and dyslipidaemic animals that received acerola (DEA), cashew (DEC) or guava (DEG) industrial processing by-products. Values are means and standard deviations represented by vertical bars (one-way ANOVA, $P \leq 0.05$, Tukey's *post hoc* test). CFU, colony-forming units. * Significant difference compared with the HC group, † significant difference compared with the DC group, ‡ significant difference compared with the DEA group.

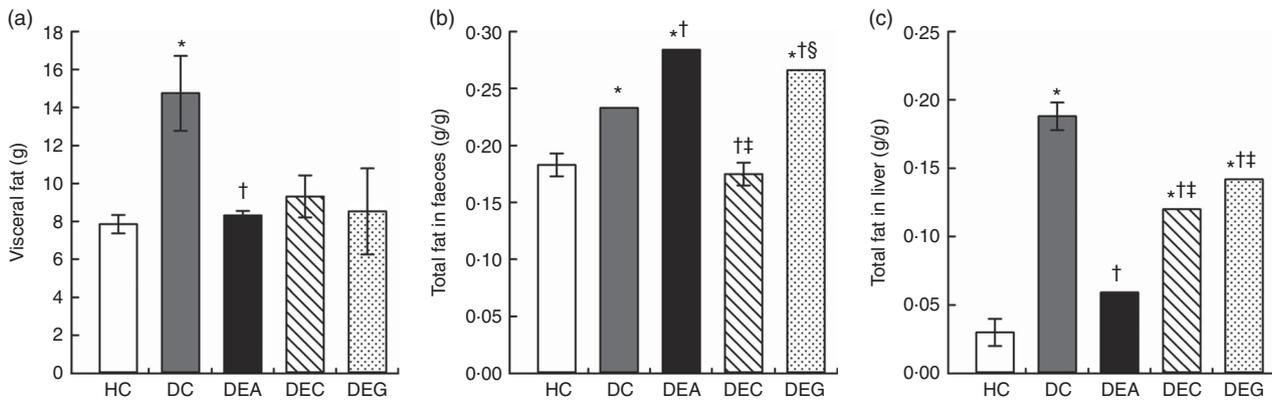


Fig. 3. Visceral fat (a), total fat in the faeces (b) and liver (c) of the healthy control animals (HC), non-treated dyslipidaemic control animals (DC) and dyslipidaemic experimental animals that received acerola (DEA), cashew (DEC) or guava (DEG) industrial processing by-products. Values are means and standard deviations represented by vertical bars (one-way ANOVA, $P \leq 0.05$, Tukey's *post hoc* test). * Significant difference compared with the HC group, † significant difference compared with the DC group, ‡ significant difference compared with the DEA group, § significant difference compared with the DEC group.

related to increased production of SCFA and antimicrobial substances in hamsters' intestine⁽³⁵⁾. Decreased luminal pH values, as observed in the DEA and DEG groups, were associated with changes in intestinal microbiota composition, hampering the growth of pH-sensitive pathogenic bacteria, such as Enterobacteriaceae⁽²⁴⁾.

Effects on visceral fat, total fat in faeces and liver

The visceral fat amount was higher in the DC group than in the DEA and HC groups ($P \leq 0.05$), indicating that supplementation with acerola by-products decreased the effects of excessive fat intake on visceral adipose tissue (Fig. 3(a)).

Fat amount in faeces was higher in the DEA and DEG group compared with the HC, DC and DEC groups ($P \leq 0.05$) (Fig. 3(b)). High total fibre content in acerola and guava by-products may have favoured a decrease in fat absorption in the intestine^(44,64) and, consequently, an increase in fat excretion in the faeces. DF and phenolic contents in bread containing an oven-dried or lyophilised extract of red grape processing

by-products has been associated with increased fat excretion in faeces and less accumulation of visceral fat in hypercholesterolaemic rats⁽⁶⁵⁾. Fat excretion in faeces may have influenced the higher weight losses in the DEA and DEG groups because energy loss through faeces impacts directly on the amount of metabolisable energy in a diet⁽⁶⁶⁾.

