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HEALTH SCIENCES

Effects of supplementation of tropical fruit processing by-products on lipid profile, retinol levels and intestinal function in Wistar rats

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Abstract: Fruits agro-industrial by-products may have a great variety of bioactive compounds that promote health. Thus, the effects of supplementation with acerola, cashew and guava processing by-products for 28 days on retinol level, lipid profile and on some aspects related to intestinal function in rats were investigated. The animals supplemented with different fruit by-products presented similar weight gain, faecal pH values and intestinal epithelial structures; however, they showed higher moisture and *Lactobacillus* spp. and *Bifidobacterium* spp. counts in faeces compared to the control group. Supplementation with the cashew by-product decreased the blood glucose, acerola and guava by-products reduced serum lipid levels and all fruit by-products tested increased serum and hepatic retinol. The results indicated that acerola and guava by-products possess a potential hypolipidemic effect. The three fruit by-products increase the hepatic retinol deposition and the faecal populations of beneficial bacterial groups and modulated aspects of intestinal function. The findings of this study can contribute to sustainable fruticulture and support future clinical studies with the supplementation of by-products.

Key words: blood glucose, by-products, gut microbiota, lipid profile, retinol status.

INTRODUCTION

Brazil produces approximately 40 million tons of tropical, subtropical and temperate fruits per year, providing a great variety of fruits throughout the year (FAO 2018). Among the more popular and most frequently processed native or exotic fruits in Brazil are acerola (*Malpighia emarginata* D.C.), cashew (*Anacardium occidentale* L.) and guava (*Psidium guajava* L.), which are greatly appreciated because of their sensory characteristics (flavour and colour), nutritional quality and bioactive compound content. Acerola is recognised as one of the greatest natural vitamin C sources, with high carotenoid and lycopene contents. Cashew is considered a source of fibres, carotenoids, vitamin C and polyphenols, while guava is an important source of vitamins A and C, fibre, pectin and potassium (Ellong et al. 2015, Vargas-Murga et al. 2016).

Frozen tropical fruit pulps have become popular worldwide due to their practical consumption (Dantas et al. 2019). However, fruit pulp processing generates a large volume of by-products such as skins, seeds and bagasse, which are often inadequately disposed into the environment (Medeiros et al. 2019). The fruit processing by-products may contain dietary fibres and different antioxidant compounds

such as phenolic compounds, which are considered dietary components associated with the modulation of bowel transit time, decreased gastric emptying, delayed glucose absorption, decreased postprandial glycaemia and reduced blood cholesterol and triacylglycerol (TAG) levels due to their physical properties that provide viscosity to the luminal content (Ayala-Zavala et al. 2011, Batista et al. 2018). In addition, by-products contain carotenoids that can exert vitamin A activity in various physiological processes such as regulation of glucose and lipid metabolisms, cell proliferation and differentiation, and the immune system (Saeed et al. 2017, García-Cayuela et al. 2018).

The intake of different fruit by-products seems to influence on maintaining the balance of the gut microbiota. Fruit by-products contain one or more components capable of selective fermentation, which promote changes in the gut microbiota composition and activity, that is, they have a potential prebiotic effect (Batista et al. 2018). These effects provide health benefits, given that the balance of commensal and pathogenic bacteria of the gut microbiota has been associated with decreased risk of developing metabolic diseases (Belizário et al. 2018).

Considering these aspects and the need for a sustainable destination of fruit processing by-products, the present study aimed to evaluate the effects of supplementation with acerola, cashew and guava by-products on the retinol levels, lipid profile and some parameters associated with intestinal function in rats.

MATERIALS AND METHODS

Pulp fruit processing by-products

Acerola (*Malpighia emarginata* D.C.), cashew (*Anacardium occidentale* L.) and guava (*Psidium guajava* L.) by-products were grown in Alhandra, PB, Brazil (latitude 07º 26' 19" S, longitude 34º 54' 52" W; altitude 49 m) during September and October 2018. By-products were donated by the *Polpa Ideal Indústria Ltda.* (João Pessoa, PB, Brazil). The total sample contained 20 kg of each by-product (skin, seeds and bagasse) homogenised from different batches. Each by-product was subjected to lyophilisation (L-101 lyophilizer, LIOTOP, São Carlos, SP, Brazil) at - 47 °C, with a vacuum pressure below 150 μHg and a lyophilisation rate of 1 m/h, for approximately 12 h. The freeze-dried by-products were ground in a domestic blender (average particle size < 1.0 mm) and stored under refrigeration (-10°C) and protected from light.

For total carotenoids quantification, initially 18 mL of acetone P.A. was added to 0.5 g of by-products samples and after homogenization, the samples were read on a spectrophotometer (Genesys 10S UV-Vis Spectrophotometer model, Madison, USA) at wavelengths of 470 nm, 645 nm, and 662 nm in the absence of light at a temperature of 25°C (Lichtenthaler & Buschmann 2001). The results were expressed in mg/100 g of sample dry weight.

The ascorbic acid concentration was determined using the Tillmans titrimetric method (2,6-Dichlorophenolindophenol sodium) (method 967.21) (AOAC 2016). A solution of 2,6-dichlorophenol-indophenol was discoloured by ascorbic acid in 0.5 g of by-product sample using a standard ascorbic acid solution. The spectrophotometer (Genesys 10S UV-Vis Spectrophotometer model, Madison, USA) was calibrated at 100% transmittance using 5 mL 2% HPO_3 blank solution and 10 mL of water. Samples were read at a wavelength of 518 nm and results were expressed as mg/100g of sample dry weight.

Animals and diets

The experimental method was approved under protocol number no. 050514 by the Animal Experimentation Ethics Committee (*Comissão de Ética no Uso de Animais – CEUA*), Federal University of Paraíba (*Universidade Federal da Paraíba – UFPB*) (João Pessoa, PB, Brazil), and all experiments followed the standards of the Brazilian Society of Science in Laboratory Animals (*Sociedade Brasileira de Ciência em Animais de laboratório - SBCAL*). The rats were acclimatized for one week at the Experimental Nutrition Laboratory - LANEX (UFPB) and were maintained in cages (4 animals/cage) at 21 ± 1°C, relative humidity 50-55% and 12 hr light-dark cycles, with water and standard diet (Presence, Paulínea, SP, Brazil) provided *ad libitum*.

