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Campus de São José do Rio Preto

Tais Fernanda Borgonovi

**Biocompostos de buriti e maracujá: perfil, aplicação em leite  
fermentado probiótico e modulação da microbiota *in vitro***

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Augusto Cury, 2004.

## Resumo

Devido ao aumento da demanda por alimentos funcionais, as indústrias de alimentos estão investindo em produtos que possuam apelo saudável e propriedades funcionais. Os produtos lácteos, principalmente os fermentados, são os produtos mais comercializados com estas características. Efeitos benéficos adicionais podem ser obtidos quando probióticos e polpa de frutas são adicionados aos produtos fermentados. Neste contexto, os objetivos deste trabalho foram: (1) determinar os componentes majoritários, compostos bioativos e atividade antioxidante de polpas de buriti e de maracujá; bem como seu efeito no crescimento de bactérias lácticas (BAL); (2) avaliar o efeito de *Lactocaseibacillus casei* SJRP38 (LC), *Lactiplantibacillus plantarum* ST8Sh (LP) e *Streptococcus thermophilus* TA 080 (ST), em cultivo puro ou em co-cultivo, e selecionar a melhor cultura/combinção baseado nos parâmetros cinéticos de acidificação do leite fermentado controle (LFC), adicionado de polpa de maracujá (LFPM) ou polpa de buriti (LFPB) e na viabilidade bacteriana; (3) avaliar o efeito do leite adicionado de polpa de frutas e fermentado por cultura probiótica selecionada na modulação da microbiota intestinal, utilizando o Simulador do Ecosistema Microbiano Humano (SHIME®). A polpa de buriti apresentou maiores valores de proteína, °Brix e fibras dietéticas totais. Celulose e hemicelulose foram detectadas em ambas as polpas de frutas; entretanto, a polpa de buriti se destacou pela maior quantidade dessas fibras. A polpa de buriti também apresentou maior teor de ácidos graxos (12 g/100 g), sendo a maioria (76%) da família ômega 9. A polpa de maracujá apresentou 0,5 g de ácidos graxos/100 g, sendo a maioria da família ômega 9 (37%), seguido de ácido palmítico (29%). Os principais flavonoides presentes nas polpas de buriti e maracujá foram quercetina-3-rutinosídeo (90,76 mg/100 g de polpa) e orientina-7-O-glicosídeo (1,45 mg/100 g de polpa), respectivamente. Em relação aos carotenoides, maiores quantidades foram encontradas na polpa de buriti (153,18 mg/100 g) do que na polpa de maracujá (10,05 mg/100 g), e o composto majoritário em ambas as polpas foi o  $\beta$ -caroteno. Ambas as polpas não inibiram o crescimento das BAL. O LF obtido pela combinação das culturas SP+LC+LP destacou-se pela alta viabilidade bacteriana e pelo menor tempo de fermentação (até pH 4,6) e esta cultura foi selecionada para a fermentação nas etapas seguintes da pesquisa. Os compostos fenólicos totais e a atividade antioxidante foram maiores nos tratamentos LFPM ( $0,10 \pm 0,02$  mg EAG/100 g e  $0,33 \pm 0,13$   $\mu$ mol Trolox/100 g, respectivamente) e

LFPB ( $0,11 \pm 0,00$  mg EAG/100 g e  $0,25 \pm 0,03$   $\mu$ mol Trolox/100 g, respectivamente) em relação ao LFC ( $0,09 \pm 0,01$  mg EAG/100 g e  $0,11 \pm 0,02$   $\mu$ mol Trolox/100 g, respectivamente). Em relação ao perfil de carotenoides, esses compostos não foram detectados no LFC e o LFPB apresentou maiores quantidades de carotenoides  $\alpha$ ,  $\beta$  e totais ( $28,97 \pm 0,08$ ;  $528,42 \pm 34,49$  e  $557,39 \pm 34,33$  mg/100 g, respectivamente) em comparação ao LFPM ( $7,07 \pm 0,08$ ;  $45,72 \pm 0,11$  e  $52,79 \pm 0,20$  mg/100 g, respectivamente). Os produtos LFPB e LFPM apresentaram os maiores teores de ácidos graxos monoinsaturados e poli-insaturados, respectivamente. Os principais ácidos graxos encontrados em LFPM e LFPB foram ômega 9 ( $25,89 \pm 0,10$  e  $66,38 \pm 0,13$  g/100 g lipídeos, respectivamente) e ácido palmítico ( $29,54 \pm 0,08$  e  $20,11 \pm 0,22$  g/100 g lipídeos respectivamente). O ensaio *in vitro* utilizando o SHIME mostrou que a viabilidade das BAL probióticas no leite fermentado foram afetadas pela adição de Polpas de frutas; entretanto, atingiram o valor mínimo requerido de  $\geq 6$  log UFC/mL para consumo. Em relação à viabilidade dos micro-organismos indicadores no cólon, houve uma diferença significativa somente para o gênero *Streptococcus* sp. ( $7.35 \pm 0.18$ ,  $7.28 \pm 0.19$  and  $6.69 \pm 0.03$  CFU/mL) para LFC, LFPM and LFPB, respectivamente. Entretanto, o leite fermentado adicionado de polpa de frutas modulou a microbiota intestinal, alterou a relação dos filos Firmicutes/Bacteroidetes, aumentou Actinobacteria e reduziu o filo Proteobacteria. Em nível de gênero, em todos os tratamentos a abundância de *Bifidobacterium* sp. aumentou, e somente LFPM and LFPB reduziu *Enterobacter* sp. E estimulou *Veillonella* sp. e bactérias da família *Ruminococcaceae* em relação ao LFC e seu respectivo *washout*-controle. Após o tratamento, produção de ácido acético aumentou após a administração de todos os tipos de LF e diminuiu durante os períodos de *washout*. Além disso, quantidades significativas de ácidos propiônico e butírico foram produzidas durante o tratamento com LFPB. Todos os tratamentos com LF diminuíram os íons amônia em relação ao período inicial (estabilização) ( $557,22 \pm 9,74$  mmol/L), entretanto, os tratamentos LFPM e LFPB promoveram redução mais acentuada ( $284,89 \pm 6,04$  mmol/L LFPM;  $378,67 \pm 6,15$  mmol/L LFPB). Portanto, pode-se concluir que as polpas de maracujá e buriti podem ser utilizadas em conjunto com as BAL testadas e o leite adicionado de polpas de frutas e fermentado por probióticos pode não somente estimular o crescimento de bactérias benéficas presentes na microbiota de humanos saudáveis, como também aumentar a produção de ácidos graxos de cadeia curta. Sendo assim, produzir LF com polpa de frutas podem ser considerada uma estratégia promissora para a comercialização de LF probiótico com características funcionais.

**Palavras-chave: Alimento funcional. Composto bioativo de frutas. Probiótico. Leite fermentado. Microbiota intestinal.**

## Abstract

Due to the increasing demand for functional foods, food industries are investing in products that have a healthy appeal and functional properties. Dairy products, especially fermented ones, are the most commercialized products with these characteristics. Additional beneficial effects can be obtained when probiotics and fruit pulp are added to the fermented products. In this context, the objectives of this study were: (1) to determine the gross composition, bioactive compounds, and antioxidant activity of buriti and passion fruit pulps, as well as their effect on the growth of lactic acid bacteria (LAB); (2) evaluate the effect of *Lactocaseibacillus casei* SJRP38 (LC), *Lactiplantibacillus plantarum* ST8Sh (LP), and *Streptococcus thermophilus* TA 080 (ST), either in pure culture or co-culture, and select the best culture/combination based on the kinetic parameters of acidification of the control fermented milk (CFM) added of passion fruit pulp (FMPF) or buriti pulp (FMB), as well as their bacterial viability; (3) to evaluate the effect of milk added of fruit pulps and fermented by a selected probiotic culture on the modulation of the intestinal microbiota using the Simulator of Human Intestinal Microbial Ecosystem (SHIME®). Buriti pulp showed higher values of protein, °Brix and total dietary fiber. Cellulose and hemicellulose were detected in both fruit pulps; however, in buriti pulp there was a larger amount of these fibers. Buriti pulp also had a higher content of fatty acids (12 g/100 g), with the majority (76%) being from the omega 9 family. The passion fruit pulp presented 0.5 g fatty acids/100 g, most of them from the omega 9 family (37%), followed by palmitic acid (29%). The main flavonoids found in the buriti and passion fruit pulps were quercetin-3-rutinoside (90.76 mg/100 g of pulp) and orientin-7-O-glycoside (1.45 mg/100 g of pulp), respectively. Regarding carotenoids, higher amounts were found in the buriti pulp (153.18 mg/100 g) compared to the passion fruit pulp (10.05 mg/100 g), and the major compound in both pulps was  $\beta$ - carotene. None of the pulps inhibited the growth of LAB. The FM obtained by combining the ST+LC+LP cultures stood out for its high bacterial viability and for the shorter fermentation time (up to pH 4.6); therefore, this culture was selected for the fermentation in the following steps of the research. The total phenolic compounds and the antioxidant activity were higher in the treatments FMPF ( $0.10 \pm 0.02$  mg GAE/100 g and  $0.33 \pm 0.13$   $\mu$ mol Trolox/100 g, respectively) and FMB ( $0.11 \pm 0.00$  mg GAE/100 g and  $0.25 \pm 0.03$   $\mu$ mol Trolox/100 g, respectively) compared to CFM ( $0.09 \pm 0.01$  mg GAE/100 g and  $0.11 \pm 0.02$   $\mu$ mol

