



**HAL**  
open science

## Fermented Pineapple Juice Consumption Limits Metabolic Disorders Associated to Sugary Drinks on High-Fat Diet-Fed Mice

Marie Guérin, Fabienne Remize, Matthieu Bringart, Cynthia Planesse,  
Philippe Rondeau, Christine Robert-da Silva, Cyrielle Garcia

► **To cite this version:**

Marie Guérin, Fabienne Remize, Matthieu Bringart, Cynthia Planesse, Philippe Rondeau, et al.. Fermented Pineapple Juice Consumption Limits Metabolic Disorders Associated to Sugary Drinks on High-Fat Diet-Fed Mice. *Molecular Nutrition and Food Research*, 2023, 67 (11), 10.1002/mnfr.202200670 . hal-04468272

**HAL Id: hal-04468272**

**<https://hal.science/hal-04468272>**

Submitted on 12 Jul 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Fermented Pineapple Juice Consumption Limits Metabolic Disorders Associated to Sugary Drinks on High-Fat Diet-Fed Mice

Marie Guérin, Fabienne Remize, Matthieu Bringart, Cynthia Planesse, Philippe Rondeau, Christine Robert-Da Silva, and Cyrielle Garcia\*

**Scope:** Lactic acid fermentation (LAF) modulates the composition of food, leading to changes in safety, sensory, and nutritional properties. The effects of lactic fermented pineapple juice (FJ) supplementation on energetic metabolism of high-fat diet (HFD) fed mice are compared with either water (control), sweetened water (SW), bacteria in SW, and pineapple juice (J) supplementation.

**Methods and results:** Drink consumption and body weight are measured during the 6 weeks of experiment, whereas glycemia and lipid content are determined at the beginning and at the end of the experiment. Total energy intake is similar between all groups though the volume of juice consumed is lower than that of SWs. Weight gain is higher for mice provided with sugary drinks ( $5.65 \pm 1.32$  to  $7.74 \pm 2.98$  g) compared to water ( $4.68 \pm 0.93$  g). The FJ is less detrimental to blood carbohydrate regulation than other sugary drinks. Triglyceride (TG) and total cholesterol content are not modified following fermented juice or water consumption, contrarily to other sugary drinks. Whatever the drink, intestinal permeability is preserved. Lactic acid bacterium (LAB) population in feces is not affected by the beverage but species composition is modified.

**Conclusion:** From a health perspective, FJ is preferable to other sugary drinks to limit metabolic disorders related to HFD.

pineapple (*Ananas comosus*) has a high sugar content and presents a good balance of sugar with acidity, which contributes to its appreciated flavor.<sup>[3]</sup> This fruit can be consumed either fresh or transformed mainly in juice, fresh-cut, and cans. Pineapple global production was above 25 million tons in 2014, and this fruit is in the top 4 of tropical fruit in volume and value for export.<sup>[4,5]</sup> Lactic acid fermentation (LAF) is a valuable method to extend shelf-life and to enhance nutritional and sensory properties of food, especially F&V, while preserving their micronutrients.<sup>[6,7]</sup> Fermented F&V beverages are mostly developed with *Lactobacillus* starters, specially *Lactiplantibacillus plantarum* species that demonstrated a short latency in acidic environment and strong acidification capacity.<sup>[8]</sup> Applied to pineapple juice (J), LAF modulates sensory profile depending on the bacterial starter used, leading to either dairy flavors or spicy ones.<sup>[8]</sup> Moreover, LAF can increase significantly total phenolic content, radical scavenging activity and reduces hemolysis of blood cells exposed to free radicals.<sup>[8]</sup>

The positive effects on health of lactic fermented F&V result from the combination of F&V properties, in particular for their content in fibers and phenolics, and changes of composition due to LAF. They rely on different mechanisms, apart from a direct decrease of sugar content and increased safety due to inhibition of foodborne pathogens by acidity. Bacterial enzymes are able to separate phenolic compounds into monomers to improve their

## 1. Introduction

Unhealthy diet is one of the main risk factors responsible for obesity and related metabolic diseases. Fruit and vegetable (F&V) consumption can contribute to health enhancement.<sup>[1]</sup> Indeed, they contain various micronutrients, such as vitamins, minerals, and phenolic compounds, which offer antiproliferative, antiinflammatory, and antioxidant properties.<sup>[2]</sup> Among those,

 The ORCID identification number(s) for the author(s) of this article can be found under <https://doi.org/10.1002/mnfr.202200670>

[+]Present address: Sciences pour l'œnologie (SPO), Univ Montpellier, Univ La Réunion, INRAE, Institut Agro, Montpellier, France

© 2023 The Authors. Molecular Nutrition & Food Research published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

DOI: 10.1002/mnfr.202200670

M. Guérin, F. Remize<sup>[+]</sup>, C. Garcia  
QualiSud  
Université de La Réunion, CIRAD, Université Montpellier, Institut Agro  
Montpellier, IRD, Université Avignon  
Montpellier, France  
E-mail: cyrielle.garcia@univ-reunion.fr

M. Guérin, M. Bringart, C. Planesse, P. Rondeau, C. Robert-Da Silva  
Diabète athérothrombose Thérapies Réunion Océan Indien (DéTROI),  
Inserm, UMR 1188  
Université de La Réunion  
Saint Denis, France

bioavailability and thus, induce beneficial effects.<sup>[9]</sup> Hence, a decrease of glycemia peak can be expected from the inhibition of digestive enzymes,<sup>[10]</sup> by plant polyphenols, possibly of modified composition after LAF. In addition, a modulation of gut microbiota can be expected from ingestion of a high population of viable bacteria<sup>[11]</sup> but also can result from bioactive metabolites from F&V.<sup>[12]</sup> Numerous positive effects of bacterial metabolites are reported, among those lactate and exopolysaccharides, which exert an antioxidant effect.<sup>[11]</sup>

Improvement of diet-induced metabolic syndrome in mice, as well as enrichment of gut microbial abundance, has been demonstrated with the consumption of fermented fruit beverages using yeast probiotics combined with lactic acid bacterium (LAB) but did not allow to highlight the specific effects assignable to the tested bacterial strains.<sup>[13]</sup> A beneficial effect of the consumption of LAB fermented apple juices was only showed on streptozocin-induced diabetic mice.<sup>[14,15]</sup> In this study, the aim was to determine if chemical and microbial composition of fermented pineapple juice (FJ) was able to induce early observable effects on mouse metabolic disorder apparition. To achieve that, mice were fed with a high-fat diet (HFD) and were provided with different beverages, including water, sweetened water (SW), and J, fermented or not, during a 6-week experiment.

## 2. Experimental Section

### 2.1. Microorganisms

The bacterial strains used, *Leuconostoc pseudomesenteroides* 56 and *Lactiplantibacillus plantarum* 75, previously isolated and genotyped, were reactivated then cultured in De Man, Rogosa and Sharpe (MRS) broth and incubated for 48 h at 30 °C prior to inoculation of juice.

### 2.2. Preparation and Fermentation of Pineapple Juice

Fresh pineapples (*A. comosus* var. "Queen Victoria") were washed, peeled, and cut. The juice was extracted using Philips Micro-Masticating juice extractor, filtered, and bottled. J was pasteurized in a water bath at 80 °C during 10 min, prior to cooling to room temperature within 2 h. The following steps were performed under sterile conditions. From *L. pseudomesenteroides* 56 and *L. plantarum* 75 bacterial cultures, an inoculation level of 0.05 uOD mL<sup>-1</sup>, corresponding to 1.10<sup>7</sup> bacteria mL<sup>-1</sup> according to McFarland standards, was calculated for each strain from turbidity at 600 nm. The adequate volume was collected and centrifuged at 5000 × g for 5 min at 10 °C. The cell pellet was washed twice and resuspended in sterile distilled water. Each bacterial culture was separately inoculated in J. Inoculated and not inoculated juices were incubated at 30 °C for 48 h. Then, the Js fermented with each strain were mixed and maintained at 4 °C for 12 days. The level of inoculated LAB in pineapple fermented juice allowed to be above the amount of 1.10<sup>6-7</sup> bacteria mL<sup>-1</sup> described as having beneficial effect for probiotics according to FDA/WHO.<sup>[1]</sup> Following a ½ dilution in sterile water, juices were stored at -20 °C until use. Beverages were thawed at 4 °C the day before administration.