The biological assay was performed during 28 days using 32 adult *Wistar* male rats (average initial weight 290.00 ± 10.00 g) at ±80 days old randomised into four groups which received saline solution (control group; CG, n=8), acerola processing by-product (acerola by-product experimental group; AEG, n=8), cashew processing by-product (cashew by-product experimental group; CEG, n=8) and guava processing by-product (guava by-product experimental group; GEG, n=8) (Figure 1). The

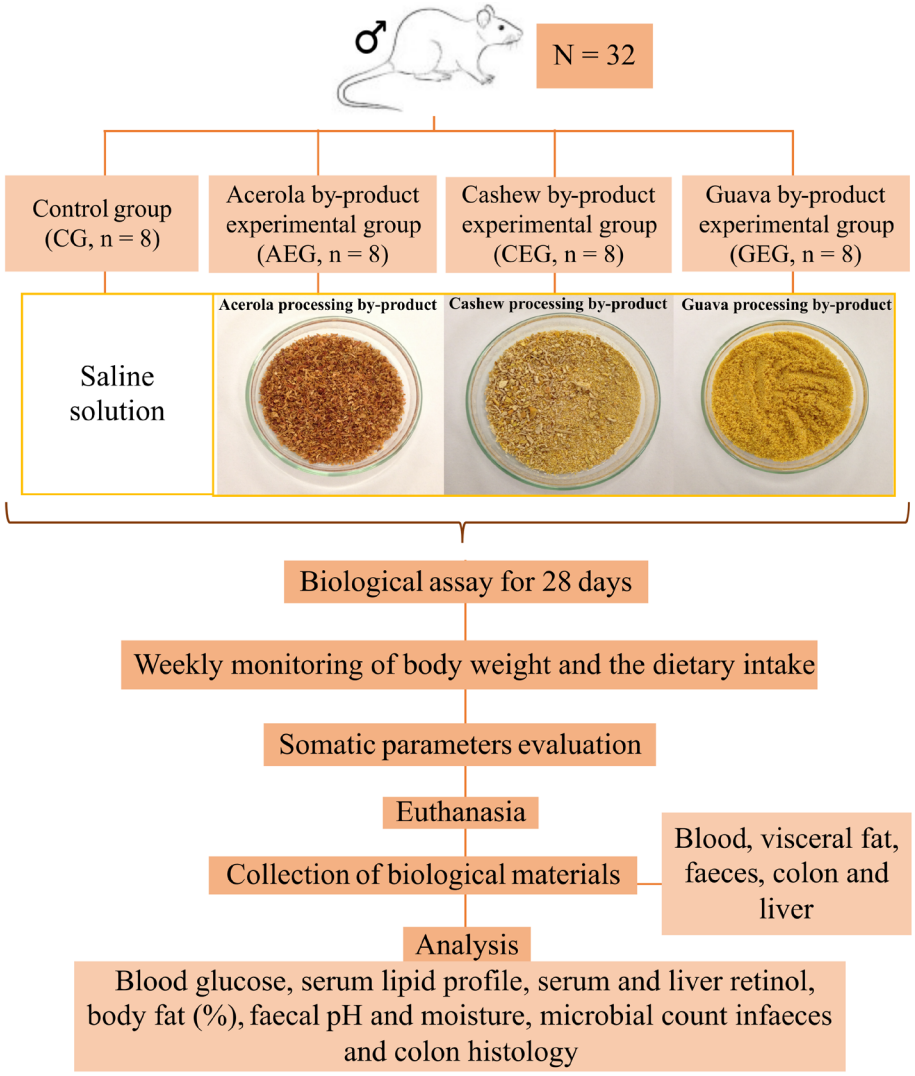

Figure 1. Flowchart of the experimental study with rats supplemented or not with acerola, cashew, or guava by-products. CG = control group; AEG= acerola by-product experimental group; CEG = cashew by-product experimental group; GEG= guava by-product experimental group.

freeze-dried by-products were administered to the AEG, CEG, and GEG groups at the dose of 400 mg/kg of animal weight. The fruit by-products were diluted in saline solution (1.6%, w/v) and gavage was performed twice a day at 4 h interval (Batista et al. 2018). The body weight and the dietary intake measurements were performed weekly using a digital electronic scale (Prix III, Toledo, São Bernardo do Campo, SP, Brazil).

Somatic parameters, lipid profile and blood glucose

After 28 days, the animals were fasted for 8 h and anaesthetised intraperitoneally using 75 mg of ketamine hydrochloride per kg of body weight and 5 mg of xylazine hydrochloride per kg of body weight. Somatic parameters were evaluated with anaesthetized animals, as follows: body weight; chest circumference measured immediately before the hind paw, abdominal circumference measured immediately behind the front leg, and naso-anal length measured using metric tape. The body mass index (BMI) and Lee index (Novelli et al. 2007) were calculated through the equations: BMI = body weight (g)/length (cm) squared; Lee index = cube root of body weight (g)/length (cm).

The anaesthetized animals were then euthanised via cardiac puncture. Blood samples were collected to perform glycaemia level using a glucometer (model Performa, Accu-check, Jaguaré, SP, Brazil) and to obtain serum by centrifugation (MPW-351R centrifuge, MPW-Med. Instrument, Warsaw, Poland) at 1,000 x g for 10 min at 4°C for determining lipid profile using commercial kits (Labtest, Lagoa Santa, MG, Brazil) and LabMax 240 Premium automatic analyzer (Labtest). Sample analyses were performed at 505 nm for TAG level; 500 nm for total cholesterol (TC); 600 nm for very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). The visceral fat samples were collected, weighed to calculate the percentage of this fat in relation to the body weight of the animal. The carcass was used to determine total fat in the rats' body (Folch et al. 1957). The colon was removed for histological analysis, and the liver was removed to quantify hepatic retinol.

Serum and liver retinol determination

Retinol was extracted from serum and liver homogenate (Aquino et al. 2016). The retinol levels were determined by high-performance liquid chromatography (UltiMate 3000, Thermo Scientific Dionex, Waltham, MA, USA) using a Dionex chromatograph containing a C18 column measuring 4.60 x 2.50 mm x 5.00 µm, a pre-column, a detector set to 325 nm, and a mobile phase flow rate (methanol) of 1.50 mL for min.

Faecal analyses

Animal faeces were collected on the 26th to 28th days of the experiment to obtain samples in representative quantities for analysis (Batista et al. 2018, Tavares et al. 2021). Part of the faecal samples was collected fresh for bacterial count analysis and part was stored in a freezer -20°C for pH and moisture analysis. The pH was determined using a digital potentiometer (Q400AS, Quimis, São Paulo, SP, Brazil) and the moisture was determined following the 934.01 method (AOAC 2016).

For analysis of bacteria count in faeces, the fresh faecal samples were diluted in sterile peptone (1:9, w/v). Then, 20 µL aliquots were inoculated using the micro drop technique (Miles et al. 1938) on selective agar for *Lactobacillus* spp. (de Man, Rogosa and Sharpe – MRS, HiMedia, Mumbai, MH, India), *Bifidobacterium* spp. (*Bifidobacterium* agar, HiMedia) and *Enterobacteriaceae* (MacConkey, HiMedia). Plates were incubated under anaerobiosis (*Lactobacillus* spp. and *Bifidobacterium* spp.;

Anaerobic System Anaerogen, Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) or aerobiosis (*Enterobacteriaceae*) at 37°C for 48 hr. Results are expressed as $\log_{10}$ colony-forming units (CFU)/g (APHA 2001).