Trolox/100 g, respectively). Regarding the carotenoid profile, these compounds were not detected in the CFM and the FMPB showed higher amounts of  $\alpha$ ,  $\beta$  and total carotenoids ( $28.97 \pm 0.08$ ;  $528.42 \pm 34.49$  and  $557.39 \pm 34.33$  mg/100 g, respectively) compared to FMPF ( $7.07 \pm 0.08$ ;  $45.72 \pm 0.11$  and  $52.79 \pm 0.20$  mg/100 g, respectively). FMB and FFPF showed the highest levels of monounsaturated and polyunsaturated fatty acids, respectively. The main fatty acids found in FMPF and FMB were omega 9 ( $25.89 \pm 0.10$  and  $66.38 \pm 0.13$  g/100 g lipids, respectively) and palmitic acid ( $29.54 \pm 0.08$  and  $20.11 \pm 0.22$  g/100 g lipids, respectively). The *in vitro* assay using SHIME show that the viability of probiotic LAB in fermented milk was affected by the addition of fruit pulps; however, it meets the minimum required value of  $\geq 6$  log CFU/mL for consumption. Regarding the viability of indicator microorganisms in the colon, there was a significant difference only in the genus of *Streptococcus* sp. ( $7.35 \pm 0.18$ ,  $7.28 \pm 0.19$  and  $6.69 \pm 0.03$  CFU/mL) for FMC, FMPF and FMB, respectively. On the other hand, fermented milk added of fruit pulp modulated the intestinal microbiota, changed the balance of Firmicutes/Bacteroidetes phyla, increasing Actinobacteria and decreasing Proteobacteria phyla. At the genus level, in all treatments the abundance of *Bifidobacterium* sp. increased, and only FMPF and FMB decreased *Enterobacter* and stimulated *Veillonella* genera and *Ruminococcaceae* family in relation to FMC and their respective washout-control. After treatment, the production of acetic acid increased after the administration of all types of fermented milk and it decreased during the washout periods. Additionally, a significant and remarkably high amounts of propionic and butyric acids were produced during the treatment of FMB. All fermented milk treatments decreased the ammonium ions compared to control (stabilization) ( $557.22 \pm 9.74$  mmol/L), however fermented milk with fruit pulps promoted a greater decrease ( $284.89 \pm 6.04$  FMPF;  $378.67 \pm 6.15$  FMB mmol/L). In conclusion, passion fruit and buriti pulps can be used together with the tested LAB and milk added of fruit pulps and fermented by probiotics can stimulate not only the growth of beneficial bacteria present in the microbiota of healthy humans, but also the production of short chain fatty acids. Therefore, producing FM with fruit pulp can be considered a promising strategy for the commercialization of probiotic FM with functional characteristics.

**Keywords: Functional food. Bioactive fruit compound. Probiotic. Fermented milk. Intestinal microbiota.**

## Lista de Figuras

### CAPÍTULO I: Fruit bioactive compounds: effect on lactic acid bacteria and on intestinal microbiota

**Figure 1.** Health benefits of bioactive compounds on gut microbiota..... 30

### CAPITULO II: Buriti and passion fruit pulps are sources of bioactive compounds and stimulate the growth of potentially probiotic strains

**Figure 1.** Chromatograms of passion fruit (A) and buriti (B) pulps. A) 2: orientin (luteolin 8-c-glucoside), 4: vitexin, 7: orientin-7-o-glucoside; B) 1: glycosylated quercetin; 2: quercetin-3-rutinoside; 3: isorhamnetin-rutinoside; 4: quercetin-3-glucoside..... 100

**Figure 2.** Chromatograms of buriti (A) and passion fruit (B) pulps. 1: Lutein; 2: Zeaxanthin; 3: Cryptoxanthin; 4:  $\alpha$ -carotene; 5:  $\beta$ -carotene..... 103

**Figure 3.** Effect of fruit pulps on the growth of *L. plantarum* ST8Sh (A) and *Lacticaseibacillus rhamnosus* GG (B). Different capital letters in the same column denote a significant difference ( $p < 0.05$ ) among the pulps, for the same strain. Different lower-case letters in the same row denote a significant difference ( $p < 0.05$ ) over time for the same pulp and strain..... 106

### CAPITULO III: Lactic acid bacteria and fruit pulps influence the kinetics of acidification and bacterial viability of potentially probiotic fermented milk

**Figure 1.** Acidification curves of fermented milk without fruit. A) Milk fermented by pure cultures. B) Milk fermented by combination of cultures..... 129

**Figure 2.** Acidification curves of fermented milk without fruit. A) Milk fermented by pure cultures. B) Milk fermented by combination of cultures..... 130

<b>Figure 3.</b> Acidification curves of fermented milk without fruit. A) Milk fermented by pure cultures. B) Milk fermented by combination of cultures.....	<b>132</b>
--	------------

**CAPITULO IV: Functional fermented milk with fruit pulps modulates the *in vitro* intestinal microbiota**

<b>Figure 1.</b> SHIME <sup>®</sup> system and conditions of each reactor. R1: stomach - pH 2.0-2.5; R2: small intestine - pH 4.3-4.8; R3.1: ascending colon - pH 5.6-5.9; R3.2: ascending colon - pH 5.6-5.9; R3.3: ascending colon - pH 5.6-5.9.....	<b>150</b>
--	------------

<b>Figure 2.</b> Microbiota composition in the reactors representing the ascending colon of SHIME <sup>®</sup> at phylum level. Control – microbiota control; FMC – Fermented milk control (without fruit pulp); WFMC – Washout Fermented milk control (without fruit pulp); FMPF – Fermented milk with passion fruit; WFMPF – Washout Fermented milk with passion fruit; FMB – Fermented milk with buriti; WFMB – Washout Fermented milk with buriti.....	<b>157</b>
--	------------

<b>Figure 3.</b> Microbiota composition in the reactors representing the ascending colon of SHIME <sup>®</sup> at genus level. Control – microbiota control; FMC – Fermented milk control (without fruit pulp); WFMC – Washout Fermented milk control (without fruit pulp); FMPF – Fermented milk with passion fruit; WFMPF – Washout Fermented milk with passion fruit; FMB – Fermented milk with buriti; WFMB – Washout Fermented milk with buriti.....	<b>159</b>
---	------------

<b>Figure 4.</b> Main component analysis.....	<b>165</b>
---	------------

## Lista de Tabelas

### **CAPITULO I: Fruit bioactive compounds: effect on lactic acid bacteria and on intestinal microbiota**

<b>Table 1.</b> Effect of bioactive compounds and fruits on LAB and on gut microbiota.....	<b>31</b>
<b>Table 2.</b> Effect of fruits on LAB and on gut microbiota.....	<b>34</b>
<b>Table 3.</b> Effect of dietary fibers, fruit by-products and polysaccharides on LAB and on gut microbiota.....	<b>39</b>
<b>Table 4.</b> Effect of polyphenols (PP) on LAB and on gut microbiota.....	<b>52</b>

### **CAPITULO II: Buriti and passion fruit pulps are sources of bioactive compounds and stimulate the growth of potentially probiotic strains**

<b>Table 1.</b> Centesimal composition, pH, acidity, °Brix, ascorbic acid and fibers of buriti and passion fruit pulps.....	<b>97</b>
<b>Table 2.</b> Carbohydrate composition of the buriti and passion fruit pulps.....	<b>98</b>
<b>Table 3.</b> Fatty acid profile (g/100 g lipids) of buriti and passion fruit pulps.....	<b>98</b>
<b>Table 4.</b> Composition of phenolic compounds, yellow flavonoids and antioxidant activity in the buriti and passion fruit pulps.....	<b>99</b>
<b>Table 5.</b> Flavonoid profile (mg/100 g pulp) in buriti and passion fruit pulps.....	<b>100</b>
<b>Table 6.</b> Carotenoid profile of buriti and passion fruit pulps.....	<b>102</b>

### **CAPITULO III: Lactic acid bacteria and fruit pulps influence the kinetics of acidification and bacterial viability of potentially probiotic fermented milk**

<b>Table 1.</b> Kinetics parameters of acidification obtained during fermentation of fermented milk without pulp (FMWP), fermented milk with passion fruit pulp (FMPF) and fermented milk with buriti pulp (FMB).....	<b>127</b>
<b>Table 2.</b> Viability of <i>Lactobacilli</i> and <i>Streptococcus thermophilus</i> (CFU/mL) in milk fermented without pulp (FMWP), fermented milk with passion fruit pulp (FMPF) and fermented milk with buriti pulp (FMB) by LAB cultures.....	<b>133</b>
<b>Table 3.</b> Composition, pH value and titratable acidity of milk fermented by selected culture.....	<b>135</b>
<b>Table 4.</b> Phenolic compounds and antioxidant activity (DPPH <sup>-</sup> ) and carotenoids profile of milk fermented by selected culture.....	<b>136</b>
<b>Table 5.</b> Fatty acids profile of milk fermented by selected culture.....	<b>137</b>