### 2.3. Fermented Pineapple Juice Characteristics

LAB enumeration was performed on MRS agar. Briefly, serial decimal dilutions of juices were prepared into sterile peptone water solution and dropped on MRS plates before incubation at 30 °C for 48 h. Yeast and mold counts were performed on glucose chloramphenicol agar plates. Each determination was performed in triplicate.

Total soluble solids (TSS) of fermented and not-fermented juices were determined with ATAGO PAL-3 pocket refractometer (Atago USA Inc., WA, USA) and expressed as °Brix (±0.1 °B). Fermented and not-fermented juice fiber content was described to be 1.4% from standardized analysis.<sup>[16]</sup> The Folin-Ciocalteu assay was used to measure the total polyphenol content of J and FJ as previously described<sup>[17]</sup> using microplate reader (Infinite M200 PRO; Tecan, Männedorf, Switzerland), and the results were expressed as gallic acid equivalents (GAEs).

The pH of fermented and not-fermented juices was determined with an uncertainty of 0.1 with a Eutech pH2700 apparatus (EUTECH Instruments, IL, USA).

### 2.4. α-Glucosidase Inhibition Assay of Pineapple Juices

The α-glucosidase inhibitory activity was determined according to Zhang et al.<sup>[18]</sup> and was assayed with type I α-glucosidase from baker's yeast in a 0.1 M sodium phosphate buffer (pH 6.9). A buffer solution containing 1 U mL<sup>-1</sup> α-glucosidase solution was incubated with J at 37 °C under shaking during 2 min. Inhibition control was performed with 0.5 mg mL<sup>-1</sup> voglibose solution. A 2 mM *p*-nitrophenol-α-D-glucopyranoside solution (12.5%) was added as substrate and the absorbance was monitored every minute for 20 min using a microplate reader (Infinite M200 PRO, Tecan, Männedorf, Switzerland) to follow the released of the product colored compounds. The α-glucosidase specific activity was calculated from the OD at 405 nm slope, divided by the enzyme concentration, expressed as OD/(min U).

### 2.5. Preparation of Sweetened Water Containing LAB

Sucrose concentration in SW was chosen to be similar to that of J, which exhibited 17.4 °Brix, diluted in water. Under sterile conditions, a solution at 87.2 g L<sup>-1</sup> of sucrose was prepared. Inoculation was performed as for Js using *L. pseudomesenteroides* 56 and *L. plantarum* 75 bacterial cultures with an inoculation level of 0.05 uOD mL<sup>-1</sup>. SW, with or without LAB, was stored at -20 °C until use. LAB enumeration, TSS, and pH were carried out as previously described.

### 2.6. Experimental Parameters

#### 2.6.1. Mouse Model

Forty-six male C57BL/6J mice were used in this study. Animals were housed in a controlled environment, under constant temperature (21 ± 2 °C), humidity (50% ± 5%), and 12 h light-dark cycle. All experimental procedures were conducted in compliance with animal protocols approved by the French Ministry of

Higher Education and Research (project # 019011015097480). After 5 weeks of acclimatization, mice (12 weeks old and  $25.3 \pm 1.7$  g at the beginning of the experiment, Janvier Labs, Saint Berthevin, France) were randomly divided into one group of six mice (control –C– corresponding to water proposed ad libitum) and four groups of ten mice receiving ad libitum either SW, LAB in SW (BSW), J, or FJ. Mice receiving sugary drinks were housed by 5 and those from the control group were housed by 3, and received food and water ad libitum. All groups were fed with an HFD corresponding to a 23.5% fat semipure rodent diet (SF04-001, Specialty Feeds, Glen Forrest, Western Australia) during the 6-weeks experiment, leading to a preobesity state. Food and sugary beverage intakes were determined once-a-week and three times-a-week, respectively, from the difference between proposed and remaining quantities. The amount of HFD consumed also included the food losses that may occur when mice consumed the solid diet. Energy intake was then estimated per mouse and per day using the energetic value of each food, neglecting bacteria. Digestible energy of HFD was  $19.0$  kJ g<sup>-1</sup> according to the feed manufacturer. The used value for J was  $1.2$  kJ mL<sup>-1</sup> and was obtained from the average of the values found in commercial Js from Reunion Island taking into account dilution. For SW, the energy value of saccharose was used ( $1.5$  kJ mL<sup>-1</sup>). Total energy intake was calculated by addition of energy intake from HFD and beverage consumption.

Before the beginning of the experiment (baseline values) and at several evaluation points, body weight was measured, and blood samples and feces were collected. During this experiment, two mice of the SW group died on day 25 and one mouse of the BSW group died on day 29. At the end of the experiment, mice were sacrificed by cardiac puncture under general anesthesia with isoflurane (2%), and intestine, liver, and adipose tissue were collected, weighted, and/or measured, and kept for later analysis at  $-80$  °C.

### 2.6.2. Assessment of Blood Parameters

After a 12 h fast, glycemia was assessed from the tail of mice at the beginning and at the end of the experiment using a commercial glucometer (OneTouch Verio Flex meter, OneTouch, USA). An oral glucose tolerance test (OGTT) was performed on day 39 of the experiment after a 6 h fast. Glucose (30% of body weight) was administered by gavage and blood sugar was measured at 0 (baseline), 15, 30, 45, 60, 90, and 120 min.

At the beginning and at the end of the experiment, mice were fasted for 4 h and blood samples were collected by retro-orbital sampling and stored at  $-80$  °C. Then, triglyceride (TG) and total cholesterol concentrations were determined using Triglycerides FS 5' and Cholesterol FS 10' kits (DiaSys Diagnostic Systems GmbH, Holzheim, Germany) according to manufacturer's instructions. Assessment of blood parameters using these assay kits were within the range previously reported in the literature.<sup>[19–21]</sup>

### 2.6.3. Measurement of Zonula Occludens-1 (ZO-1) in Small Intestines

ZO-1 levels were determined using a mouse Tight Junction Protein 1 (TJP 1) ELISA kit (Wuhan Fine Biotech Co., Ltd, Wuhan,

China). Briefly, 200 mg of small intestine (ileum) sample was mixed with 500  $\mu$ L of PBS 1X buffer containing a protein kinase inhibitor, lysed, and centrifuged. The supernatant was collected, and the assay was performed following the manufacturer's instructions. Results were normalized using total protein contents measured by bicinchoninic acid assay (BCA). Briefly, samples were incubated with the BCA reagent at 37 °C for 1 h and absorbance was read at 562 nm.

### 2.6.4. Histopathological Evaluation

The small intestine was rapidly removed after sacrifice, weighed, and divided into duodenum, proximal jejunum, distal jejunum, and ileum (1:1:1:1 ratio) and flushed with cold saline solution. Proximal section of the duodenum was fixed in 4% paraformaldehyde, embedded in paraffin, and dissected in 5- $\mu$ m sections using Eprelia™ HM 340E Electronic Rotary Microtome (ThermoFisher Scientific, Waltham, MA, USA). Then, the sections were stained with hematoxylin and eosin (H&E). Pictures were obtained using NanoZoomer S60 digital slides scanner (Hamamatsu Photonics, Hamamatsu City, Japan).