Histological colon evaluation

Colon samples collected for histological evaluation were processed using routine histological techniques and stained with Haematoxylin and Eosin (H&E). Stasis, leukocyte migration, haemorrhage, vasodilation, necrosis, epithelial preservation, hypertrophy and hyperplasia of the outer muscular layer were evaluated at a total 40x magnification using an optical microscope (Motic BA 200, Kowloon Bay, Kowloon, Hong Kong) (Batista et al. 2018).

Statistical analysis

The sample size (32 animals randomized into four groups, n = 8) was calculated to meet a minimum statistical power of 80%, with a minimally detectable effect size of 1.0 and a significance level of 0.05 (a = 0.05). Results were expressed as the mean and standard deviation. The data showed normal distribution by the Shapiro-Wilk test and were therefore, subjected to parametric one-way Analysis of Variance (ANOVA) and the Tukey's post hoc test at a 5% significance level ($P \leq 0.05$), when there was a difference between the obtained data. The analysis and graphic design were carried out using SigmaPlot 12.5 for Windows (Systat Software Inc., San Jose, CA, USA) statistical software.

RESULTS AND DISCUSSION

The by-products of acerola and cashew presented the highest amount of carotenoids, however, the by-product of acerola stood out from the others by containing almost one hundred times more ascorbic acid (Table I). The by-products of acerola, cashew and guava have high amounts of ascorbic acid and carotenoids compared to other fruit by-products such as melon, orange, mandarin, and grapefruit (Rico et al. 2020, Reynoso-Camacho et al. 2021). However, not only the amounts of antioxidant compounds in each by-products should be considered to assess the benefits of their consumption. The carotenoid fractions and the interfering factors in the bioavailability of ascorbic acid present in each by-product also impacts the bioactivity in body, demonstrating the importance of carrying out animal model interventions to study the fruit by-products (Gómez-García et al. 2020, Mieszczakowska-Frąc et al. 2021).

The cashew by-product experimental group (CEG) presented higher dietary intake in comparison to the control group (CG), acerola by-product experimental group (AEG) and guava by-product experimental group (GEG) ($P \leq 0.05$; Figure 2a). Although all experimental groups (AEG, CEG and GEG) consumed a greater amount of diet, they presented similar weight gain to CG ($P > 0.05$) (Figure 2b). These results can be

Table I. Total carotenoids and ascorbic acid contents in acerola, cashew and guava processing by-products.

Bioactive compounds (mg/100 g of dry weight)	By-products		
	Acerola	Cashew	Guava
Total carotenoids	4.66±0.11[a]	4.82±0.14[a]	4.29±0.06[b]
Ascorbic acid	149.40±5.30[a]	1.69±0.20[b]	1.41±0.20[b]

Note: [a,b] Different lowercase letters in the same row indicate a significant difference between the mean ± standard deviation of each by-product (one-way ANOVA, $P \leq 0.05$, Tukey's *post hoc* test).

explained by an increase in gastrointestinal motility with consequent reduction in the amount of absorbed and metabolized energy, promoted by supplementation with the fruit by-products (Batista et al. 2018).

Despite the higher dietary intake of the CEG group, the cashew by-product maintained the body weight of the supplemented rats, providing similar final body weight to the other groups ($P > 0.05$) (Table II). The acerola and guava by-products have also been shown to significantly reduce the final body weight of the AEG and GEG groups ($P \leq 0.05$). The beneficial effects of these fruit by-products on body weight and body composition are suggested when it is noted that the body length, abdominal and chest circumferences, visceral and body fat were similar between experimental and control

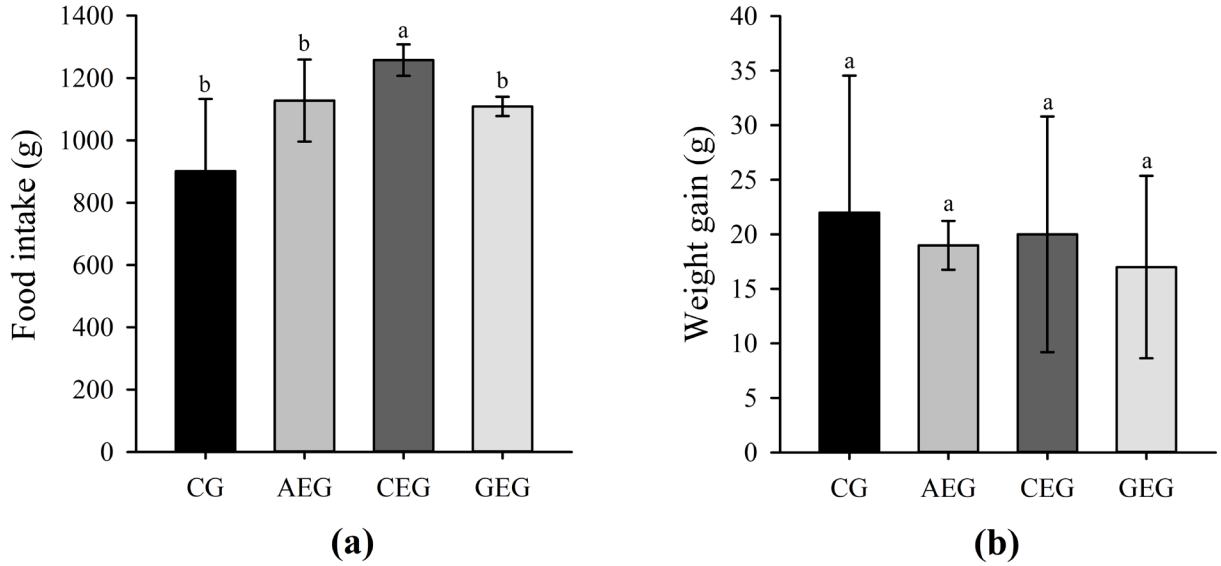

Figure 2. Food intake (a) and weight gain (b) of rats supplemented or not with acerola, cashew, or guava by-products. [a,b] Different lowercase letters in vertical bars indicate a significant difference between the mean ± standard deviation of each rat group (one-way ANOVA, $P \leq 0.05$, Tukey's *Post hoc* test; $n = 8$ rats/group). CG = control group; AEG= acerola by-product experimental group; CEG = cashew by-product experimental group; GEG= guava by-product experimental group.