The DC group exhibited higher amounts of liver fat than the HC, DEA, DEC and DEG groups ($P \leq 0.05$) (Fig. 3(c)). High-fat diet consumption can lead to the development of NAFLD, such as hepatic steatosis, which is characterised by fat accumulation in more than 5% of liver cells⁽⁶⁷⁾. In hepatic steatosis, there is increased formation of reactive oxygen species that damage hepatic tissue and cause mitochondrial dysfunction, reduction of β -oxidation and elevation of TAG production, which could justify the elevation of serum TAG levels in the DC group, as previously discussed⁽⁶⁸⁾.

The lowest amounts of liver fat were found in the DEA group ($P \leq 0.05$), indicating a hepatoprotective effect from supplementation with the acerola by-products. The hepatoprotective effects induced by the acerola by-products may also be related

to the majority phenolics (catechin, myricetin, quercetin, rutin, 2,5-dihydroxybenzoic acid, syringic acid and salicylic acid) found in this material (Table 1). These compounds induce reduced liver fat deposition by decreasing fatty acid synthesis and lipogenic enzyme activity, as well as promoting cellular β -oxidation and lipophagy^(3,69). In addition, a lower amount of liver fat in the DEA group could indicate decreased activity of the lipogenic enzymes, such as lipoprotein lipase, involved in metabolism and transport of TAG-rich lipoproteins (chylomicrons and VLDL)⁽⁷⁰⁾.

Effects on histopathological characteristics of intestine and liver

Histopathological examination of H&E-stained colon samples revealed that consumption of dyslipidaemic diet caused epithelial destruction (Fig. 4(b), \blacktriangle) with the loss of organ integrity and epithelial conservation (\blackleftarrow). Colon samples stained with PAS showed loss of organ integrity, as evidenced by reduced goblet cell number (Fig. 5(b), \blacktriangle) in the DC group colonic mucosa. The HC group exhibited a normal colonic epithelial structure (Fig. 4(a) and 5(a)), and colon in the DEA, DEC and DEG groups (Fig. 4(c)–(e) and Fig. 5(c)–(e)) did not show morphological changes induced by the dyslipidaemic diet. The DEA, DEC and DEG groups showed maintenance of integrity of crypts, goblet cells and epithelial cells, indicating improved colon health. Goblet cells produce intestinal mucus and are among the most abundant cell types in colonic mucosa, exerting an important role in colon health⁽⁷¹⁾. DF and fructan amounts in acerola, cashew and guava by-products may have protected colon tissue from damage caused by a dyslipidaemic diet, as previously observed in rats fed a diet added with yacon root (rich in fructo-oligosaccharide) or fed a diet containing resistant starch⁽⁵³⁾. The production of SCFA from the intestinal microbial fermentation of

DF and phenolic compounds contained in acerola, cashew and guava by-products may have positively affected intestinal health, as SCFA are the main source of energy for the colonocytes⁽²⁶⁾.

The liver architecture of the HC group was normal (Fig. 6(a)). However, the dyslipidaemic diet led to the fat accumulation in hepatocytes, and both hepatic steatosis (arrowhead) and blood stasis (black arrow) were observed in the DC group (Fig. 6(b)). The DEC (Fig. 6(d)) and DEG groups (Fig. 6(e)) exhibited reduction in steatosis compared with the DC group. The DEA group presented minor degenerative changes in the liver (Fig. 6(c)) compared with the DC, DEC and DEG groups. Previous studies on histopathological examinations of liver samples have found increased fat deposits in the livers of rodents fed a high-fat diet. These changes were reduced, however, after the animals received a high-fat diet with dehydrated *Rosa laevigata* Michx fruit, which contains a high amount of flavonoids⁽⁷²⁾, as well as with dried oranges, strawberries and pomegranates⁽⁴⁴⁾. These hepatic protective effects were related to the existence of potential synergism among phenolic compounds present in tested materials. Fruit rich in phenolic compounds have also presented a protective effect against increased hepatic fat deposits in animals fed a high-fat diet^(73–75). Phenolic compounds can minimise hepatic steatosis by regulating the activity of the antioxidant enzyme paraoxonase, which protects against the development of hypercholesterolaemia and atherogenesis⁽⁷⁴⁾, as well as by decreasing hepatic lipogenesis through the activation of the hepatic cyclic AMP (30,50-monophosphate) (cAMP)/protein kinase A/cAMP-responsive element binding protein pathway⁽²⁶⁾.