### 2.6.5. Assessment of Fecal Microbial Population

LAB population was determined in the collected feces at the beginning, at half, and at the end of the experiment. Serial dilutions of the samples were made in sterile 0.9% sodium chloride and spread on MRS plates before incubation at 37 °C for 48 h. Each determination was performed in triplicate. Bacterial colonies were subcultured and isolates were preserved in Protect Microorganism Preservation System tubes (Technical Service Consultants Ltd, Lancashire, UK) at  $-80$  °C.

For DNA extraction, InstaGene Matrix (Bio-Rad Laboratories, Marne-la-Coquette, France) was used according to supplier recommendations. Identification was performed on feces collected at D41 by sequencing of the region coding 16S RNA according to Fessard et al. (2017).<sup>[22]</sup> The used primers were RD1m (5'-GGMTACCTACGAYTTC-3') and FD1m (5'-AGAGTTTGATCCTGGCTCAG-3') (Eurogentec, Seraing, Belgium). The reaction mixture contained 1X buffer, 0.2 mM dNTP, 0.3  $\mu$ M per primer, 2.5 mM MgCl<sub>2</sub>, 0.05 U  $\mu$ L<sup>-1</sup> Taq polymerase (Eurogentec, Seraing, Belgium), and DNA solution. PCR was performed with Bio-Rad S1000 Thermal Cycler (Bio-Rad Laboratories, Marne-la-Coquette, France). PCR products were then separated by electrophoresis (30 min at 100 V) on 0.8% agarose gel in 0.5X TAE. DNA was stained with ethidium bromide and detected using a UV-illuminator system. PCR product purification was performed using QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany). Sequencing was achieved by Eurofins Genomics (Ebersberg, Germany) and the resulting sequences were compared with NCBI Nucleotide database with the BLASTN program. To characterize the genotype of bacterial strains a Rep-PCR method based on (GTG)<sub>5</sub> primer (5'-GTGGTGGTGGTGGTGGTGG-3') (Eurogentec, Seraing, Belgium) was performed according to Fessard and Remize (2019).<sup>[23]</sup> PCR products were then separated by electrophoresis (3 h at 50 V) on 0.8% agarose gel in 0.5X TAE.

**Table 1.** Juice (J) and fermented juice (FJ) bacterial population, pH, total soluble solids (TSS) content, and inhibition of  $\alpha$ -glucosidase activity (%).

	J		FJ	
	Day 0	Day 7	Day 0	Day 7
Bacterial population [log CFU mL <sup>-1</sup> ]	0.0 ± 0.0	0.0 ± 0.0	5.7 ± 1.2	11.8 ± 0.6
pH	3.4	3.4	3.5	3.3
TSS [°Brix]	16.8	17.0	17.9	17.8
Activity inhibition [%]				
Dilution 1/5		76.0 ± 5.2 a (21.5)		58.5 ± 12.1 a (29.1)
1/10		65.6 ± 1.9 a (5.6)		49.8 ± 17.5 a (34.9)
1/20		60.2 ± 12.3 a (30.9)		56.9 ± 10.9 a (25.4)
1/40				45.4 ± 2.0 a (3.6)
1/80				42.2 ± 6.4 a (11.1)

Inhibition by voglibose 0.5 mg mL<sup>-1</sup>: 63.4% ± 1.5%. Results are presented as mean ± SD (CV%). Different letters correspond to significantly different values from two-way analysis of variance (ANOVA),  $p < 0.05$ .

## 2.7. Statistics

To quantify the sample size, a power analysis was realized with an  $\alpha$ -level of 0.05 and a  $\beta$ -level of 0.1 based on previous study investigating the effect of yeast fermented juice consumption on mice lipid metabolism.<sup>[24]</sup> Sample size was adjusted to ten animals per group for the anticipated animal losses. All values are expressed as mean ± standard deviation. The statistical analysis was performed using XLSTAT (Addinsoft). Analysis of variance (ANOVA) was performed with the Ryan–Einot–Gabriel–Welsch (REGWQ) test and comparison to control according to Dunnett's test. Differences were considered as significant for indicated  $p$ -values.

## 3. Results

### 3.1. Pineapple Juice Properties

Bacterial population increased from  $5.7 \pm 1.2$  log of colony-forming unit (CFU) mL<sup>-1</sup> to  $11.8 \pm 0.6$  log CFU mL<sup>-1</sup> between day 0 and day 7 after inoculation, confirming a proper fermentation of the juice (Table 1). On the same period, no yeast or mold were detected thus showing the absence of contamination. The pH values were not significantly modified during fermentation, as expected since the initial pH of pineapple was already low. TSS content was not significantly reduced by fermentation.

### 3.2. Effect of Pineapple Juices on a Digestive Enzyme Activity

J, fermented or not, inhibited  $\alpha$ -glucosidase, involved in carbohydrate hydrolysis during digestion. The inhibition of the enzyme tended to decrease with dilutions of J, fermented or not (Table 1) ( $p$ -value < 0.0001).

### 3.3. Drink Consumption, Energy Intake, and Weight Gain of Mice

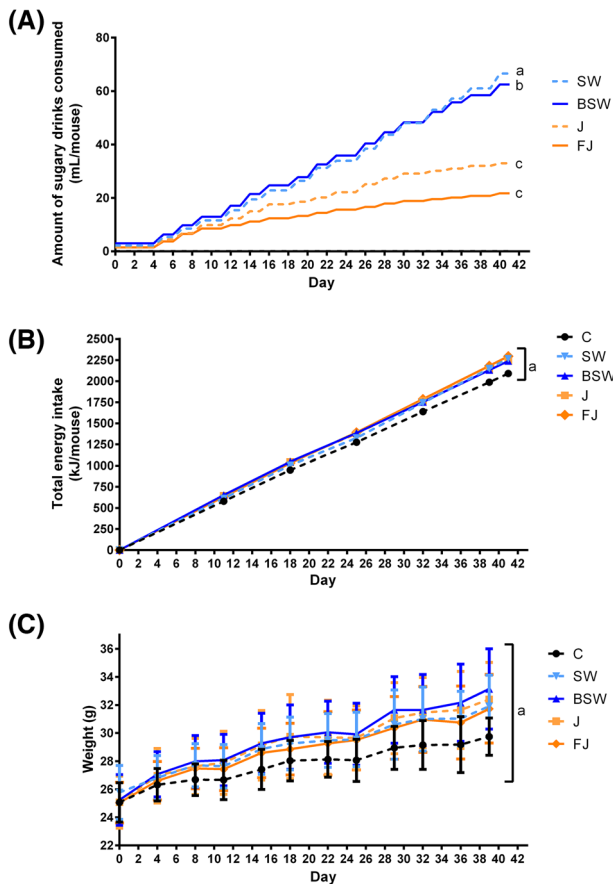
During the 6-week experiment, solid food intake volume and energy were the same regardless of the drink administered (Table

S1). The volume of sugary drink consumption depended on the beverage proposed. FJ and J consumptions were lower than SW and BSW consumption (Figure 1a). The drink consumption was stable for J and increased significantly for SW and BSW, compared to FJ leading to significantly different consumption of SW and BSW to that of FJ after 22 days and to that of J after 29 days.