**CAPITULO IV: Functional fermented milk with fruit pulps modulates the *in vitro* intestinal microbiota**

<b>Table 1.</b> Viability (Log CFU/mL) of fermented milk applied in SHIME®.....	<b>154</b>
<b>Table 2.</b> The counts of colony forming units (log CFU/mL) of <i>Lactobacilli</i> , <i>Streptococcus</i> spp., <i>Bifidobacterium</i> spp., <i>Clostridium</i> spp. and total anaerobic bacteria .....	<b>155</b>
<b>Table 3.</b> Short chain fatty acid levels (mmol/L) in the vessels corresponding to the ascending colon during the experimental period .....	<b>168</b>

## Sumário

<b>INTRODUÇÃO E JUSTIFICATIVA.....</b>	<b>20</b>
<b>REFERÊNCIAS.....</b>	<b>22</b>
<b>OBJETIVOS.....</b>	<b>24</b>
<b>ORGANIZAÇÃO DOS CAPÍTULOS.....</b>	<b>25</b>
<b>CAPÍTULO I - REVISÃO BIBLIOGRÁFICA: Fruit bioactive compounds: effect on lactic acid bacteria and on intestinal microbiota.....</b>	<b>26</b>
<b>Abstract.....</b>	<b>26</b>
<b>Introduction.....</b>	<b>27</b>
<b>Beneficial effect of fruit compounds on lactic acid bacteria and on intestinal microbiota.....</b>	<b>27</b>
<i>Description of LAB, probiotics and gut microbiota.....</i>	<b>28</b>
<i>Fruit bioactive compounds and major effects.....</i>	<b>33</b>
<i>Dietary fibers and prebiotics.....</i>	<b>33</b>
<i>Phenolic compounds or polyphenols (PP).....</i>	<b>50</b>
Fruit polyphenols and their metabolization by microbiota.....	<b>52</b>
<i>Fatty acids (FA).....</i>	<b>62</b>
<i>Carotenoids.....</i>	<b>65</b>
<i>Vitamins.....</i>	<b>67</b>
<b>Final remarks.....</b>	<b>70</b>
<b>Acknowledgments.....</b>	<b>71</b>
<b>Declaration of interest statement.....</b>	<b>71</b>
<b>References.....</b>	<b>71</b>
<b>CAPÍTULO II - Buriti and passion fruit pulps are sources of bioactive compounds and stimulate the growth of potentially probiotic strains.....</b>	<b>88</b>
<b>Abstract.....</b>	<b>88</b>
<b>1. Introduction.....</b>	<b>89</b>

<b>2. Materials and methods</b> .....	<b>91</b>
<b>2.1 Passion fruit and buriti pulps preparation</b> .....	<b>91</b>
<b>2.2 Physicochemical characterization</b> .....	<b>91</b>
<b>2.3. Determination of lignin and carbohydrates in the fruit pulps</b> .....	<b>91</b>
<b>2.4 Bioactive compounds and antioxidant activity</b> .....	<b>92</b>
2.4.1 <i>Total phenolic compounds and yellow flavonoids</i> .....	<b>92</b>
2.4.2 <i>Flavonoids profile</i> .....	<b>93</b>
2.4.3 Carotenoids profile.....	<b>94</b>
2.4.4 Antioxidant activity.....	<b>95</b>
<b>2.5 Effect of fruit pulps on the growth of lactic acid bacteria (LAB)</b> .....	<b>95</b>
<b>2.6 Statistical analysis</b> .....	<b>96</b>
<b>3. Results and discussion</b> .....	<b>96</b>
<b>3.1 Chemical characterization of fruit pulps</b> .....	<b>96</b>
<b>3.2 Characterization of bioactive compounds and antioxidant activity of fruit pulps</b> .....	<b>99</b>
<b>3.3 Effect of fruit pulp on the growth of LAB strains</b> .....	<b>104</b>
<b>4. Conclusion</b> .....	<b>108</b>
<b>5. References</b> .....	<b>108</b>
<b>CAPITULO III - Lactic acid bacteria and fruit pulps influence the kinetics of acidification and bacterial viability of potentially probiotic fermented milk</b> .....	<b>119</b>
<b>Abstract</b> .....	<b>119</b>
<b>1. Introduction</b> .....	<b>120</b>
<b>2. Materials and methods</b> .....	<b>122</b>
<b>2.1 Passion fruit and buriti pulps preparation</b> .....	<b>122</b>
<b>2.2 Lactic acid bacteria (LAB) cultures</b> .....	<b>122</b>
<b>2.3 Milk characterization</b> .....	<b>123</b>
<b>2.4 Preparation of fermented milk</b> .....	<b>123</b>

<b>2.5 Preparation of cultures.....</b>	<b>123</b>
<b>2.6 Fermentation conditions.....</b>	<b>124</b>
<b>2.7 LAB viability.....</b>	<b>124</b>
<b>2.8 Physicochemical characterization of milk fermented by selected culture.....</b>	<b>124</b>
<b>2.9 Total phenolic compounds and antioxidant activity of milk fermented by selected culture.....</b>	<b>125</b>
<b>2.10 Carotenoid profile of milk fermented by selected culture.....</b>	<b>125</b>
<b>2.11 Fatty acid profile of milk fermented by selected culture.....</b>	<b>125</b>
<b>2.12 Statistical analysis.....</b>	<b>126</b>
<b>3. Results and Discussion.....</b>	<b>126</b>
<b>3.1 Milk characterization and preparation of probiotic fermented milk.....</b>	<b>126</b>
<b>3.2 Fermentation kinetic parameters and LAB viability.....</b>	<b>126</b>
<b>3.3 Centesimal composition, pH and acidity of milk fermented by selected culture.....</b>	<b>134</b>
<b>3.4 Phenolic compounds and antioxidant activity of milk fermented by selected culture.....</b>	<b>135</b>
<b>3.5 Fatty acid profile.....</b>	<b>136</b>
<b>4. Conclusion.....</b>	<b>137</b>
<b>5. References.....</b>	<b>138</b>
<b>CAPITULO IV - Functional fermented milk with fruit pulps modulates the <i>in vitro</i> intestinal microbiota.....</b>	<b>146</b>
<b>Abstract.....</b>	<b>146</b>
<b>1. Introduction.....</b>	<b>147</b>
<b>2. Materials and Methods.....</b>	<b>148</b>
<b>2.1 Preparation of fermented milk.....</b>	<b>148</b>
<b>2.2 Lactic acid bacteria viability in fermented milk.....</b>	<b>149</b>
<b>2.3 Simulator of Human Intestinal Microbial Ecosystem (SHIME®).....</b>	<b>149</b>
<b>2.3.1 Composition of the SHIME® feed medium.....</b>	<b>149</b>

2.3.2 Microbiota colonization.....	150
2.3.3 Experimental protocol.....	151
<b>2.4 Production of metabolites in SHIME®.....</b>	<b>151</b>
<b>2.5 Viability of microorganisms.....</b>	<b>152</b>
<b>2.6 Microbial DNA extraction and 16S rRNA gene-based Illumina MiSeq sequencing.....</b>	<b>152</b>
<b>2.7 Bioinformatics and Statistical analysis.....</b>	<b>153</b>
<b>3. Results and Discussion.....</b>	<b>153</b>
<b>3.1 LAB viability in fermented milk.....</b>	<b>153</b>
<b>3.2 Viability of microorganisms in the SHIME® .....</b>	<b>154</b>
<b>3.3 Microbiota composition evaluation by gene sequencing .....</b>	<b>156</b>
<b>3.4 Short-chain fatty acids and ammonium ions .....</b>	<b>166</b>
<b>4. Conclusion.....</b>	<b>170</b>
<b>References.....</b>	<b>170</b>
<b>CAPITULO IV - CONCLUSÕES GERAIS.....</b>	<b>182</b>

## INTRODUÇÃO E JUSTIFICATIVA

Uma alimentação saudável deve ser baseada em alimentos que forneçam os nutrientes necessários a manutenção da saúde diária, e incluir algumas fontes de micronutrientes, fibras e compostos bioativos (ANJO, 2004). Alguns alimentos, além da nutrição e do apelo saudável, podem apresentar efeito benéfico à saúde, e são conhecidos como alimentos funcionais. Estes alimentos têm ganhado grande destaque nas pesquisas na área de Ciência e Tecnologia de Alimentos, devido à demanda dos consumidores por alimentos saudáveis (STORTI, FERREIRA, PEREIRA, 2014) e à necessidade de entender melhor os efeitos que os biocompostos presentes nos alimentos exercem sobre a saúde.

A propriedade funcional do alimento é relativa ao papel metabólico ou fisiológico que o nutriente ou não nutriente tem no crescimento, desenvolvimento, manutenção e outras funções normais do organismo humano (BRASIL, 1999). Os ingredientes que possuem alegações funcionais são: ácidos graxos - omega-3 ( $\omega$ -3), carotenóides (licopeno, luteína e zeaxantina), psílio ou psyllium, quitosano,  $\beta$ -glucano, fitosteróis, proteína de soja, manitol, xilitol, sorbitol, fibras (fibras resistentes à dextrina, lactulose, goma de guar parcialmente hidrolisada e polidextrose), inulina e microorganismos probióticos (BRASIL, 1999). Estas substâncias bioativas (nutrientes) presentes em alimentos podem agir em espécies reativas ou estimular sistemas de defesa endógeno. Este efeito protetor foi atribuído à presença de compostos com atividade antioxidante, tais como vitaminas, minerais, compostos fenólicos e carotenoides (ALMEIDA et al., 2011).