Table II. Murinometric parameters, quantification of visceral and body fats of Wistar rats supplemented or not with acerola, cashew or guava by-products.

Parameters	Groups			
	CG	AEG	CEG	GEG
Final body weight (g)	318.33±7.64[a]	297.50±8.66[b]	306.67±5.77[ab]	293.00±7.58[b]
Body length (cm)	24.67±0.61[a]	23.86±0.90[a]	23.75±0.60[a]	24.36±0.47[a]
BMI (g/cm^2)	0.51±0.01[ab]	0.49±0.02[ab]	0.52±0.02[a]	0.47±0.03[b]
Lee index	0.27±0.00[b]	0.27±0.00[ab]	0.28±0.01[a]	0.27±0.01[b]
Abdominal circumference (cm)	16.12±0.85[a]	16.10±0.60[a]	16.44±1.18[a]	16.62±0.48[a]
Chest circumference (cm)	14.17±0.82[a]	14.17±1.25[a]	14.19±1.10[a]	14.00±0.35[a]
Visceral fat (%)	4.07±0.17[a]	3.27±0.91[a]	4.11±0.89[a]	3.20±0.55[a]
Body fat (%)	4.09±0.49[a]	3.40±0.32[a]	4.17±0.77[a]	3.33±0.16[a]

Note: [a,b] Different lowercase letters in the same row indicate a significant difference between the mean ± standard deviation of each group (one-way ANOVA, $P \leq 0.05$, Tukey's *post hoc* test).

groups ($P > 0.05$) (Table II), and the BMI and Lee index are within normal range, 0.45-0.68 g/cm^2 and < 0.30, respectively (Novelli et al. 2007).

Regarding the serum lipid profile, the AEG and GEG showed lower levels of TAG, TC, LDL and VLDL than CG ($P \leq 0.05$) (Figure 3a, b, c and d). Supplementation with by-products in healthy rats did not interfere with serum HDL ($P > 0.05$) (Figure 3e). These results suggest that specific compounds, for example dietary fibres, phenolic compounds (Batista et al. 2018), carotenoids and ascorbic (Table I), present in acerola and guava by-products may have directly or indirectly favoured some physiological mechanisms such as delayed gastric emptying associated with the increase of glucagon-like peptide-1; digestion/absorption delay of TAG and cholesterol by reducing the action of pancreatic lipase (key enzyme in TAG digestion) and bile salts in fat droplets; reduction of hepatic synthesis of cholesterol, VLDL and TAG (Relevy et al. 2015, Macho-González et al. 2018).

The blood glucose was lower in the CEG group animals than in the CG ($P \leq 0.05$) (Figure 3f). The insoluble dietary fibre content of the cashew by-product (Batista et al. 2018) may partly explain glycaemia reduction. The insoluble fibres may be a physical barrier that promotes the acceleration of intestinal transit time (Wu et al. 2020). Besides, they may increase the system viscosity, reducing α-amylase activity and enzyme-substrate interaction, thereby making

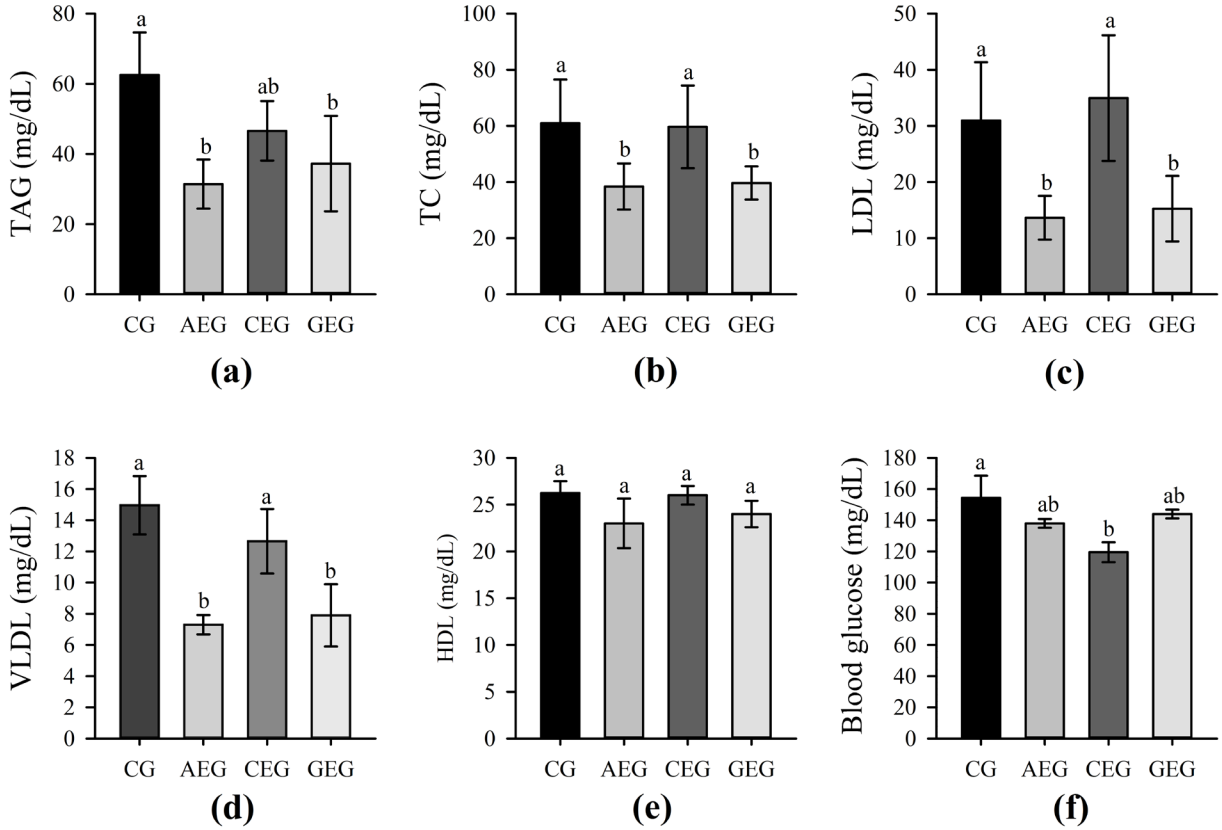

Figure 3. Lipid profile (a-e) and blood glucose (f) of rats supplemented or not with acerola, cashew, or guava by-products. [a,b] Different lowercase letters in vertical bars indicate a significant difference between the mean ± standard deviation of each rat group (one-way ANOVA, $P \leq 0.05$, Tukey's *post hoc* test; $n = 8$ rats/group). CG = control group; AEG= acerola by-product experimental group; CEG = cashew by-product experimental group; GEG= guava by-product experimental group.

glucose diffusion and absorption difficult (Qi et al. 2016, Carvalho et al. 2018).