Energy intake of drinks represented 5.1%–10.7% of the total energy intake for SW and BSW, whereas it was in the range 7.6%–9.3% for J and 4.8%–7.2% for FJ (Figure S1). The ratio of energy provided by drink to food became different between water and other drinks after 11 days ( $p$ -value = 0.008), between SW, BSW plus J and FJ after 32 days ( $p$ -value = 0.001) and the difference increased ( $p$ -value = 0.0003) thereafter. However, the groups which consumed sugary drinks showed a total energy intake similar to that of the control group, because the energy provided by the drink remained below 11% of the total energy intake (Figure 1b). The weight gain of mice, measured three-times a week, did not show statistical difference according to the groups receiving different sugary drinks (Figure 1c). The four groups gained more weight than the control group (water), though slightly significant ( $p$ -value = 0.025 after 39 days). Fat epididymal mass of the BSW group increased significantly compared to the control group, but this was not found for other groups receiving sugary drinks (Table S2). Fat mass of the FJ group was only 10.8% above that of the control. This result suggests that weight gain was not primarily directed toward fat weight. Adipose tissue of the control and FJ groups weighted  $0.8 \pm 0.3$  g and  $0.9 \pm 0.2$  g respectively at D41. Conversely, J consumption increased adipose tissue weight to  $1.2 \pm 0.4$  g although not significant compared to the control group, and a significant increase of adipose tissue weight was obtained only for the BSW group compared to the control group. Weight of the liver was higher for all groups compared to the control.

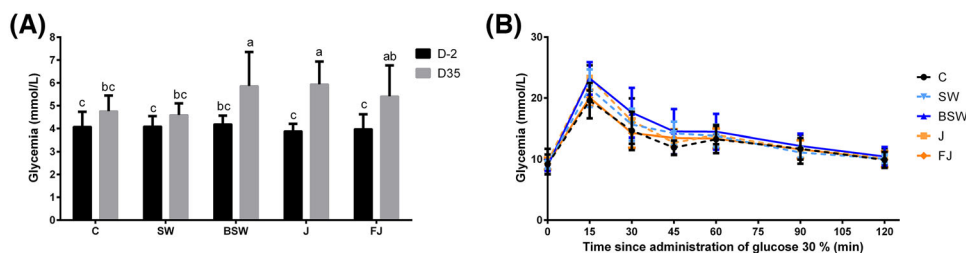
### 3.4. Effect of Fermented Juice on Carbohydrate Metabolism

Glycemia significantly increased after a 6-week supplementation of BSW and J, by 23.2% and 24.7% respectively compared to the control group (Figure 2a). OGTT showed a similar regulation for



**Figure 1.** Evolution of sugary drink consumption a), energy intake b), and mice weight c) during the experimentation. Control (C, black curve with round symbols) corresponded to mice receiving only high-fat diet (HFD) and water ad libitum. BSW, LAB in sweetened water (blue curve with triangle symbols); FJ, fermented pineapple juice (orange curve with diamond symbols); J, pineapple juice (dashed orange curve with square symbols); SW, sweetened water (dashed blue curve with inverted triangle symbols). Different letters correspond to significantly different values at day 41 from two-way analysis of variance (ANOVA),  $p < 0.0001$ .

the FJ group compared to the control group (Figure 2b). This regulation appeared to be less efficient for the other groups, especially for BSW and SW, which exhibited higher glycaemic curves. A difference of the maximum of glycaemia reached at 15 min



**Figure 2.** Glycemia at days 2 and 35 a) and oral glucose tolerance test (OGTT) after 39 days of experimentation b). Control (C, black curve with round symbols) corresponded to mice receiving only high-fat diet (HFD) and water ad libitum. BSW, LAB in sweetened water (blue curve with triangle symbols); FJ, fermented pineapple juice (orange curve with diamond symbols); J, pineapple juice (dashed orange curve with square symbols); SW, sweetened water (dashed blue curve with inverted triangle symbols). Different letters correspond to significantly different values from two-way analysis of variance (ANOVA),  $p < 0.0001$ .

**Table 2.** Values from oral glucose tolerance test (OGTT) after 39 days of experimentation.

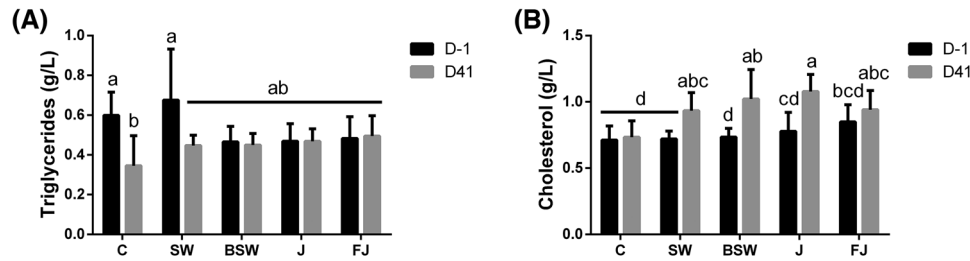
	Glycemia [mmol L <sup>-1</sup> ] after administration of glucose 30%			
	15 min	30 min	45 min	AUC [mmol L <sup>-1</sup> min <sup>-1</sup> ]
C	19.6 ± 2.9 b	14.6 ± 3.1 a	11.9 ± 1.1 a	457.5 ± 226.4 a
SW	21.6 ± 3.1 ab	15.6 ± 2.6 a	14.2 ± 1.9 a	542.7 ± 89.6 a
BSW	23.2 ± 2.7 a	17.6 ± 4.1 a	14.5 ± 3.7 a	663.5 ± 250.8 a
J	23.3 ± 2.1 a	16.2 ± 3.7 a	12.7 ± 2.0 a	475.4 ± 275.4 a
FJ	20.1 ± 1.3 b	14.2 ± 2.4 a	13.5 ± 1.4 a	424.2 ± 158.6 a

Control (C) corresponded to mice receiving only high-fat diet (HFD) and water ad libitum. BSW, LAB in sweetened water; FJ, fermented pineapple juice; J, pineapple juice; SW, sweetened water. Different letters correspond to significantly different values from two-way analysis of variance (ANOVA),  $p < 0.0001$ .

was observed, with values of 23.3 mm for BSW and J, 21.8 mm for SW, and 19.5 mm for the control. AUC increased by 45.0% for the BSW group, 18.6% for the SW group, and 3.9% for the J group compared to the control. Although not significant ( $p$ -value = 0.154), our results showed a decrease of AUC for FJ group compared to other groups (Table 2).

### 3.5. Sugary Drink Consumption Effect on Lipid Metabolism

After ingestion of an HFD supplemented with sugary drinks, our results showed a TG concentration at the end of the experiment (day 41) not significantly increased for groups supplemented with sugary drinks compared to the control (Figure 3a). For an unknown reason, groups C and SW exhibited a large discrepancy of TG content at day -1. This result is due to an intragroup difference that could not be explained. Hence, the use of these values as references should be done cautiously and do not allow us to precisely calculate TG evolution. Sugary drink consumption induced a significant increase of total cholesterol concentration whatever the drink compared to the control group at the end of the 6-week supplementation experiment (Figure 3b). Indeed, blood cholesterol concentration ranged between 0.9 and 1.1 g L<sup>-1</sup> for the groups which consumed sugary drinks, versus 0.7 g L<sup>-1</sup> for the control. A difference of cholesterol concentration between day 1 and day 41 was observed for SW, BSW, and J groups, but not for the control and FJ group.



**Figure 3.** Triglyceride a) and total cholesterol b) concentrations in blood at day –1 and day 41 for the five groups of mice. Control (C) corresponded to mice receiving only high-fat diet (HFD) and water ad libitum. BSW, LAB in sweetened water; FJ, fermented pineapple juice; J, pineapple juice; SW, sweetened water. Different letters correspond to significantly different values from two-way analysis of variance (ANOVA),  $p < 0.0001$ .