Conclusions

In general, supplementation with acerola, cashew or guava industrial processing by-products reduced weight gain, faecal

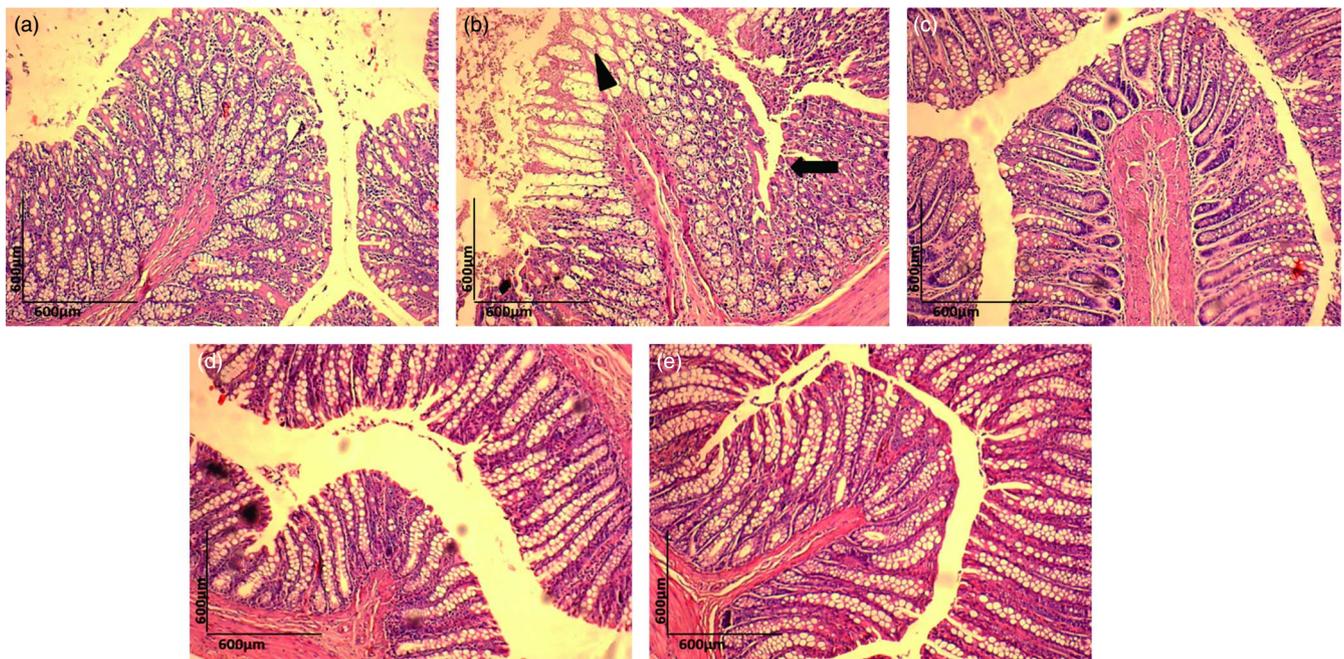


Fig. 4. Haematoxylin–eosin (H&E) staining (10 \times) for histopathological examination of the colon in the healthy control animals (a), non-treated dyslipidaemic control animals (b) and dyslipidaemic animals that received acerola (c), cashew (d) or guava (e) industrial processing fruit by-products.