Serum retinol was higher in AEG, CEG and GEG than in the CG group ($P \leq 0.05$) (Figure 4a). The CEG group, particularly, showed the highest serum retinol levels ($P \leq 0.05$), probably due to the differences in the pro-vitamin A fractions found in each studied by-product (de Abreu et al. 2013, Vargas-Murga et al. 2016).

Retinol is the precursor of retinoic acid, which in turn is the active metabolite of vitamin A that is required for proliferation, differentiation, and functional integrity of mucosal membrane cells, immune regulation, and glucose and lipid metabolism (Biesalski 2016, Saeed et al. 2017). The lower glycemia measured in the CEG group (Figure 3f) may also be related to the elevated serum carotenoid level in this group, since blood carotenoids are inversely associated with glycated haemoglobin levels and insulin resistance (Wang et al. 2017). Carotenoids can promote expression of peroxisome proliferator-activated receptor-gamma (Roohbakhsh et al. 2017), a regulator of glucose and lipid metabolisms (Wang et al. 2017).

The AEG, CEG and GEG groups presented higher hepatic retinol deposition than the CG group ($P \leq 0.05$), especially in AEG (Figure 4b). Soluble fibres found in large quantity in the acerola by-product (Batista et al. 2018) can protect oxidation carotenoids by the gastric pH, preventing its absorption in the small intestine (García-Cayuela et al. 2018). Thus, they favour the arrival of dietary carotenoids to the large intestine, where carotenoids are released from fibres by the gut microbiota's action, and it can be exported through the lymphatic system or portal vein to the liver to be stored (Saeed et al. 2017).

No differences were observed among experimental and control groups regarding faecal pH ($P \geq 0.05$) (Figure 5a). These results are interesting because changes in the faecal pH could be associated with specific diets or

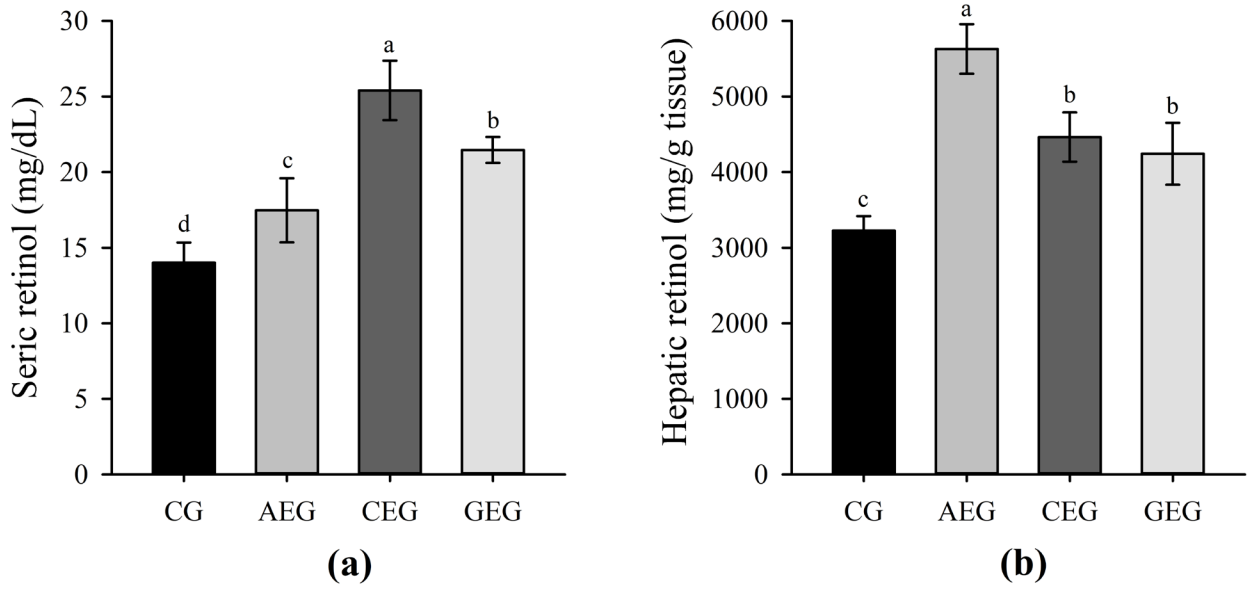

Figure 4. Serum (a) and hepatic retinol (b) of rats supplemented or not with acerola, cashew, or guava by-products. [a,b,c,d] Different lowercase letters in vertical bars indicate a significant difference between the mean ± standard deviation of each rat group (one-way ANOVA, $P \leq 0.05$, Tukey's *post hoc* test; $n = 8$ rats/group). CG = control group; AEG= acerola by-product experimental group; CEG = cashew by-product experimental group; GEG= guava by-product experimental group.

dietary compounds and could indicate dysbiosis in disease models (Nie et al. 2017, Batista et al. 2018). The three experimental groups presented higher ($P \leq 0.05$) faecal moisture than the CG (Figure 5b), likely because the consumption of the fruit by-product increased the water content in the faeces due to their fibre content (Batista et al. 2018). Insoluble fibre causes a mechanically irritating effect on the large bowel mucosa, stimulating water and mucus' secretion. In contrast, the soluble fibre has a high water-holding capacity that resists dehydration in the large bowel (McRorie & McKeown 2017).

AEG and GEG groups presented higher *Lactobacillus* spp. counts ($P \leq 0.05$) then CG and CEG groups ($P \leq 0.05$) (Figure 5c). The experimental groups presented higher *Bifidobacterium* spp. counts concerning the CG ($P \leq 0.05$). The increase in *Lactobacillus* spp. and *Bifidobacterium* spp. counts verified in the present study corroborate the results reported by Huang et al. (2014), who observed average increases of ~1.0–2.4 log CFU/g in the counts of these bacterial groups in the caecum contents of hamsters fed diets supplemented with pineapple peel (2.5 g, 5 g and 10 g). Huang et al. (2014) suggested that the modulatory effects on beneficial bacteria counts could be associated with the fibre and phenolic compounds present in the fruit by-products.