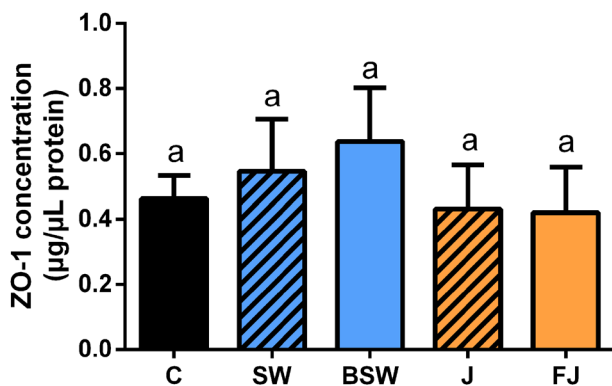
### 3.6. Sugary Drink Consumption Effect on Intestinal Physiology

Obesity is correlated with an increased intestinal permeability due to a deficiency in proteins involved in tight junction formation and function, including the ZO-1 proteins.<sup>[25]</sup> As we induced a preobesity state by HFD consumption, ZO-1 concentration was investigated (Figure 4). This parameter did not change regardless of the drink consumed ( $p$ -value = 0.076).

Intestine weights were not significantly different between the five studied groups (Table S2). Histopathology of intestinal sections was performed using H&E staining (Figure 5). BSW group histological sections were not exploitable due to improper preparation and were not included in the analysis. No morphological differences were observed regardless of the sugary drink consumed compared to the control group. Measurements of the sections showed that crypts depth significantly increased following FJ consumption compared to SW (Figure 6). No significant differences were obtained regarding villus length and villus length/crypt depth ratio.

### 3.7. Evolution of Lactic Bacteria Colonization Following Sugary Drink Consumption

Given that some of the groups of this study received daily LAB supplementation, we looked at this population size in the feces



**Figure 4.** Zonula occludens-1 (ZO-1) concentrations in the small intestine determined by ELISA for the five groups of mice. Control (C) corresponded to mice receiving only high-fat diet (HFD) and water ad libitum. BSW, LAB in sweetened water; FJ, fermented pineapple juice; J, pineapple juice; SW, sweetened water. Different letters correspond to significantly different values from two-way analysis of variance (ANOVA),  $p < 0.0001$ .

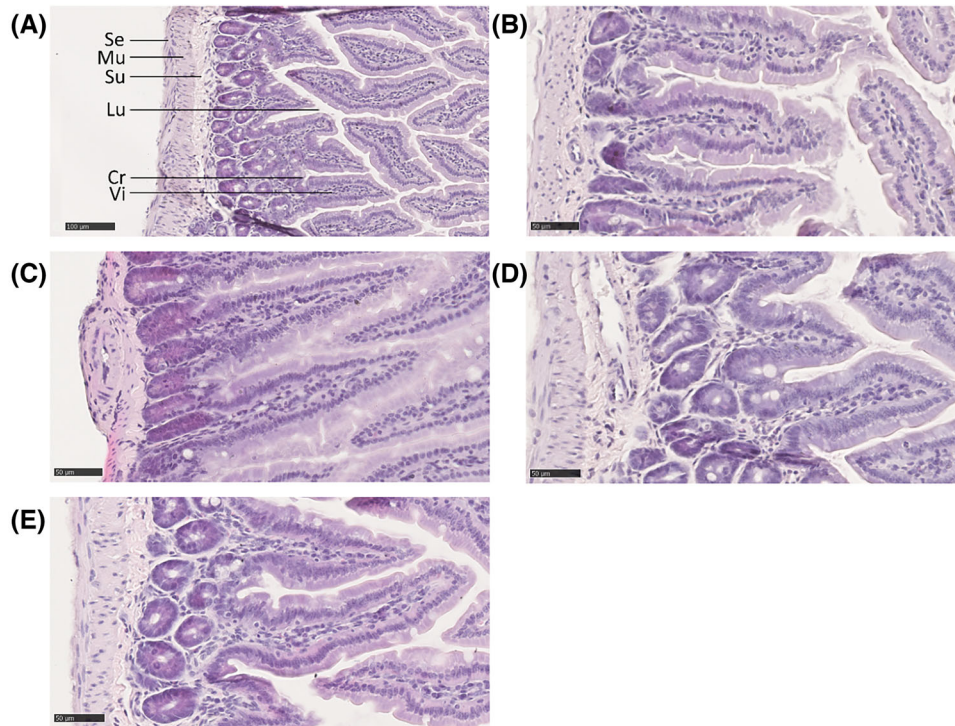
at the beginning of, at half and at the end of the 6-week experiment (Figure 7a). For the control group receiving only a HFD, we observed an increase in LAB population during the experimentation, as well as for the SW group. However, for the group receiving LAB alone (BSW), the J and LAB and juice together (FJ), LAB population remained stable. LAB species identification was then performed for C and FJ groups (Figure 7b). The control group had a majority of *L. animalis/murinus* (28 identifications out of 43 isolates, i.e., 65.1%) whereas the FJ showed a greater abundance of *Enterococcus faecalis* (34 identifications out of 49 isolates, i.e., 69.4%). *E. faecalis* was isolated on MRS agar: this culture medium is supposed to be selective to *Lactobacilli*, but the growth of *E. faecalis* was previously reported.<sup>[26–28]</sup> Interestingly, the group receiving the fermented beverage showed an increased frequency of *L. plantarum* detection compared to the control group (+13.1%). The species *L. pseudomesenteroides* was not detected in any group.

By comparing sequencing results with the profiles obtained by Rep-PCR(GTG)<sub>5</sub>, we hypothesized that the P1 profile corresponded to *L. murinus/animalis* isolates (75.6% in C and 18.4% in FJ by Rep-PCR), the P2 profile to *E. faecalis* isolates (24.4% in C and 73.5% in FJ by Rep-PCR), the P3 profile to *L. plantarum* isolates (0.0% in C and 6.1% in FJ by Rep-PCR), and the P4 profile to *L. reuteri* isolates (0.0% in C and 2.0% in FJ by Rep-PCR) (Figure 7c). The Rep-PCR profiles of strains 75 and 56 were not found in the analyzed isolates.

Hence, the microbiota composition in mice was modified by FJ consumption, but microbiota was not colonized by the ingested bacteria.

## 4. Discussion

Extensive studies using models of HFD were conducted on pathobiology of obesity-related diseases since high energy diet results in obesity and associated metabolic disorders.<sup>[29]</sup> Metabolic disorder symptoms cover intraabdominal and intraorgan fat deposition, glucose intolerance, insulin resistance, peripheral inflammation, leptin signaling dysregulation, and increased gastrointestinal permeability.<sup>[29]</sup> Addressing eating behavior by F&V consumption, specially through functional fermented food, can be an efficient prevention tool.<sup>[6,30]</sup> In this study, mice were fed with a high-caloric diet during a 6-week period to induce a preobesity state, the onset of metabolic disorders. No statistical differences were observed regarding food intake, but weight gain was slightly higher for mice supplemented with sugary drinks instead of water. However, the volume of sugary drink consumed varied depending on the beverage proposed. These variations may be

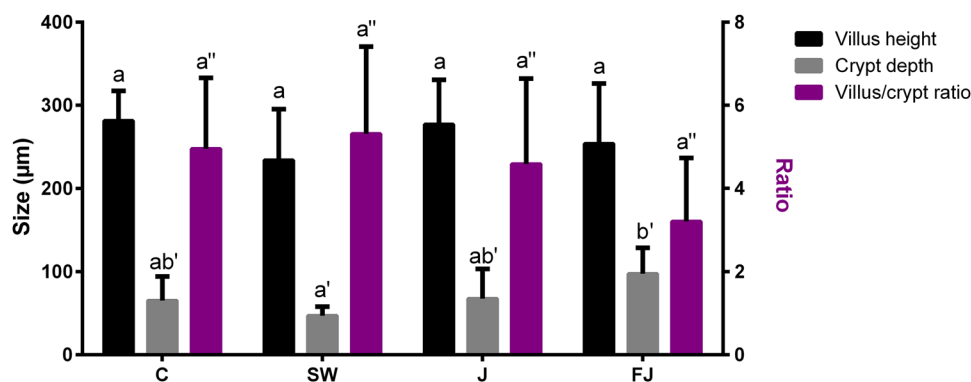


**Figure 5.** Hematoxylin and eosin (H&E) staining for pathohistological evaluation of intestinal permeability. Structure presentation a), Control (C) corresponded to mice receiving only high-fat diet (HFD) and water ad libitum b). SW, sweetened water c); J, pineapple juice d); FJ, fermented pineapple juice e). Se, serosa; Mu, muscularis externa; Su, submucosa; Lu, lumen; Cr, crypt; Vi, villus.