Para o desenvolvimento de alimentos funcionais, o leite e os produtos lácteos estão entre as principais matérias-primas utilizadas, uma vez que são consumidos regularmente, apresentam alto valor nutricional e muitos benefícios para a saúde: são ricos em cálcio, podem prevenir a osteoporose, possuem proteínas, aminoácidos essenciais e lipídios, que são ricos em ácidos graxos essenciais, e são fontes de vitaminas, entre outros benefícios.

Entre os produtos lácteos funcionais, os produtos fermentados são apontados como o tipo mais importante (MARCO et al., 2021). O uso de bactérias probióticas para fermentação é uma estratégia para atender à exigência dos consumidores por alimentos benéficos à saúde. Outra tendência no desenvolvimento de lácteos funcionais é adicionar

polpas de frutas, que podem melhorar as propriedades sensoriais, como sabor, aroma, textura e cor, diversificando os produtos disponíveis no mercado; estas são algumas das razões de sua popularidade (SANTOS, 2009).

As polpas de frutas tropicais, especialmente as não convencionais, contribuem para o apelo saudável e, ao mesmo tempo, aumentam o valor nutricional do produto pelos compostos bioativos presentes naturalmente nas polpas de frutas. Muitos destes compostos bioativos apresentam propriedades antioxidantes, que podem contribuir para aumentar a vida útil dos produtos, e que estão relacionados à baixa incidência de certas doenças (GENOVESE et al., 2008; SANTOS, 2009; GONÇALVES; LAJOLO; GENOVESE, 2010; ALMEIDA et al., 2011).

Os biocompostos das polpas de buriti e de maracujá foram avaliados em estudos anteriores e as polpas foram aplicadas em leite fermentado (BORGONOVI et al., 2021). Para avaliar o efeito das polpas no crescimento das BAL foram usadas cepas de *Streptococcus thermophilus* SJRP107, *Lactobacillus delbrueckii* subsp. *bulgaricus* SJRP57, *Lactocaseibacillus casei* SJRP38, *Limosilactobacillus fermentum* SJRP30 e SJRP43, *Enterococcus durans* SJRP29, *Enterococcus faecium* SJRP20 e SJRP65, isoladas de muçarela de búfala, previamente identificadas a partir do sequenciamento do gene 16S rRNA (SILVA, 2010) e anteriormente caracterizadas quanto à segurança, potencial tecnológico e probiótico. Dentre as cepas testadas, *L. casei* SJRP38, potencialmente probiótico (SALOTTI-SOUZA et al., 2019) apresentou maior crescimento na presença de polpas e foi selecionado para aplicação em leite fermentado com adição de polpa de buriti e de maracujá. O leite fermentado apresentou resultados promissores (estabilidade na degradação de compostos fenólicos, atividade antioxidante e aumento da população de lactobacilos durante o período de prateleira), o que estimula a continuidade desses estudos.

Por outro lado, *Lactiplantibacillus plantarum* (anteriormente conhecido como *Lactobacillus plantarum*) é uma espécie muito versátil e faz parte da composição de diversos alimentos, tais como produtos lácteos fermentados, vegetais fermentados, produtos cárneos, cereais, frutas e bebidas. Além disso, *L. plantarum* tem sido tradicionalmente usado como cultura em fermentações de alimentos, como produtora de substâncias antimicrobianas e algumas cepas podem apresentar propriedades probióticas. A cepa *L. plantarum* ST8Sh foi caracterizada como produtora de bacteriocina da família da pediocina PA-1, com atividade contra diversos microorganismos: *Enterococcus* spp., *Klebsiella pneumoniae*, *Listeria* spp., e contra alguns patógenos que causam doenças em

humanos e estão presentes em alguns alimentos. Também foi considerada potencialmente probiótica pela elevada resistência às condições do trato gastrointestinal, produção de  $\beta$ -galactosidase, desconjugação de sais biliares, elevada hidrofobicidade, propriedades de agregação e adesão celular (TODOROV, HOLZAPFEL, NERO, 2016; TODOROV et al., 2017).

Diante deste cenário, o estudo *in vitro* do efeito de leite adicionado de polpas de frutas e fermentado por bactérias ácido lácticas (*L. casei* SJRP38, *L. plantarum* ST8Sh, isoladas ou em co-cultura com *S. thermophilus*) poderá ampliar os conhecimentos sobre a ação dos alimentos funcionais na microbiota intestinal e o metabolismo dos nutrientes.

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## OBJETIVOS

Esta pesquisa foi realizada para atender os seguintes objetivos principais:

- a) Determinar a composição dos componentes majoritários, compostos bioativos e atividade antioxidante em polpas de buriti e de maracujá; bem como seu efeito no crescimento de bactérias lácticas (BAL);
- b) Avaliar o efeito de *Lacticaseibacillus casei* SJRP38 (LC), *Lactiplantibacillus plantarum* ST8Sh (LP) e *Streptococcus thermophilus* TA 080 (ST), em cultivo puro ou em co-cultivo, sobre os parâmetros cinéticos de acidificação do leite fermentado (LF) adicionado de polpa de maracujá (FMPF) ou polpa de buriti (FMPB) e na viabilidade bacteriana;
- c) Avaliar o efeito do leite fermentado sem adição de polpas de frutas - controle (LFC), adicionado de polpa de maracujá (FMPF) ou polpa de buriti (FMPB) na modulação da microbiota intestinal usando o Simulador do Ecossistema Microbiano Humano (SHIME<sup>®</sup>), assim como avaliar a produção de ácidos graxos de cadeia curta e de íons amônia.

## **ORGANIZAÇÃO DOS CAPÍTULOS**

O presente trabalho foi organizado em cinco capítulos para melhor apresentação e entendimento dos assuntos abordados.

O Capítulo I consiste na revisão bibliográfica do tema abordado da tese intitulada “Fruit Bioactive Compounds: Effect on Lactic Acid Bacteria and on Intestinal Microbiota”, submetido à publicação no periódico Food Research International, classificado como A1 pelo Qualis/CAPES. Após a análise, os revisores apontaram que o trabalho poderá ser aceito se forem realizadas pequenas alterações, que foram atendidas e submetidas novamente dia 23 de abril de 2022.

Os Capítulos II, III e IV também foram redigidos na forma de artigos científicos, a serem submetidos à publicação em periódicos internacionais com elevada classificação pelo Qualis/CAPES. O Capítulo V trata das conclusões gerais deste trabalho.

## CAPÍTULO I - REVISÃO BIBLIOGRÁFICA

### **Fruit Bioactive Compounds: Effect on Lactic Acid Bacteria and on Intestinal Microbiota**

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#### **Abstract**

The benefits of bioactive compounds to human health have been highly explored in recent years; they are widely distributed in nature, mainly in fruits. In this review, the effect of the main fruit bioactive compounds (FBC) on lactic acid bacteria (LAB) and on gut microbiota composition was discussed. The fruit dietary fibers, phenolic compounds, fatty acids, carotenoids, and vitamins have important health benefits. Furthermore, they can interact with LAB and modulate the human intestinal microbiota, which favor the diversity of beneficial bacterial groups, thus providing several benefits to human health, such as reduction of weight gain, improvement the mucosal barrier function of gastrointestinal (GI) tract against pathogens, decrease the chronic inflammation and the incidence of diseases, such as cardiovascular, diabetes, hypertension and chronic diseases. Additionally, FBC are able to change the Firmicutes/Bacteroidetes ratio and inhibit the putrefactive bacteria in the gut. Due to the complex composition of human gut microbiota and variations among individuals, additional research must be carried out to elucidate the mechanism of interaction between the bioactive compounds and the human microbiota.

**KEYWORDS:** functional food; immunomodulation; intestinal health; prebiotics; health benefit.

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## **Introduction**

Fruits are rich in numerous bioactive compounds, such as carotenoids, phenolic compounds, fibers, vitamins, minerals, fatty acids, and bioactive peptides, which can provide health benefits (Septembre-Malaterre, Remize, & Poucheret, 2018). Therefore, the increase in fruit consumption is encouraged among Western populations in order to reduce weight gain, cardiovascular diseases, diabetes, and hypertension.

Fruits are included within functional foods, which should be safe for consumption without compulsory medical supervision. Functional food and bioactive compounds have potential to modulate gut microbiota and has gained attention over the last years. Modulating the gut microbiota to a more favorable profile, keeping it in homeostasis, has been related to a reduced risk of developing a wide range of metabolic, immunological and neurological disorders (Li, Ma, & Fu, 2017). The intestinal microbiota may also control the strengthening of the intestinal barrier function, decreasing chronic inflammation, shaping the lipid and glycemic metabolism, body weight gain, and accumulation of fat in the liver and adipose tissue (Corrêa, Rogero, Hassimotto, & Lajolo, 2019). In this regard, bioactive compounds derived from the diet may also promote gut health, by interacting with factors and/or signaling pathways associated with the intestinal immune function (Zimmermann & Wagner, 2021).