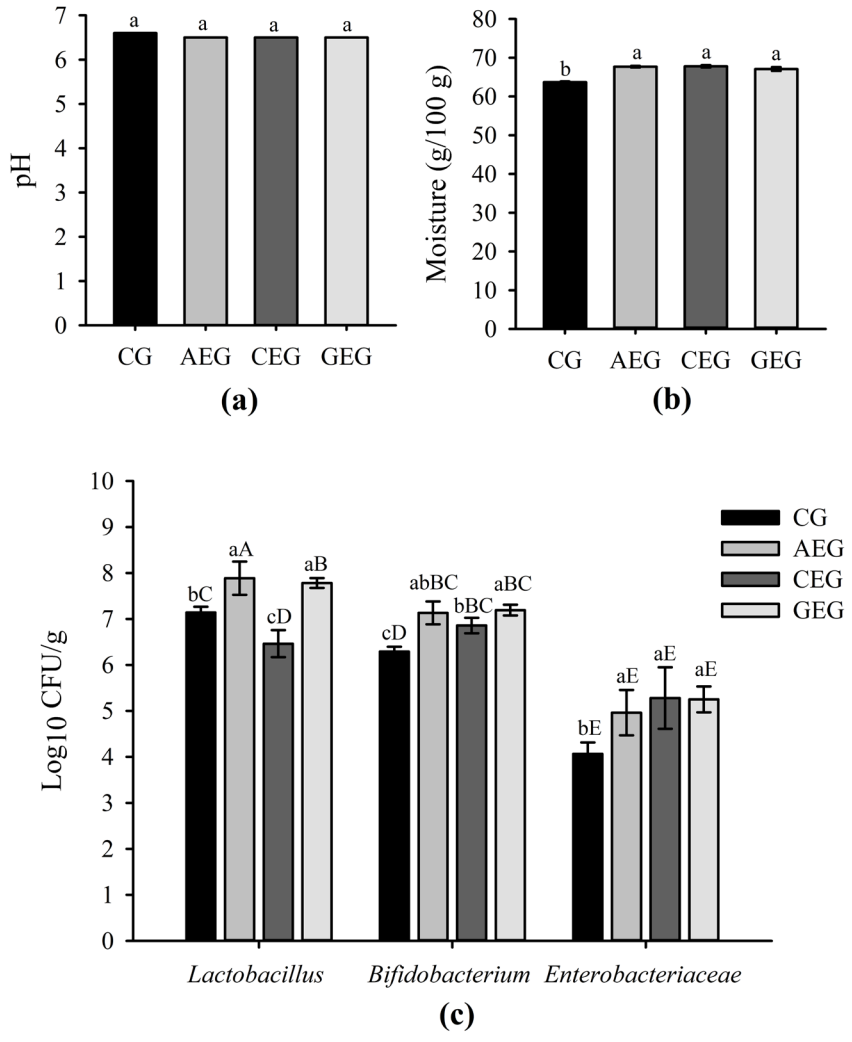

Figure 5. pH (a), moisture (b) and viable cell count of *Bifidobacterium* spp., *Lactobacillus* spp. and *Enterobacteriaceae* (c) in faeces of rats supplemented or not with acerola, cashew, or guava by-products. [a,b,c] Different lowercase letters in vertical bars indicate a significant difference between the mean ± standard deviation of each rats group and [A,B,C,D,E] different uppercase letters in vertical bars indicate a significant difference between bacterial groups (one-way ANOVA, $P \leq 0.05$, Tukey's *post hoc* test; $n = 8$ rats/group). CG = control group; AEG= acerola by-product experimental group; CEG = cashew by-product experimental group; GEG= guava by-product experimental group.

The supplementation with acerola, cashew and guava by-products likely increased the availability of fibre and phenolics in the colon, increasing fermentation products which are substrates for microbial growth (Huang et al. 2014, Paturi et al. 2017). Acerola, cashew, and guava by-products have different amounts of phenolic compounds. The major compounds common to these by-products are myricetin, 3,4-dihydroxybenzoic acid and salicylic acid, as well as different fractions of soluble, insoluble and total fibres, as previously characterized by Batista et al. (2018). *Lactobacillus* and *Bifidobacterium* species may provide various health benefits, such as inhibiting intestinal colonisation by pathogens through competitive exclusion, serum cholesterol reduction, intestinal cancer prevention, innate and cellular immune stimulation (Kechagia et al. 2013, Grom et al. 2020).

The faeces of the experimental groups (AEG, CEG and GEG) presented similar *Enterobacteriaceae* counts ($P > 0.05$). The increase in faecal *Enterobacteriaceae* counts in experimental groups (da Silva et al. 2013) compared to those found in the CG ($P \leq 0.05$) could be associated with the high diversity of bioactive compounds present in the fruit processing by-products under study. Nevertheless, although higher *Enterobacteriaceae* counts are observed in the AEG, CEG and GEG experimental groups, special attention should be paid to the fact that these counts are always lower than the *Lactobacillus* spp. and *Bifidobacterium* spp. counts ($P \leq 0.05$), demonstrating positive effects on the intestinal microbiota composition. However, the selective stimulation of beneficial microorganisms that form the intestinal microbiota to the detriment of pathogenic microorganisms (i.e. enterobacteria) is the main property of prebiotic components, which would most likely be best evidenced in the results obtained over a longer study period.

Furthermore, the consumption and metabolization of the carotenoids found in fruit by-products (Table I) by the animals may have avoided higher growth of *Enterobacteriaceae*, influencing the production by Paneth intestinal cells of defensins that control the bacterial population density at the intestinal mucosa surface (Biesalski 2016); however, the effects of carotenoid metabolism in intestinal health is not well established in the literature (Lyu et al. 2018).

Histological examination demonstrated that all analysed colon tissues presented a standard of normality (Figure 6), indicating that supplementation with the acerola, cashew and guava processing by-products did not cause alterations in the intestinal epithelium during the experimental period. Studies have reported the efficacy of fruit bioactive compounds in preserving the cell structure of the intestinal epithelium in healthy rats (Ramiro-Puig et al. 2008) and in recovering from damage caused by dyslipidaemia (Batista et al. 2018), colitis (Scarminio et al. 2012) and colon cancer (Romualdo et al. 2015).

As limitations of the present study, we observed that other doses of the product could have been administered to establish a dose-response curve. Also, long-term effects could have been evaluated. However, the study was conducted based on the dose of 400 mg of by-product/kg of rat weight, which shows a plausible dose for human consumption. Calculating the dose conversion from rodents to humans is equivalent to 3.64 g (Nair & Jacob 2016). According to our results, fruit by-products can be considered a good alternative for functional food, easily accessible to the population, and low cost that can be processed on an industrial scale for human consumption,