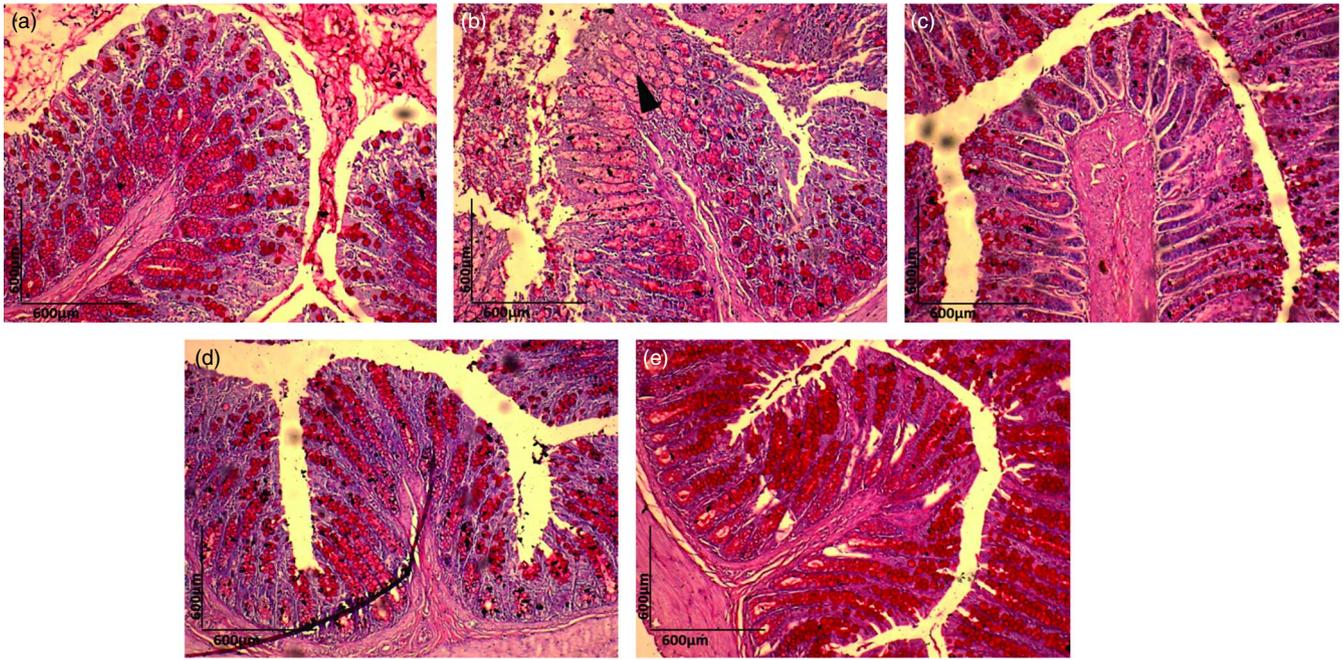


Fig. 5. Periodic acid–Schiff (PAS) staining (10×) for histopathological examination of the colon in the healthy control animals (a), non-treated dyslipidaemic control animals (b) and dyslipidaemic animals that received acerola (c), cashew (d) or guava (e) industrial processing fruit by-products.