due to a stronger appetite for SWs suggesting a greater palatability compared to Js. Possibly, fibers contained in the juices provided satiety.<sup>[31]</sup> Js were prepared from both the pulp and the core. Pineapple core is especially rich in dietary fiber: it was previously estimated at 64.19 g/100 g of dry weight (d.w.) with two third of insoluble dietary fiber, mainly cellulose and hemicellulose, and one third of soluble dietary fiber.<sup>[32,33]</sup> Consumption of high fiber content enhances satiation and satiety. Fibers stimulate the release of gastric juice and satiety hormones.<sup>[34]</sup> They delay gastric emptying and gastric distension resulting in slower digestion and absorption in the gastrointestinal tract.<sup>[34–36]</sup> Soluble fibers possess a high water-binding capacity, have an important swelling

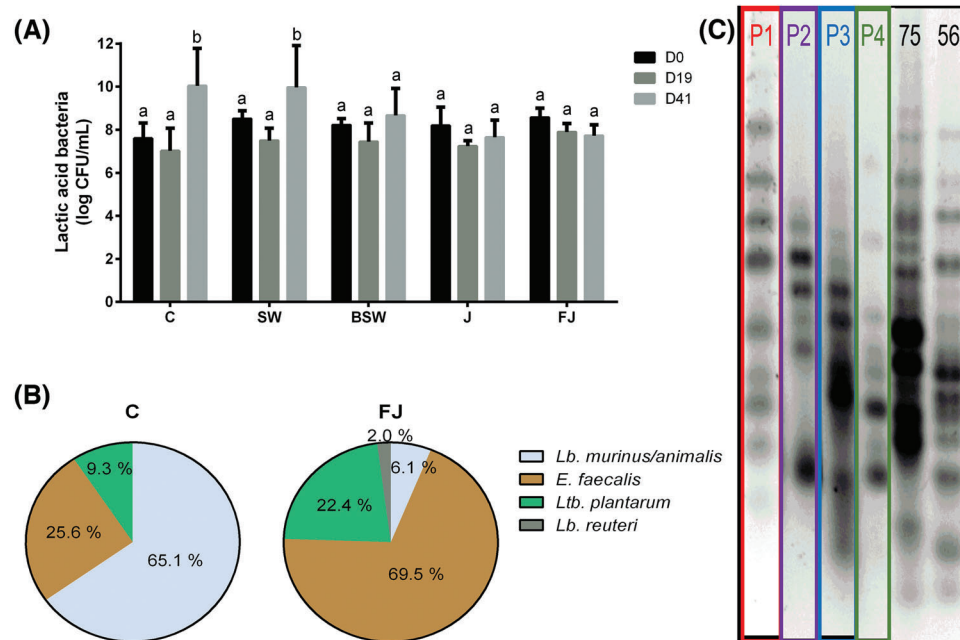
capacity, and stimulate satiety by activating vagal afferents.<sup>[37]</sup> They are also more susceptible to fermentation by colonic bacteria, generating short chain fatty acids whose affect appetite regulation.<sup>[38]</sup>

As metabolic disorders linked to HFD lead to impaired carbohydrate and lipid metabolism, blood constants were investigated. Whatever the beverage, glycemia of mice increased after 6 weeks of HFD. Compared to the control group having water, BSW and J group glycemia was higher after 35 days. The FJ and control groups showed a similar regulation of glucose concentration according to the OGTT whereas the other groups showed a tendency to higher glycemic curves. All together



**Figure 6.** Histological measurements of intestines. Purple bars values refer to the right y axis. Different letters within a measurement correspond to significantly different values from two-way analysis of variance (ANOVA),  $p < 0.05$ .





**Figure 7.** Lactic acid bacterium (LAB) population at days 0, 19, and 41 in mouse feces a); proportion of bacterial species identified from isolates of control (C) and fermented juice (FJ) mouse group feces at day 41 b); Rep-PCR profiles of LAB isolates at day 41 c). Control (C) corresponded to mice receiving only high-fat diet (HFD) and water ad libitum. BSW, LAB in sweetened water; FJ, fermented pineapple juice; J, pineapple juice; SW, sweetened water. Species graphically represented the major ones for 16S rRNA coding region sequencing. Different letters correspond to significantly different values from two-way analysis of variance (ANOVA),  $p < 0.0001$ .

these results suggest that FJ consumption, contrarily to other sugary drinks, does not increase glucose regulation disorders. Postprandial carbohydrate absorption depends on the amount of carbohydrate ingested and to their hydrolysis. The inhibition of hydrolytic enzymes can modulate digestion rate and exert an antihyperglycemic effect.<sup>[39]</sup> Silva et al. (2018) suggested that polyphenols can bind and precipitate digestive enzymes and that polyphenol extract bioactivity depends on the polyphenol composition.<sup>[40]</sup> Guaraná digests showed a dose-independent inhibitory activity on  $\alpha$ -glucosidase, possibly influencing glucose metabolism and showing an antihyperglycemic potential.<sup>[40]</sup> The activity of  $\alpha$ -glucosidase was inhibited by both J and FJ. This inhibition could induce a slowing-down of carbohydrate digestion and a spreading of the glycemic curve in the postprandial state. It could result from polyphenol composition of the J, but the impact of fermentation was not evidenced.<sup>[41]</sup> Papaya (*Carica papaya*) purée fermented with *L. pseudomesenteroides* 56 or *L. plantarum* 75 inhibited  $\alpha$ -glucosidase activity by 37% or 17%, respectively.<sup>[42]</sup> Similarly, fermented mango (*Mangifera indica* L. cv. "Cogshall") purée was more inhibitory than the not-fermented one.<sup>[43]</sup> The polyphenol composition of pineapple, different from that of papaya and mango, probably explained a more limited effect on  $\alpha$ -glucosidase inhibition of lactic fermented pineapple.<sup>[44]</sup> Probiotics can enhance fasting and postprandial blood glucose, glucose tolerance, and hepatic glycogen synthesis.<sup>[45]</sup> Hence, the observed effect of FJ on carbohydrate regulation could result from the presence of active bacteria.<sup>[45,46]</sup> A causal link has been previously demonstrated between HFD diet and altered glucose regulation with higher AUC values for OGTT, in the same range as observed here.<sup>[46]</sup>

Our results are consistent with observations on diabetic mice receiving Aksu apple juice in addition of HFD diet, which showed a reduced fasting blood glucose level compared to mice receiving the not-fermented juice and the control group, although not significant.<sup>[14]</sup>

To study energy metabolism, lipid profile was measured. TG concentration was measured since excess carbohydrates are stored as TGs and both plasma TG and total cholesterol concentration are dysregulated in metabolic disorders. C57BL/6 mice fed with HFD did not show any change of TG and total cholesterol levels compared to mice with chow diet.<sup>[46]</sup> TG and cholesterol levels did not change during the experiment for HFD mice supplemented with water. On the opposite, an increase of circulating lipid content was observed for mice supplemented with SW, BSW, and J. FJ consumption maintained TG and cholesterol levels and adipose tissue weight. Concomitantly, a decrease in total bacteria population has been reported for HFD compared to low fat diet.<sup>[47]</sup> Consumption of high caloric diets lead to shifts in the diversity of species belonging to the *Firmicutes* and *Bacteroidetes* phyla, reducing the microbiota diversity.<sup>[48]</sup> Conversely, the administration of noni fruit phenolic extract restored gut microbiota structure.<sup>[49]</sup> FJ consumption did not change LAB population but did change species composition in feces. Indeed, the control group mainly contained *L. animalis/murinus* whereas the FJ group had a greater abundance of *E. faecalis* and a higher proportion of *L. plantarum*.