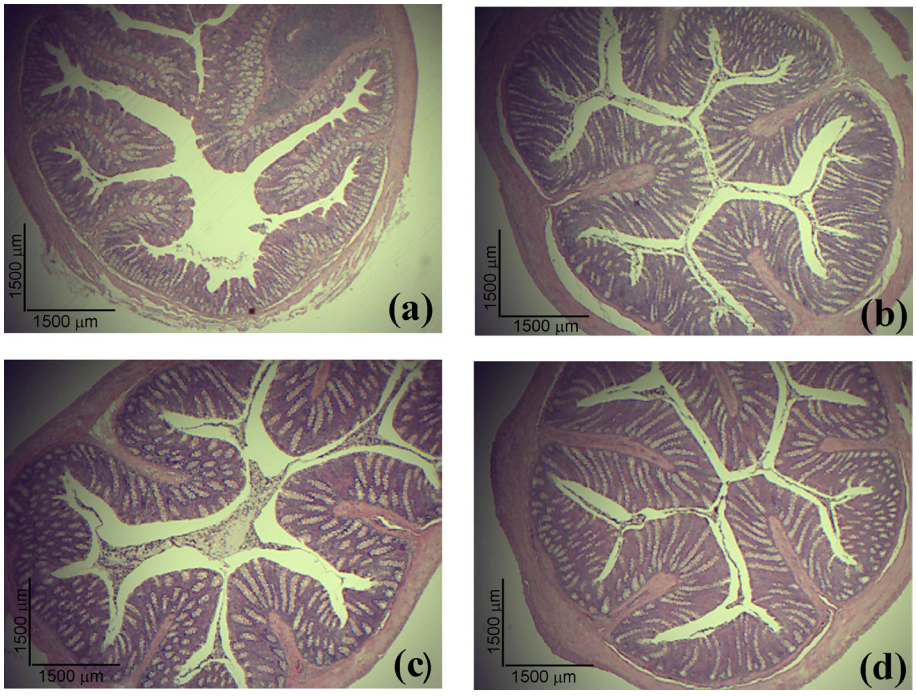

Figure 6. Photomicrographs (H&E) of the colons of rats of the control group (a) and groups supplemented with acerola (b), cashew (c) or guava (d) by-products.

aiming to sustain the chain's sustainability. To this end, conducting translational studies with the healthy population and having chronic disease is suggested.

CONCLUSION

The supplementation of fruit by-products, especially the by-products of acerola and guava, favourably modulated the intestinal function, lipid profile and retinol status in healthy rats, while the supplementation with the cashew by-product decreased the blood glucose levels, thus the effects that varied according to the respective bioactive composition. Further studies are needed to establish the role of bioactive compounds of acerola, cashew, and guava by-products on lipid, glycidic and vitamin A metabolisms, both in healthy animals and animals with metabolic diseases.

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REFERENCES

AOAC. 2016. Official methods of analysis of AOAC international. 20th edition, Washington: AOAC.

APHA - AMERICAN PUBLIC HEALTH ASSOCIATION. 2001. Compendium of methods for the microbiological examination of foods. 4th edition, Washington: APHA.

AQUINO JS, VASCONCELOS MHA, PESSOA DCNP, SOARES JKB, PRADO JPS, MASCARENHAS RJ, MAGNANI M & STAMFORD TLM. 2016. Intake of cookies made with buriti oil (*Mauritia flexuosa*) improves vitamin A status and lipid profiles in young rats. Food Funct 7: 4442-4450.

AYALA-ZAVALA JF, VEGA-VEGA V, ROSAS-DOMÍNGUEZ C, PALAFOX-CARLOS H, VILLA-RODRIGUEZ JA, SIDDIQUI MDW, DÁVILA-AVIÑA JE & GONZÁLEZ-AGUILAR GA. 2011. Agro-industrial potential of exotic fruit by-products as a source of food additives. Food Res Int 44: 1866-1874.

BATISTA KS ET AL. 2018. Beneficial effects of consumption of acerola, cashew or guava processing by-products on

intestinal health and lipid metabolism in dyslipidaemic female Wistar rats. Br J Nutr 119: 30-41.

BELIZÁRIO JE, FAINTUCH J & GARAY-MALPARTIDA M. 2018. Gut microbiome dysbiosis and immunometabolism: new frontiers for treatment of metabolic diseases. Mediators Inflamm 2018: 1-12.

BIESALSKI HK. 2016. Nutrition meets the microbiome: micronutrients and the microbiota. Ann N Y Acad Sci 1372: 53-64.

CARVALHO DV, SANTOS FA, DE LIMA RP, VIANA AFSC, FONSECA SGC, NUNES PIG, DE MELO TS, GALLÃO MI & DE BRITO ES. 2018. Influence of low molecular weight compounds associated to cashew (*Anacardium occidentale* L.) fiber on lipid metabolism, glycemia and insulinemia of normal mice. Bioact Carbohydr Diet Fibre 13: 1-6.

DA SILVA JK ET AL. 2013. Antioxidant activity of aqueous extract of passion fruit (*Passiflora edulis*) leaves: *in vitro* and *in vivo* study. Food Res Int 53: 882-890.

DANTAS AM, MAFALDO IM, OLIVEIRA PML, LIMA MS, MAGNANI M & BORGES GSC. 2019. Bioaccessibility of phenolic compounds in native and exotic frozen pulps explored in Brazil using a digestion model coupled with a simulated intestinal barrier. Food Chem 274: 202-214.

DE ABREU FP, DORNIER M, DIONISIO AP, CARAIL M, CARIS-VEYRAT C & DHUIQUE-MAYER C. 2013. Cashew apple (*Anacardium occidentale* L.) extract from by-product of juice processing: a focus on carotenoids. Food Chem 138: 25-31.

ELLONG EN, BILLARD C, ADENET S & ROCHEFORT K. 2015. Polyphenols, carotenoids, vitamin c content in tropical fruits and vegetables and impact of processing methods. Food Nutr Sci 06: 299-313.

FAO - FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS. 2018. FAOSTAT - Compare data. Available from http://www.fao.org/faostat/en/#compare [accessed 19 September 2018].

FOLCH J, LESS M & STANLEY S. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226: 497-509.

GARCÍA-CAYUELA T, NUÑO-ESCOBAR B, WELTI-CHANES J & CANO MP. 2018. *In vitro* bioaccessibility of individual carotenoids from persimmon (*Diospyros kaki*, cv. Rojo Brillante) used as an ingredient in a model dairy food. J Sci Food Agric 98: 3246-3254.

GÓMEZ-GARCÍA R, CAMPOS DA, AGUILAR CN, MADUREIRA AR & PINTADO M. 2020. Valorization of melon fruit (*Cucumis melo* L.) by-products: phytochemical and biofunctional properties with emphasis on recent trends and advances. Trends Food Sci Technol 99: 507-519.

GROM LC ET AL. 2020. Postprandial glycemia in healthy subjects: Which probiotic dairy food is more adequate? J Dairy Sci 103: 1110-1119.

HUANG YL, TSAI YH & CHOW CJ. 2014. Water-insoluble fiber-rich fraction from pineapple peel improves intestinal function in hamsters: evidence from cecal and fecal indicators. Nutr Res 34(4): 346-354.

KECHAGIA M, BASOULIS D, KONSTANTOPOULOU S, DIMITRIADI D, GYFTOPOULOU K, SKARMOUTSOU N & FAKIRI EM. 2013. Health benefits of probiotics: a review. ISRN Nutr 2013: 1-7.