The interactions between food components and intestinal populations need to be taken into account in order to better understand the beneficial effects of fruit bioactive compounds (FBC) on the overall well-being of the host and to plan effective dietary interventions. However, the mechanisms of action through which bioactive compounds change the gut composition have just started to be explained (Dou, Chen, & Fu, 2019; Luca et al., 2019; Molinari et al., 2021) and some of them remain unclear. Therefore, further studies aiming at investigating these mechanisms should be carried out. Moreover, it is also important to evaluate whether these phytochemicals positively or negatively affect beneficial microorganisms, such as lactic acid bacteria (LAB), considering that they are usually employed to modulate the gut microbiota.

In the light of accumulating evidence linking bioactive compounds and gastrointestinal (GI) health, in this review, we outline the effects of FBC on LAB and on intestinal microbiota.

## **Beneficial effect of fruit bioactive compounds on lactic acid bacteria and on intestinal microbiota**

### ***Description of LAB, probiotics and gut microbiota***

LAB are generally recognized as safe (GRAS) and associated with the probiotic properties of some commercial products. Probiotic is established as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (Hill et al., 2014). Such microorganisms have several health benefits and they have been used to improve gut health for several decades. Their efficacy is strain dependent, i.e., the beneficial properties of each specific strain must be proven through clinical trials (Jäger et al., 2018).

Unlike probiotics, the definition of postbiotic as "preparation of inanimate microorganisms and/or their components that confers a health benefit on the host" was published as a consensus issued by the International Scientific Association of Probiotics and Prebiotics (ISAPP) (Salminen et al., 2021); however, in the literature different terms have been proposed, such as "paraprobiotics", "non-viable microbial cells", "fermented infant formulas", "ghost probiotics", "inactivated probiotics", "non-viable microbial cells" and "metabolic probiotics" (Wegh, Geerlings, Knol, Roeselers, & Belzer, 2019; Teame et al., 2020; Salminen et al., 2021). The postbiotics refer to bioactive compounds produced by food-grade microorganisms as microbial cells, cellular constituents or fractions, peptidoglycans and many kinds of metabolites, as short chain fatty acids (SCFA), functional (secreted) proteins and extracellular polysaccharides (EPS) (Wegh, Geerlings, Knol, Roeselers, & Belzer, 2019), bacteriocins and organic acids (Teame et al., 2020).

Postbiotics can have different beneficial effects that can mediate the positive general effect on the host, such as immunomodulatory, antitumor, antimicrobial, and barrier-preserving effects (Teame et al., 2020), can also relieve symptoms colic in children, atopic dermatitis in adults, and different causes of diarrhea (Wegh, Geerlings, Knol, Roeselers, & Belzer, 2019).

*Lactobacilli*, *Bifidobacterium* spp., some members of the genera *Enterococcus* (*E. faecium*), yeast from *Saccharomyces* genera (*S. boulardii*) and strains of spore-forming bacteria, such as *Bacillus* sp., are the mostly used ones as probiotic. More recently, in order to increase the range of probiotics in the industrial market, researchers are intensifying their search for new microorganisms. Currently the most studied strains are intestinal health-associated bacteria belonging to the genera *Bacteroides*, *Clostridium*, *Faecalibacterium*, *Akkermansia*, *Eubacterium*, *Propionibacterium* and *Roseburia*

(Sanders, Merenstein, Reid, Gibson, & Rastall, 2019; Carmo et al., 2020), as well as genetically modified (GM) strains (usually *Lactococci* carrying a new health-giving characteristic) (Saarela, 2018). Some of the genera or species studied had never been used for food production, much less on an industrial scale (Saarela, 2018; Carmo et al., 2020).

These new microorganisms are called the next generation of probiotics (NGP) (Saarela, 2018; Sanders, Merenstein, Reid, Gibson, & Rastall, 2019; Carmo et al., 2020). However, the proof of these microorganisms as probiotics has many challenges, such as: understanding their efficacy, safety for consumption and technical robustness, their use in the production of new food products, as well as the industrial scale production (Carmo et al., 2020; Saarela, 2018). Most of these strains have metabolic characteristics that make difficult their cultivation, and some strains are very demanding nutritionally and strictly anaerobic, which make unfavorable their production on an industrial scale (O'Toole, Marchesi, & Hill, 2017; Saarela, 2018; Sanders, Merenstein, Reid, Gibson, & Rastall, 2019).

The human gut microbiota composition is incredibly dynamic and varies widely among individuals (Wang, Yao, Lv, Ling, & Li, 2017; da Silva, Casarotti, Oliveira, & Penna, 2020). Three bacterial phyla are the most abundant ones in the adult human microbiota: Firmicutes (Gram-positive), Bacteroidetes (Gram-negative) which compose 85-90% of the total microbiota and, in lower abundance, Actinobacteria (Gram-positive), Proteobacteria (Gram-negative) and Verrucomicrobia (Gram-negative) are frequent, but generally in lower frequency (Cani, Moens de Hase, & Van Hul, 2021). However, the microbiota can be easily altered due to several factors, e.g., individual's diet, and physicochemical gut properties, such as oxygen availability, pH, concentration of bile and mucus barrier, and production of antimicrobial peptides (Leeming, Johnson, Spector, & Roy, 2019; Cani, Moens de Hase, & Van Hul, 2021).

Additionally, the phytochemicals present in fruit have gained attention due to their action on both probiotic LAB for food production and human gut microbiota. Depending on the phytochemicals dose, these compounds can exert both beneficial and deleterious effects on these groups of microorganisms (Holst & Williamson, 2008); they can affect food fermentation process and LAB viability, as well as the intestinal human microbiota. Studies have demonstrated that the effect of these compounds on bacterial development is subject to the compound's structure and concentration, on the consumer's living habits, on the host's diet and, especially, on the resident's intestinal microbiota (Espín, González-Sarrías, & Tomás-Barberán, 2017). The intrinsic differences of the cell wall structure of

Gram-negative and Gram-positive bacteria are likely to be associated with higher resistance of Gram-negative microorganisms due to the presence of outer membrane that act as a barrier to certain bioactive compounds and antibiotics (Owusu, Afedzi, & Quansah, 2021). Additionally, food phytochemicals and nutritional molecules can display a potent protective effect on intestinal mucosal regeneration and barrier function, which prevents gut-derived bacteria and endotoxins from being translocated to other organs (Chen et al., 2021).

Considering the health benefits of dietary fibers (DF) and prebiotics, polyphenols (PP), fatty-acids, carotenoids, and vitamins on microbiota, which are described in this review, it is suggested that FBC may potentially contribute to improve the host's microbiota balance and health (Figure 1). The effects of some FBC and fruits on LAB and intestinal microbiota are shown in Tables 1 and 2, respectively. Currently, the investigations have been concentrated in some fruits, such as tropical fruits (banana, jabuticaba, apple, mango) and berries (goji berry, mulberry, raspberry, cherry). These fruits have the potential to stimulate the growth of some LAB with probiotic properties such as *Lactobacillus* (*L. casei*, *L. plantarum*, *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*) and *Bifidobacteria* (*B. animalis* subsp. *lactis*, *B. longum*). They can also modulate the gut microbiota beneficially changing the Firmicutes/Bacteroidetes ratio by increasing the diversity of beneficial bacteria, and stimulating some bacteria, such as *Lactobacillus* spp., *Akkermansia* spp., *Bifidobacterium* spp., *Faecalibacterium* spp., *Ruminococcaceae* spp., *Lachnospira* spp., and inhibiting putrefactive bacteria, such as *E. coli*, *Clostridium* spp., *Salmonella* spp., *Klebsiella* spp., *Ruminoclostridium* spp.

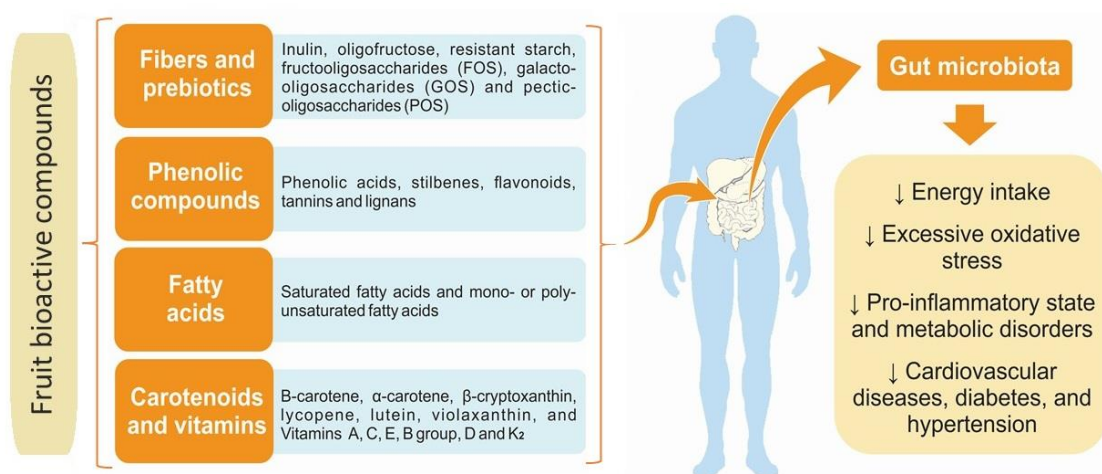


Figure 1. Health benefits of bioactive compounds on gut microbiota.