These observations can be related to recent findings showing that streptozotocin-induced diabetic mice receiving fermented apple juice showed lower serum TG and total cholesterol

levels than mice receiving a nonfermented juice, indicating a better blood lipid regulation in diabetic mice following FJ administration.<sup>[14]</sup> Moreover, LAF has a beneficial health effect through microbiota interactions, promoting gastro-intestinal tract health, improving antiinflammatory potential, and controlling oxidative stress.<sup>[11,50]</sup> Interestingly, an increased abundance of *E. faecalis* was shown to promote a cholesterol-lowering effect.<sup>[51]</sup> Also, HFD mice exhibited a proportion of *Firmicutes* reaching 62.56%, among those *Lactobacillus* abundance increased, compared to the control group fed with a normal chow.<sup>[14]</sup> *Lactobacillus* abundance increased further when fermented apple juice was consumed, possibly because of LAB added with the fermented juice.<sup>[50]</sup>

Intestinal permeability is impaired by metabolic disorders, as shown through a decrease in tight junction protein concentration or morphological changes of intestinal surface.<sup>[52]</sup> HFD consumption is known to decrease ZO-1 concentration and to reduce the length of intestinal villi and the depth of intestinal crypts compared to mice fed with a regular diet.<sup>[53–55]</sup> Although it has been demonstrated that polyphenol extracts increased tight-junction protein concentration and thus, enhanced intestinal barrier integrity, we did not observed modifications of ZO-1 content by juice consumption compared to the control group.<sup>[49,53,54]</sup> Administration of orange juice-derived nanovesicles to mice fed with a high-caloric diet resulted in an increase in villi size in mice and thus an improved absorptive area.<sup>[56]</sup> This is consistent with the observation of a difference in crypt depth between SW and FJ, but morphological changes of the intestine were not significantly different between groups.

Together our results show that FJ consumption does not increase metabolic disorders resulting from HFD, contrarily to other sugary drinks. Despite high amounts of sugar, fruit juices are rich in fibers and phenolic compounds.<sup>[2]</sup> In addition, LAF has an impact on phenolic composition.<sup>[8]</sup> The differences in health indicators observed between FJ and other sugary drink consumed possibly results from a combination of pineapple and LAF positive beneficial effects.

## Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

## Acknowledgements

M.G. was a recipient of a fellowship grant from Région Réunion. This work was supported by the BIOST federation of research and by the European regional found FEDER PO 2014-2020. All experiments were carried out in strict compliance with the protocol for the Animal Ethics Committee of CYROI Réunion Island who approved the in vivo protocol.

## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

M.G. contributed to the research project, performed the experiments, collected the data, performed the statistical analysis, and wrote the paper.

M.B. supervised and performed the in vivo study. C.P. performed the in vivo study. P.R. supervised the in vivo study. F.R. supervised the research project, performed the statistical analysis, and wrote the paper. C.R.-D.S. conceptualized the research project, supervised the project, contributed to data analysis, and wrote the paper. C.G. conceptualized the research project, supervised the project, contributed to data analysis, and wrote the paper.

## Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Keywords

bacterial colonization, beverages, fruit, lactic fermentation, metabolic disorders

Received: October 5, 2022

Revised: March 17, 2023

Published online: May 19, 2023

- [1] C. Garcia, M. Guerin, K. Souidi, F. Remize, *Beverages* **2020**, 6, 1.
- [2] A. Septembre-Malaterre, G. Stanislas, E. Douraguia, M. P. Gonther, *Food Chem.* **2016**, 212, 225.
- [3] G. M. Sun, X. M. Zhang, A. Soler, P. A. Marie-Alphonsine, *Nutr. Compos. Fruit Cultiv.* **2016**, 609.
- [4] G. Sanewski, D. P. Bartholomew, R. E. Paull, *The Pineapple: Botany, Production and Uses*, CAB International, Wallingford, UK **2018**.
- [5] FAO, "Principaux fruits tropicaux : quantités exportées", **2022**. <https://www.fao.org/publications/card/fr/c/CB6897FR/> (accessed: September 2022).
- [6] A. Septembre-Malaterre, F. Remize, P. Poucheret, *Food Res. Int.* **2018**, 104, 86.
- [7] N. Shiferaw Terefe, M. A. Augustin, *Crit. Rev. Food Sci. Nutr.* **2020**, 60, 2887.
- [8] A. Fessard, A. Kapoor, J. Patche, S. Assemat, M. Hoarau, E. Bourdon, T. Bahorun, F. Remize, *Microorganisms* **2017**, 5, <https://doi.org/10.3390/microorganisms5020023>.
- [9] M. R. Swain, M. Anandharaj, R. C. Ray, R. Parveen Rani, *Biotechnol. Res. Int.* **2014**, 2014, 1.
- [10] L. Sun, M. Miao, *Crit. Rev. Food Sci. Nutr.* **2020**, 60, 541.
- [11] M. L. Marco, D. Heeney, S. Binda, C. J. Cifelli, P. D. Cotter, B. Folligné, M. Gänzle, R. Kort, G. Pasin, A. Pihlanto, E. J. Smid, R. Hutkins, *Curr. Opin. Biotechnol.* **2017**, 44, 94.
- [12] J. Luo, X. Lin, M. Bordiga, C. Brennan, B. Xu, *Int. J. Food Sci. Technol.* **2021**, 56, 2055.
- [13] H. Li, J. Liang, M. Han, X. Wang, Y. Ren, Y. Wang, J. Huang, S. Li, C. Liu, Z. Wang, T. Yue, Z. Gao, *Food Res. Int.* **2022**, 156, 111180.
- [14] X. Wang, Y. Wang, M. Han, J. Liang, M. Zhang, X. Bai, T. Yue, Z. Gao, *Food Res. Int.* **2022**, 155, 110998.
- [15] H. Gao, J. J. Wen, J. L. Hu, Q. X. Nie, H. H. Chen, T. Xiong, S. P. Nie, M. Y. Xie, *Food Res. Int.* **2019**, 121, 367.
- [16] CIQUAL-ANSES, "Anses Ciqua – Table de composition nutritionnelle des aliments," **2020**, <https://ciqua.anses.fr/> (accessed: February 2023).
- [17] M. G. Managa, S. A. Akinola, F. Remize, C. Garcia, D. Sivakumar, *Front. Nutr.* **2021**, 8, 649189.
- [18] L. Zhang, S. Hogan, J. Li, S. Sun, C. Canning, S. J. Zheng, K. Zhou, *Food Chem.* **2011**, 126, 466.
- [19] K. B. Kuentzel, I. Bradić, Z. N. Mihalić, M. Korbelius, S. Rainer, A. Pirchheim, J. Kargl, D. Kratky, *Int. J. Mol. Sci.* **2022**, 23, 12286.