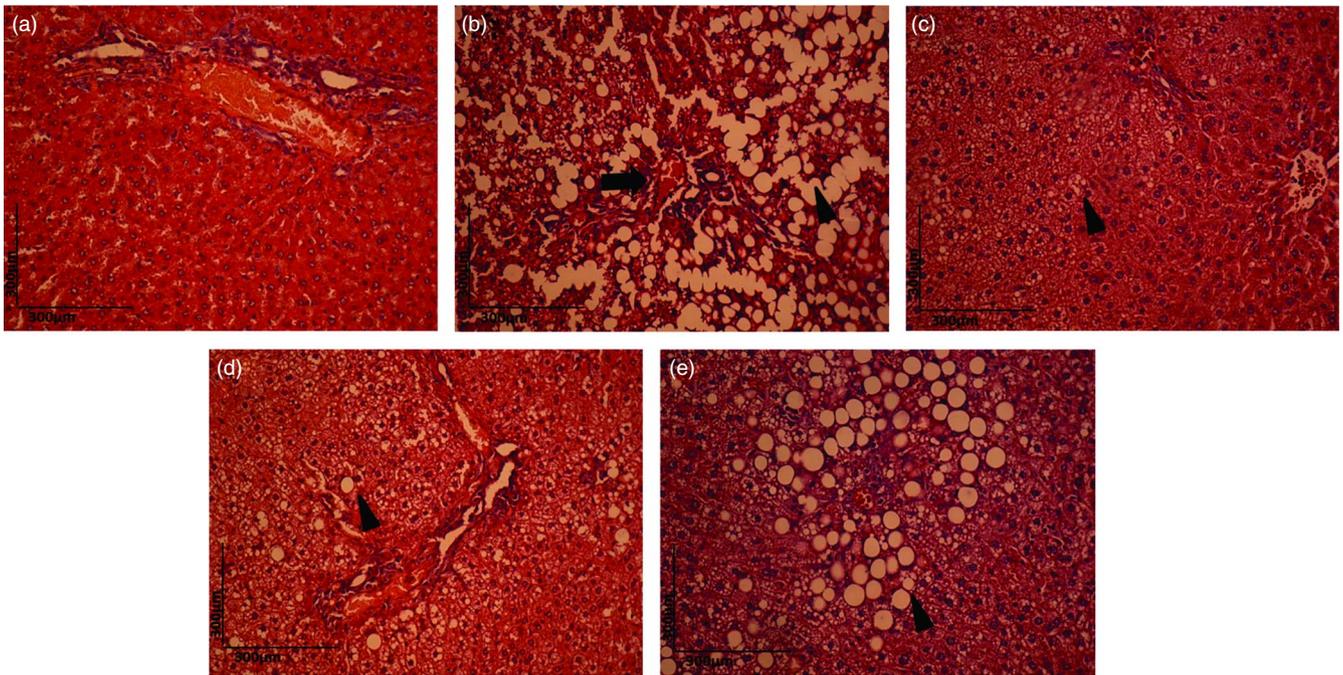


Fig. 6. Haematoxylin–eosin (H&E) staining (10×) for histopathological examination of the liver in the healthy control animals (a), non-treated dyslipidaemic control animals (b) and dyslipidaemic animals that received acerola (c), cashew (d) or guava (e) industrial processing fruit by-products.

pH, liver fat accumulation, preserved colonic epithelial integrity and liver cell structure in dyslipidaemic female Wistar rats. Acerola and cashew processing by-products increased faecal moisture, and acerola and guava by-products increased fat excretion in faeces. The consumption of acerola processing by-products had the greatest effects on the monitored parameters. Its consumption of acerola by-products by

dyslipidaemic female rats increased faecal counts of beneficial bacteria belonging to the *Bifidobacterium* and *Lactobacillus* genera, in addition to inducing higher organic acid production in the intestine, attenuating visceral fat accumulation and lipid profile changes. Protective effects of acerola by-products may be associated with its high amounts of phenolic compounds and other bioactive compounds and DF balance. These results

encourage considering the safe use of acerola, cashew and guava by-products to protect the harmful effects caused by a dyslipidaemic diet on intestinal health and lipid metabolism. This use could be either as dietary supplements or as ingredients used in food products.

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K. S. B., A. S. S., E. L. d. S. and J. d. S. A. conceived the study, performed literature searches, wrote the manuscript and reviewed the final draft. M. d. S. L., B. R. L. d. A. M. and A. M. T. d. M. performed the characterisation analyses of fruit by-products and the statistical analysis of these results. K. S. B., A. F. A., L. A. d. S., P. P. L., J. A. d. S. G., L. T. T. and M. L. d. C. performed the biological assay and literature searches. K. S. B., M. L. d. C. and L. T. T. performed statistical analyses of the data and wrote the manuscript.

The authors declare that there are no conflicts of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit <https://doi.org/10.1017/S0007114517003282>

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