**Table 1.** Effect of bioactive compounds and fruits on LAB and on gut microbiota.

Bioactive compounds or ingredients	Main effects	References
<b><i>In vitro</i> effects on LAB</b>		
Dietary fibers and polysaccharides	↑ growth of probiotic strains, such as <i>Lactobacillus</i> , <i>Bacillus</i> , and <i>Bifidobacterium</i> .	Majeed et al. (2018), Gómez et al. (2019), Pereira et al. (2020), Huang et al. (2019), Guo, Zhang, Wang, Li, & Ding (2020), Shalini, Abinaya, Saranya, & Antony (2017), Zhang, Zhang, Li, Chen, & Ding (2019)
Fruits and its by-products	↑ growth of <i>Lactobacillus</i> , <i>Bacillus</i> , <i>Bifidobacterium</i> , and <i>Streptococcus</i> .	Vieira, Bedani, Albuquerque, Biscola, & Saad (2017), Sah, Vasiljevic, McKechnie, & Do (2016), Powthong, Jantrapanukorn, Suntornthiticharoen, & Laohaphatanalert (2020), Mahore & Shirolkar (2018), Kaprasob, Kerdchoechuen, Laohakunjit, & Somboonpan (2018), Öztürk, Demirci, & Akin (2018), Barat & Ozcan (2018), Skenderidis, Mitsagga, Lampakis, Petrotos, & Giavasis (2019)
Polyphenols	↑ <i>Lactobacillus</i> and <i>Bifidobacterium</i> population.	Tabashsum et al. (2019), Coman et al. (2018), Ahmad et al. (2020), Pacheco-Ordaz et al. (2018), Gu et al. (2019)
<b><i>In vitro</i> effects on gut microbiota</b>		
Dietary fibers and polysaccharides	↓ F/B ratio, ↑ <i>Lactobacillus</i> and <i>Prevotella</i>	Wang, Li, Huang, Fu, & Liu (2019), Li et al. (2020)
Fruits and its by-products	↑ <i>Lactobacillus</i> , <i>Bifidobacterium</i> , <i>Akkermansia</i> , <i>Clostridium</i> , and <i>Ruminococcaceae</i> . Distinct effects on <i>Clostridium</i> .	Freire et al. (2017), Parkar et al. (2018), Dalu, Nurhayati, & Jayus (2019), Attri & Goel (2018), Cheng et al. (2020), Campos, Ribeiro, Teixeira, Pastrana, & Pintado (2020)

Polyphenols	Distinct effects on <i>Lactobacillus</i> , <i>Clostridium</i> and <i>Bifidobacterium</i>	Mayta-Apaza et al. (2018), Burgos-Edwards et al. (2020), Wu et al. (2018)
<b><i>In vivo</i> effects on gut microbiota</b>		
Dietary fibers and polysaccharides	↑ <i>Lactobacillus</i> and <i>Bifidobacterium</i> ; distinct effects on <i>Clostridium</i> and <i>Bacteroides</i> .	Massot-Cladera et al. (2015), Zhang et al. (2017), Pansai et al. (2020), Ji et al. (2020)
Fruits and its by-products	↑ <i>Lactobacillus</i> , <i>Bifidobacterium</i> , and <i>Akkermansia</i>	Elkahoui, Levin, Bartley, Yokoyama, & Friedman (2019), Paturi, Butts, Monro, & Hedderley (2018), Kim et al. (2020), Grant et al. (2019), Lima et al. (2019), Fidéliz, Milenkovic, Sivieri, & Cesar (2020)
Polyphenols	↓ F/B ratio, ↑ bacterial diversity and improved dysbiosis.	Gu et al. (2019), Mayta-Apaza et al. (2018), Estruel-Amades et al. (2019), Huang et al. (2016), Etxeberria et al. (2015), Peng et al. (2020), Cires et al. (2019), Masumoto et al. (2016), Rodríguez-Daza et al. (2020), Zhao et al. (2019), Collins et al. (2016), Jiao et al. (2019), Bai et al. (2019)

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F/B ratio – Firmicutes/Bacteroidetes ratio.

Most of the studies in this field are concentrated on investigating DF and PP; however, more recently, studies involving other fruit compounds have been published, such as carotenoids and fatty acids (Martini et al., 2022; López-Salazar et al., 2021). Additionally, some studies present discrepant results, probably because the release of compounds with biological activities from different foods is complex and depends on many factors, including their synergistic effects with the food matrix, differences in bioavailability, bioaccessibility, degradation of the compound, interaction with other foods in the diet, and the host's health status. Moreover, only a few studies have tested the biological effects of bioactive compounds, or the metabolites produced in the gut from either whole fruit or fruit extracts combined with a model of GI tract digestion process with or without fermentation in the colon, nor have compared the bioactivity of the metabolites produced to the former compound (Table 2). Therefore, this would be a fruitful area for further studies aiming to elucidate the mechanisms of action of FBC on the intestinal microbiota, and to establish their positive association.

### ***Fruit bioactive compounds and major effects***

#### ***Dietary fibers and prebiotics***

Dietary fibers (DF) are the edible polysaccharides of fruits and vegetables, which are not metabolized by the enzymes from the upper intestine (Salehi & Aghajanzadeh, 2020; Thumann, Pferschy-Wenzig, Moissl-Eichinger, & Bauer, 2019). The importance of DF to the human health is already well-documented and accepted by the scientific community. Therefore, a healthy diet should include adequate daily amounts of DF (Valdés-Varela, Ruas-Madiedo, & Gueimonde, 2017; Zhang et al., 2020). The daily recommendation for adults of both sexes may vary according to age group, ranging from 21 to 38 g/day (Institute of Medicine, 2005).

**Table 2.** Effect of fruits on LAB and on gut microbiota.

	Study design	Effect	Reference
<b><i>In vitro</i> effects on LAB</b>			
Banana powder (Saba, Pisang Awak and Silver bluggoe)	Strains ( <i>L. acidophilus</i> , <i>L. casei</i> , <i>L. fermentum</i> , <i>S. thermophilus</i> , obtained from the Microbiology Laboratory of Thailand Institute of Scientific and Technological Research) were inoculated in MRS broth supplemented with 1-6% banana powder, followed by incubation at 37 °C/72 h.	MRS broth containing banana powder seemed to promote the growth of four probiotic strains, but no significant differences in the number of probiotic bacterial groups were detected in response to different types and concentrations of bananas after 72 h of fermentation.	Powthong, Jantrapanukorn, Suntornthiticharoen, & Laohaphatanalert (2020)
Banana pulp flour	<i>L. acidophilus</i> NCIM-5426 was inoculated in mMRS broth containing banana flour (prepared from processed and processed raw, ripe and processed ripe) as a sole carbon source at 5%, followed by incubation at 37 °C/48 h. MRS with glucose was used as control.	<i>L. acidophilus</i> showed the fastest growth and the shorter doubling time in the presence of raw banana flour, which proved the ability of this flour to support the growth of the lactobacilli strain.	Mahore & Shirolkar (2018)
Cashew apple juice	<i>L. acidophilus</i> TISTR 1338, <i>L. casei</i> TISTR 390, <i>L. plantarum</i> TISTR 543, <i>B. longum</i> TISTR 2195, and <i>L. mesenteroides</i> TTISTR 053 were inoculated into 20 g of pasteurized cashew apple juice. The fermentation process was performed at 37 °C/48 h.	All strains ↓ growth during fermentation. <i>L. casei</i> and <i>L. plantarum</i> showed the highest survival at the end of fermentation, whereas <i>B. longum</i> had the lowest population.	Kapasob, Kerchoechuen, Laohakunjit, & Somboonpanyakul (2018)
White and dark blue fruits of <i>Myrtus communis</i>	Dark blue and white pulps were added (12%) to probiotic goat milk ice cream formulations containing <i>L. casei</i> 431. A control treatment without fruit was prepared. The mixes were incubated at 30 °C overnight.	After fermentation and at the end of ice cream storage (8 weeks), <i>L. casei</i> 431 counts were about 0.5 log CFU/mL and 0.2 log CFU/mL, respectively, higher in mixes with fruits than in control treatment.	Öztürk, Demirci, & Akın (2018)
Black mulberry, red grape and cornelian cherry	Milk fermented by <i>S. thermophilus</i> , <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> , <i>L. acidophilus</i> and <i>B. animalis</i> subsp. <i>lactis</i> . was mixed with fruit juices (black mulberry, red grape or	Fermented milk containing red grape juice ↑ counts of <i>S. thermophilus</i> and <i>L. acidophilus</i> cells, while <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> and <i>B. animalis</i> subsp. <i>lactis</i> counts ↑ in the fermented milk containing mulberry	Barat & Ozcan (2018)