- [20] M. Bijnen, T. Josefs, I. Cuijpers, C. J. Maalsen, J. Van De Gaar, M. Vroomen, E. Wijnands, S. S. Rensen, J. W. M. Greve, M. H. Hofker, E. A. L. Biessen, C. D. A. Stehouwer, C. G. Schalkwijk, K. Wouters, *Gut* **2018**, *67*, 1317.
- [21] R. Shiri-Sverdlov, K. Wouters, P. J. V. Gorp, M. J. Gijbels, B. Noel, L. Buffat, B. Staels, N. Maeda, M. Van Bilsen, M. H. Hofker, *J. Hepatol.* **2006**, *44*, 732.
- [22] A. Fessard, E. Bourdon, B. Payet, F. Remize, *Can. J. Microbiol.* **2016**, *62*, 550.
- [23] A. Fessard, F. Remize, *Int. J. Food Microbiol.* **2019**, *301*, 61.
- [24] B. Escudero-López, G. Berná, Á. Ortega, G. Herrero-Martín, I. Cerriño, F. Martín, M. S. Fernández-Pachón, *Food Chem. Toxicol.* **2015**, *78*, 78.
- [25] S. Y. Lee, S. S. Sekhon, J. H. Ko, H. C. Kim, S. Y. Kim, K. Won, J. Y. Ahn, K. Lee, Y. H. Kim, *Toxicol. Environ. Health Sci.* **2018**, *10*, 11.
- [26] A. S. Carvalho, J. Silva, P. Ho, P. Teixeira, F. X. Malcata, P. Gibbs, *J. Appl. Microbiol.* **2003**, *94*, 947.
- [27] M. Al-Balawi, F. M. Morsy, *Front. Microbiol.* **2020**, *11*, 2017.
- [28] J. M. Miranda, C. M. Franco, B. I. Vázquez, C. A. Fente, J. Barros-Velázquez, A. Cepeda, *Let. Appl. Microbiol.* **2005**, *41*, 153.
- [29] S. Krishna, Z. Lin, C. B. de La Serre, J. J. Wagner, D. H. Harn, L. M. Pepples, D. M. Djani, M. T. Weber, L. Srivastava, N. M. Filipov, *Physiol. Behav.* **2016**, *157*, 196.
- [30] J. L. Slavin, B. Lloyd, *Adv. Nutr.* **2012**, *3*, 506.
- [31] CIQUAL-ANSES, "Ciqual: Table de composition nutritionnelle des aliments," **2020**, [https://ciqual.anses.fr/#/aliments/13200/mangue-jose-pulpe-crue-prelevee-a-la-reunion-\(mangifera-indica-l.\)](https://ciqual.anses.fr/#/aliments/13200/mangue-jose-pulpe-crue-prelevee-a-la-reunion-(mangifera-indica-l.)), (accessed: October 2022).
- [32] M. E. S. Pardo, M. E. R. Cassellis, R. M. Escobedo, E. J. García, M. E. S. Pardo, M. E. R. Cassellis, R. M. Escobedo, E. J. García, *J. Agric. Chem. Environ.* **2014**, *3*, 53.
- [33] X. Rico, B. Gullón, J. L. Alonso, R. Yáñez, *Food Res. Int.* **2020**, *132*, 109086.
- [34] D. G. Costa, C. Almeida, C. Cavadas, S. Carmo-Silva, *Nutr. Rev.* **2022**, *80*, 1942.
- [35] N. Bosch-Sierra, R. Marqués-Cardete, A. Gurrea-Martínez, C. G. Del Valle, C. Morillas, A. Hernández-Mijares, C. Bañuls, *Nutrients* **2019**, *11*, 3014.
- [36] H. Dong, L. J. Sargent, Y. Chatzidiakou, C. Saunders, L. Harkness, N. Bordenave, I. Rowland, J. P. E. Spencer, J. A. Lovegrove, *Appetite* **2016**, *107*, 478.
- [37] C. Tan, H. Wei, X. Zhao, C. Xu, Y. Zhou, J. Peng, *Nutrients* **2016**, *8*, 615.
- [38] P. E. S. Munekata, J. Á. Pérez-Álvarez, M. Pateiro, M. Viuda-Matos, J. Fernández-López, J. M. Lorenzo, *Trends Food Sci. Technol.* **2021**, *113*, 397.
- [39] V. Krishnan, P. Verma, S. Saha, B. Singh, T. Vinutha, R. R. Kumar, A. Kulshreshta, S. P. Singh, T. Sathyavathi, A. Sachdev, S. Praveen, *Biocatal. Agric. Biotechnol.* **2022**, *43*, 102411.
- [40] C. P. Silva, G. R. Sampaio, R. A. M. S. Freitas, E. A. F. S. Torres, *Food Chem.* **2018**, *267*, 405.
- [41] A. Aleixandre, J. V. Gil, J. Sineiro, C. M. Rosell, *Food Chem.* **2022**, *372*, 131231.
- [42] F. Mashitola, S. Akinola, V. Manhevi, C. Garcia, F. Remize, R. Slabbert, D. Sivakumar, *Foods* **2021**, *10*, 962.
- [43] F. Mashitola, V. Manhivi, S. Akinola, C. Garcia, F. Remize, T. Shoko, D. Sivakumar, *J. Food Process. Preserv.* **2021**, *45*, e15937.
- [44] M. Alongi, G. Verardo, A. Gorassini, M. Anese, *LWT* **2018**, *98*, 366.
- [45] C. Tang, L. Kong, M. Shan, Z. Lu, Y. Lu, *Food Res. Int.* **2021**, *147*, 110490.
- [46] J. Li, H. Wu, Y. Liu, L. Yang, *Exp. Anim.* **2020**, *69*, 326.
- [47] C. B. De La Serre, C. L. Ellis, J. Lee, A. L. Hartman, J. C. Rutledge, H. E. Raybould, *Am. J. Physiol. – Gastrointest. Liver Physiol.* **2010**, *299*, G440.
- [48] B. Senghor, C. Sokhna, R. Ruimy, J. C. Lagier, *Hum. Microbiome J.* **2018**, *7–8*, 1.
- [49] R. Wang, L. Wang, S. Wang, J. Wang, C. Su, L. Zhang, C. Li, S. Liu, *Food Chem.* **2023**, *402*, 134232.
- [50] N. Mota de Carvalho, E. M. Costa, S. Silva, L. Pimentel, T. H. Fernandes, M. Estevez Pintado, *Fermentation* **2018**, *4*, 90.
- [51] Y. Zhu, T. Li, A. U. Din, A. Hassan, Y. Wang, G. Wang, *Appl. Microbiol. Biotechnol.* **2019**, *103*, 3181.
- [52] B. Lee, K. M. Moon, C. Y. Kim, *J. Immunol. Res.* **2018**, *2018*, <https://doi.org/10.1155/2018/2645465>.
- [53] M. Balakumar, D. Prabhu, C. Sathishkumar, P. Prabu, N. Rokana, R. Kumar, S. Raghavan, A. Soundarajan, S. Grover, V. K. Batish, V. Mohan, M. Balasubramanyam, *Eur. J. Nutr.* **2018**, *57*, 279.
- [54] M.-Y. Park, M. Y. Kim, Y. R. Seo, J.-S. Kim, M.-K. Sung, *J. Cancer Prev.* **2016**, *21*, 95.
- [55] Y. Xie, F. Ding, W. Di, Y. Lv, F. Xia, Y. Sheng, J. Yu, G. Ding, *Mol. Med. Rep.* **2020**, *21*, 1133.
- [56] E. Berger, P. Colosetti, A. Jalabert, E. Meugnier, O. P. B. Wiklander, J. Jouhet, E. Errazuriz-Cerda, S. Chanon, D. Gupta, G. J. P. Rautureau, A. Geloën, S. El-Andaloussi, B. Panthu, J. Rieusset, S. Rome, *Mol. Ther. Methods Clin. Dev.* **2020**, *18*, 880.