LICHTENTHALER HK & BUSCHMANN C. 2001. Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. Curr Protoc Food Anal Chem 1: F4.3.1-F4.3.8.

LYU Y, WU L, WANG F, SHEN X & LIN D. 2018. Carotenoid supplementation and retinoic acid in immunoglobulin A regulation of the gut microbiota dysbiosis. Exp Biol Med 243: 613-620.

MACHO-GONZÁLEZ A, GARCIMARTÍN A, NAES F, LÓPEZ-OLIVA ME, AMORES-ARROJO A, GONZÁLEZ-MUÑOZ MJ, BASTIDA S, BENEDÍ J & SÁNCHEZ-MUNIZ FJ. 2018. Effects of fiber purified extract of carob fruit on fat digestion and postprandial lipemia in healthy rats. J Agric Food Chem 66: 6734-6741.

MCRORIE JW & MCKEOWN NM. 2017. Understanding the physics of functional fibers in the gastrointestinal tract: an evidence-based approach to resolving enduring misconceptions about insoluble and soluble fiber. J Acad Nutr Diet 117: 251-264.

MEDEIROS IUD DE, AQUINO J DE S, CAVALCANTI NS DE H, CAMPOS ARN, CORDEIRO AMT DE M, DAMASCENO KSF DA SC & HOSKIN RT. 2019. Characterization and functionality of fibre-rich pomaces from the tropical fruit pulp industry. Br Food J 122: 813-826.

MIESZCZAKOWSKA-FRĄC M, CELEJEWSKA K & PŁOCHARSKI W. 2021. Impact of innovative technologies on the content of vitamin C and its bioavailability from processed fruit and vegetable products. Antioxidants 10: 54.

MILES AA, MISRA S & IRWIN JO. 1938. The estimation of the bactericidal power of the blood. J Hyg (Lond.) 38: 732-749.

NAIR A & JACOB S. 2016. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm 7: 27-31.

NIE Q, HU J, GAO H, FAN L, CHEN H & NIE S. 2017. Polysaccharide from *Plantago asiatica* L. attenuates hyperglycemia, hyperlipidemia and affects colon microbiota in type 2 diabetic rats. Food Hydrocoll 86: 34-42.

NOVELLI ELB, DINIZ YS, GALHARDI CM, EBAID GMX, RODRIGUES HG, MANI F, FERNANDES AAH, CICOGNA AC & NOVELLI FILHO JLVB. 2007. Anthropometrical parameters and markers of obesity in rats. Lab Anim 41: 111-119.

PATURI G, BUTTS CA, STOKLOSINSKI H, HERATH TD & MONRO JA. 2017. Short-term feeding of fermentable dietary fibres influences the gut microbiota composition and metabolic activity in rats. Int J Food Sci Technol 52: 2572-2581.

QI J, LI Y, MASAMBA KG, SHOEMAKER CF, ZHONG F, MAJEED H & MA J. 2016. The effect of chemical treatment on the *in vitro* hypoglycemic properties of rice bran insoluble dietary fiber. Food Hydrocoll 52: 699-706.

RAMIRO-PUIG E, PÉREZ-CANO FJ, RAMOS-ROMERO S, PÉREZ-BEREZO T, CASTELLOTE C, PERMANYER J, FRANCH À, IZQUIERDO-PULIDO M & CASTELL M. 2008. Intestinal immune system of young rats influenced by cocoa-enriched diet. J Nutr Biochem 19: 555-565.

RELEVY NZ, HARATS D, HARARI A, BEN-AMOTZ A, BITZUR R, RÜHR R & SHAISH A. 2015. Vitamin A-deficient diet accelerated atherogenesis in Apolipoprotein E $^{-/-}$ mice and dietary β -carotene prevents this consequence. BioMed Res Int 2015: 1-9.

REYNOSO-CAMACHO R, RODRÍGUEZ-VILLANUEVA LD, SOTELO-GONZÁLEZ AM, RAMOS-GÓMEZ M & PÉREZ-RAMÍREZ IF. 2021. Citrus decoction by-product represents a rich source of carotenoid, phytosterol, extractable and non-extractable polyphenols. Food Chem 350: 129239.

RICO X, GULLÓN B, ALONSO JL & YÁÑEZ R. 2020. Recovery of high value-added compounds from pineapple, melon, watermelon and pumpkin processing by-products: an overview. Food Res Int 132: 109086.

ROMUALDO GR, FRAGOSO MF, BORGUINI RG, DE ARAÚJO SANTIAGO MCP, FERNANDES AAH & BARBISAN LF. 2015. Protective effects of spray-dried açaí (*Euterpe oleracea* Mart) fruit pulp against initiation step of colon carcinogenesis. Food Res Int 77: 432-440.

ROOHBAKHSH A, KARIMI G & IRANSHAHI M. 2017. Carotenoids in the treatment of diabetes mellitus and its complications: a mechanistic review. Biomed Pharmacother 91: 31-42.

SAEED A, DULLART R, SCHREUDER T, BLOKZIJL H & FABER K. 2017. Disturbed vitamin A metabolism in non-alcoholic fatty liver disease (NAFLD). Nutrients 10: 1-25.

SCARMINIO V, FRUET AC, WITAICENIS A, RALL VLM & DI STASI LC. 2012. Dietary intervention with green dwarf banana flour (*Musa* sp AAA) prevents intestinal inflammation in a trinitrobenzenesulfonic acid model of rat colitis. Nutr Res 32: 202-209.

TAVARES RL, VASCONCELOS MHA, DORAND VAM, JUNIOR EUT, TOSCANO LT, QUEIROZ RT, ALVES AF, MAGNANI M, GUZMAN-QUEVEDO O & AQUINO J. 2021. *Mucuna pruriens* treatment shows anti-obesity and intestinal health effects on obese rats. Food Funct 12: 6479-6489.

VARGAS-MURGA L, DE ROSSO VV, MERCADANTE AZ & OLMEDILLA-ALONSO B. 2016. Fruits and vegetables in the Brazilian Household Budget Survey (2008–2009): carotenoid content and assessment of individual carotenoid intake. J Food Compos Anal 50: 88-96.

WANG Q, IMAM MU, YIDA Z & WANG F. 2017. Peroxisome proliferator-activated receptor gamma (PPARγ) as a target for concurrent management of diabetes and obesity-related cancer. Curr Pharm Des 23: 3677-3688.

WU L, WENG M, ZHENG H, LAI P, TANG B, CHEN J & LI Y. 2020. Hypoglycemic effect of okra aqueous extract on streptozotocin-induced diabetic rats. Food Sci Technol 40: 972-978.

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