Encapsulated Goji berry	cornelian cherry) at a concentration ratio of 1:1, stored for 28 days at 4 °C. <i>L. acidophilus</i> (Dupont), <i>B. animalis</i> subsp. <i>lactis</i> BB-12, <i>B. longum</i> Bb-46, <i>L. casei</i> , <i>L. rhamnosus</i> (Chr. Hansen) were added at a ratio of 1% in sterile mMRS broth with 0.1% (w/v) addition of Goji berry powder, followed by incubation at 37 °C/34 h. MRS with glucose was used as control.	juices; the ↓ levels of probiotics were found in the fermented milk containing cornelian cherry. Goji berry resulted in ↔ <i>L. rhamnosus</i> growth, ↑ <i>L. casei</i> (increase of 1.34 log CFU/mL), <i>L. acidophilus</i> (increase of 0.64 log CFU/mL) <i>B. longum</i> (increase of 0.26 log CFU/mL), <i>B. animalis</i> subsp. <i>lactis</i> (increase of 2.0 log CFU/mL), all compared to the control.	Skenderidis, Mitsagga, Lampakis, Petrotos, & Giavasis (2019)
<b><i>In vitro</i> effects on gut microbiota</b>			
Kiwifruit cultivars at the ‘ready to eat’ stage of ripeness	Five types of kiwi fruit (25 g each) were subjected to simulated gastrointestinal digestion before fermentation at 37 °C/16 h with fecal microbiota from ten individual donors. The positive control was inulin (1%).	All the kiwifruit and inulin ↑ <i>Bifidobacterium</i> , but only kiwi ↑ <i>Ruminococcaceae</i> and ↓ <i>Bacteroides</i> when compared to inulin. Green-fleshed kiwifruit ↑ <i>Lachnospira</i> , while the gold-fleshed kiwi fruit ↑ <i>Akkermansia</i> in comparison to inulin.	Parkar et al. (2018)
Fermented and non-fermented banana and guava juices	Juices were made from banana or red guava (fruit:water at a ratio of 1:4) fermented or not by <i>L. casei</i> . <i>In vitro</i> test was conducted by mixing juices and human fecal bacteria (1% w/v), followed by incubation at 37 °C/24 h.	Fermented and non-fermented juices ↑ total bacteria (> 2 log CFU/mL) as compared to control sample (without juice addition); ↓ <i>E. coli</i> , <i>Klebsiella</i> sp. and <i>Salmonella</i> sp.	Dalu, Nurhayati, & Jayus (2019)
Sea buckthorn berries juice	Lyophilized fraction of small intestines digested juice (250 mg) was used in the batch culture fermentation. A control treatment with undigested juice was also carried out. Both treatments used healthy human fecal microbiota as inoculum and flasks were incubated at 37 °C/72 h.	Compared to the beginning of batch fermentation, digested juice ↑ diversity <i>Bacteroides/Prevotella</i> group (71%), LAB (35%) and bifidobacteria (17%). Control group showed lower richness compared to digested juice (33% <i>Bacteroides/Prevotella</i> group, 20% LAB and 8.3% bifidobacteria).	Attri & Goel (2018)
Fermented raspberry juice (FRJ)	Juice fermented with 5% (v/v) of <i>L. casei</i> at 37 °C for 0, 18, 42 and 72 h and non-FRJ added of 5% MRS broth were submitted to <i>in vitro</i> simulated digestion and colonic fermentation.	FRJ ↑ <i>Escherichia coli</i> , butyric acid-producing bacteria, <i>Lactobacillus</i> and <i>Akkermansia</i> and ↓ <i>Bacteroides</i> and <i>Ruminococcus</i> .	Wu et al. (2021)

***In vivo* effects on gut microbiota**

Mango	Ten participants received a daily dose of 200–400 g of mango pulp for 8 weeks.	↑ <i>Lactobacillus</i> spp., <i>L. plantarum</i> , <i>L. reuteri</i> and <i>L. lactis</i> .	Kim et al. (2020)
Mango pulp	Pigs (n=10/group) were fed one of three diets: control based on wheat starch, and treatment diets where starch was partially substituted with either 15% mango pulp or 10% pectin, for 3 weeks.	Mango and pectin resulted in the highest and lowest total species abundance and diversity, respectively. <i>L. mucosae</i> ↑ in mango group. Mango promoted a more stable abundance of <i>F. prausnitzii</i> along the large intestines, while pectin ↑ <i>F. prausnitzii</i> in the proximal colon and ↓ in distal colon. <i>F. prausnitzii</i> was not detectable in low fiber control group.	Grant et al. (2019)
Orange juice	Controlled clinical study with temporal series intergroup design with 10 apparently healthy women that were evaluated after the continuous consumption of commercial pasteurized orange juice (300 mL/day) for 2 months.	After the intervention period, ↑ <i>Bifidobacterium</i> spp., <i>Lactobacillus</i> spp and total bacteria, ↔ <i>Clostridium</i> spp. PCR–DGGE of gut microbiota showed similar composition of total bacteria among women microbiota.	Lima et al. (2019)
Orange juice	Controlled non-randomized human trial with temporal series intergroup design with ten women who had a regular diet without orange juice for 30 days (OJ-free diet), followed by a regular diet + 300 mL/day orange juice for 60 days (OJ-Diet), and 30 days with a regular diet without orange juice (washout).	<i>Bifidobacteriaceae</i> , <i>Atopobiaceae</i> , <i>Coriobacteriaceae</i> , <i>Eggerthellaceae</i> , <i>Lactobacillus</i> spp., <i>Akkermansia</i> spp., and <i>Ruminococcus</i> spp. ↑ after the intervention with orange juice. <i>Bacteroidaceae</i> , <i>Barnesiellaceae</i> , <i>Muribaculaceae</i> , <i>Prevotellaceae</i> , <i>Rikenellaceae</i> , <i>Tannerellaceae</i> , <i>Lactobacillaceae</i> , <i>Leuconostocaceae</i> , <i>Clostridiaceae</i> , <i>Lachnospiraceae</i> , <i>Peptococcaceae</i> and <i>Ruminococcaceae</i> ↓ after the intervention with orange juice.	Fidélis, Milenkovic, Sivieri, & Cesar (2020)

Fermented raspberry juice (FRJ)	Four groups of male Kun Ming mice (n = 8/group) were fed with: standard diet an experimental diet (C); standard diet + 3% FRJ (L); standard diet + 6% FRJ (M); standard diet + 9% FRJ (H) for 30 days.	FRJ treatments altered the $\beta$ -diversity of mice, especially in groups L and H, and significantly decreased the Firmicutes to Bacteroidetes ratio in groups L, M, and H compared to the control group. FRJ treatments $\downarrow$ <i>Blautia</i> , <i>Ruminiclostridium_9</i> , and $\uparrow$ <i>Lactobacillus</i> (especially group M). Group L treatment markedly $\uparrow$ <i>Akkermansia</i> . Low and high dose FRJ treatments even had some reverse effects on faecal microbiota.	Wu et al. (2021)
Okra fruit powder	Male C57BL/6J mice were divided in five groups: normal diet group (mice fed with standard diet), HFD group; low dose of okra group (2.5% of okra powder replaced corn starch in HFD); middle dose of okra group (5% of okra powder replaced corn starch in HFD); high dose of okra group (10% of okra powder replaced corn starch in HFD). The in vivo assay was carried out for 12 weeks.	Okra fruit powder $\downarrow$ F/B ratio, <i>Porphyromonadaceae</i> , <i>Pasteurella</i> and <i>Lactobacillus</i> , and $\uparrow$ <i>Parabacteroides</i> , Proteobacteria and Actinobacteria abundance. Mice in groups treated with okra fruit powder showed distribution of gut bacterial community close to that observed in mice belonging to the normal diet group.	Zhang, Zhao, Ren, & Yang (2020)

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AC - ascending colon; ATCC - American Type Culture Collection; CFU - colony forming unit; DC - descending colon; DP - degree of polymerization; F/B - Firmicutes/Bacteroidetes; FOS - fructooligosaccharide; GAE - gallic acid equivalent; HFD - high-fat diet; HFHSD - high-fat high-sucrose diet; HPD - high-protein diet; LAB - lactic acid bacteria; LFD - low-fat diet; mMRS - modified de Man-Rogosa-Sharpe; MRS - de Man-Rogosa-Sharpe; MW - molecular weight; NFD - normal-fat diet; PBS -phosphate buffer saline; SHIME - Simulator of the Human Intestinal Microbial Ecosystem; TC - transverse colon;  $\leftrightarrow$  - no effect;  $\uparrow$  - increase;  $\downarrow$  - decrease.

Usually, DF are classified according its solubility as soluble, including  $\beta$ -glycans, gums, wheat dextrins, psyllium, pectin and inulin, or insoluble, such as cellulose, lignin, some pectins, some hemicelluloses and resistant starch (Slavin, 2013). Fruits can contain a mixture of both soluble and insoluble DF in different ratios (Katsirma, Dimidi, Rodriguez-Mateos, & Whelan, 2021). In addition, DF can be classified regarding its fermentability, as fermentable or non-fermentable, and its viscosity, as viscous or non-viscous (Slavin, 2013). One of the reasons explaining fiber beneficial physiological effects is its fermentation by LAB and gut microbiota. In general, due to its high viscosity, soluble fibers are more readily fermented by gut microbiota than insoluble fiber, which in turn are not or are very slowly metabolized by gut microbiota (McRorie & McKeown, 2017). The different types of fibers results in a combination of these physicochemical properties and, therefore, their effects on gut microbiota depend on the food composition (Abreu y Abreu et al., 2021).

DF is the main energy substrate for LAB and *Bifidobacterium*, which have specific enzymes that break down these complex carbohydrates, and several *in vitro* studies have shown the effects of DF on these microorganisms (Table 3). Among the fibers found in fruits, there are: inulin, oligofructose, resistant starch, fructooligosaccharides (FOS) and pectic-oligosaccharides (POS). POS obtained from lemon peel with a higher degree of polymerization was fermented by *Bifidobacterium* strains and by strains from *Lactobacillaceae* family, whereas only *B. lactis* Bb-12 was able to grow in the presence of POS from lemon peel with lower degree of polymerization (Gómez et al., 2019). In another study (Rivas et al., 2021), the highest populations of *L. casei* (HL 245, HL 233), *L. reuteri* (PL503, PL519) and *E. faecium* (SE 906, SE 920) strains were achieved in the presence of extract from grape by-product and *L. casei* HL 233 and *E. faecium* SE 920 showed moderate growth (about 40%) in the presence of pomegranate peel extract.

The ability of bacteria to breakdown DF is influenced by several factors, such as degree of polymerization, weight, chain size and the number of ramifications in the molecule. Polysaccharides that are formed by groups with a more complex structure are slower metabolized (Hamaker & Tuncil, 2014). Therefore, the type of DF can influence on the ability of bacteria to ferment this substrate as well as on the extension of this fermentation.

**Table 3.** Effect of dietary fibers, fruit by-products and polysaccharides on LAB and on gut microbiota.

	Study design	Effect	Reference
<b><i>In vitro</i> effects on LAB</b>			
Cranberry seed fiber	<i>B. coagulans</i> MTCC 5856 was inoculated into MRS broth and in cranberry seed fiber prepared in demineralized water (0.5, 1.0 and 2.0%, w/v). Flasks were incubated at 37 °C/24 h.	Cranberry seed extract at 2% between 0 and 24 h of incubation ↑ the growth by 2.3 log CFU/mL and it was comparable to the growth in MRS.	Majeed et al. (2018)
Dietary fiber (DF) from pomegranate, tomato, grape and broccoli by-products	mMRS individually supplemented (2%) with sterile-filtered extract of dietary fiber was inoculated with <i>L. casei</i> (HL 245, HL 233), <i>L. reuteri</i> (PL503, PL519) and <i>E. faecium</i> (SE 906, SE 920). Incubation was carried out at 37 °C/48 h.	The highest populations of all probiotic strains were achieved in the presence of extract from grape by-product and <i>L. casei</i> HL 233 and <i>E. faecium</i> SE 920 showed moderate growth (about 40%) in the presence of pomegranate peel extract. All strains had a slight growth in the presence of tomato peel and broccoli stem extracts.	Rivas et al. (2021)
DF from unripe and ripe papayas	DF from ripe and unripe papayas (100 mg/50 mL) were added, separately, in fermentation medium containing feces inoculum. The flasks were incubated at 37 °C/24 h.	DF from unripe papayas ↑ <i>Coprobacillus</i> , <i>Bulleidia</i> , and <i>Slackia</i> ; dietary fibers from ripe papaya ↓ <i>Prevotella</i> ; dietary fibers from both ripe and unripe papayas ↑ <i>Bacteroides</i> and <i>Clostridium</i> and ↓ <i>Blautia</i> , <i>Oscillospira</i> , <i>Roseburia</i> and <i>Sutterella</i> .	Prado, Minguzzi, Hoffmann, & Fabi (2021)
Acerola, orange, passion fruit, and mango by-products	Ten LAB cultures ( <i>Streptococcus thermophilus</i> , <i>Lactobacillus</i> spp.) and three <i>Bifidobacterium</i> spp. cultures (all from Chr. Hansen), and two pathogenic strains ( <i>E. coli</i> ATCC 8739 and <i>Clostridium perfringens</i> ATCC 13124) were individually inoculated in MRS broth supplemented with 1% fruit by-products and incubated at 37 °C/48 h.	Growth of all bacteria between 0 and 48 h of incubation ↑ in the presence of orange and passion-fruit by-products, including the pathogenic strains. Acerola by-product was the substrate that showed the highest selectivity for beneficial bacteria (LAB and <i>Bifidobacterium</i> spp.).	Vieira, Bedani, Albuquerque, Biscola, & Saad (2017)

Pineapple peel and pomace (oven dried and freeze dried)	<i>S. thermophilus</i> ASCC 1275, <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> Lb1466, <i>L. acidophilus</i> ATCC 4356, <i>L. casei</i> ATCC 393, and <i>L. paracasei</i> subsp. <i>paracasei</i> ATCC BAA52 were individually inoculated in mMRS medium with 2% of by-product and incubated at 37 °C/24 h.	In all the pineapple powders tested, all strains grew more than one log higher compared to the negative control (mMRS broth without glucose).	Sah, Vasiljevic, McKechnie, & Donkor (2016)
Digested jabuticaba by-product	mMRS broth was added (2%) with digested jabuticaba by-product, FOS or glucose, individually inoculated with <i>L. acidophilus</i> LA-05, <i>L. casei</i> LAFTI L-26 and <i>B. animalis</i> subsp. <i>lactis</i> BB-12 and incubated at 37 °C/48 h.	Probiotics strains population ↑ about 2 log CFU/mL in mMRS with digested jabuticaba by-product after 48 h of cultivation. <i>L. acidophilus</i> LA-05 had similar counts in mMRS with jabuticaba by-product or FOS. <i>L. casei</i> L-26 counts ↑ in the presence of FOS and was similar in mMRS with jabuticaba by-product or glucose. <i>B. lactis</i> BB-12 had similar counts in mMRS with jabuticaba by-product, glucose or FOS.	Massa et al. (2020)
Apple, banana and mango peels	<i>L. rhamnosus</i> LGG, <i>L. casei</i> 431 and <i>B. lactis</i> Bb-12 as pure cultures or co-culture (1:1:1 ratios) were inoculated into mMRS supplemented with fruit peels at 2% and 4%. The inoculated media were incubated at 37 °C/72 h.	Fruit peels ↑ probiotic growth and the highest populations were obtained after 24 h of incubation; there were ↔ in probiotic counts between both concentrations of fruit peels used.	Zahid, Ranadheera, Fang, & Ajlouni (2021)
Pectic oligosaccharides (POS) from lemon by-product	<i>L. plantarum</i> IFPL935, <i>L. reuteri</i> DSM17938, <i>L. rhamnosus</i> GG, <i>B. lactis</i> Bb-12, <i>B. breve</i> 26M2 were inoculated in mMRS individually supplemented (1%) with two streams enriched in POS (POS-1 and POS-2) from lemon peels with different size. Cultures were grown at 37 °C/48 h.	<i>B. lactis</i> Bb-12 was the only strain able to ferment POS-2 (MW = 738 to 5900 Da) whereas POS-1 (MW = 5900 Da) was the preferred carbohydrate source for <i>Lactobacillus</i> spp.	Gómez et al. (2019)
Fruta-do-lobo starch	<i>L. acidophilus</i> LA5, <i>L. casei</i> LC01 and <i>B. lactis</i> BLC1 were inoculated in mMRS broth supplemented with 2.0% of starch, followed by incubation for 48 h. MRS with glucose, lactose and FOS were used as controls.	BLC1 preferentially metabolized glucose and lactose but its growth rate in the presence of tested starch was ↑ than in presence of FOS. For LA5, tested starch also promoted ↑ growth than FOS. For LC01, the growth rate in the presence of starch was ↓ than in other carbon sources.	Pereira et al. (2020)

Polysaccharides from longan pulp	<i>L. fermentum</i> GIM 1.191, <i>L. acidophilus</i> GIM 1.731, <i>L. plantarum</i> GIM 1.380, and <i>L. bulgaricus</i> , GIM 1.189 were inoculated in mMRS broth with 0.5, 1.0, 1.5, 2.0, and 3.0% of polysaccharides (unfermented LP-0 and fermented until 72 h: LP-6, LP-12, LP-24, LP-48, LP-60, LP-72) from longan pulp, followed by incubation at 37 °C/48 h.	Polysaccharide at 3.0% stimulated the highest increased-fold bacterial populations. The stimulatory effect of LP-12 was the most pronounced one.	Huang et al. (2019)
Polysaccharide from <i>Crataegus pinnatifida</i>	Polysaccharide solution (100 µL at 2.5 mg/mL or 5 mg/mL) was used as sole carbon source in 96 well plates containing bacterial cells ( <i>Bacteroides thetaiotamicron</i> ATCC 29148, <i>B. ovatus</i> ATCC 8483 and <i>B. longum</i> , donated by Jiangnan University, China, <i>L. rhamnosus</i> GG and <i>L. reuteri</i> BNCC 186563). Cultures were grown at 37 °C/72 h.	At 5 mg/mL, polysaccharide ↑ growth of <i>B. thetaiotamicron</i> ATCC 29148, <i>B. ovatus</i> ATCC 8483 and <i>B. longum</i> . Effect on <i>L. rhamnosus</i> GG and <i>L. reuteri</i> BNCC 186563 growth was less pronounced.	Guo, Zhang, Wang, Li, & Ding (2020)
Fructans from Nendran banana	<i>L. casei</i> ATCC11578, <i>L. acidophilus</i> ATCC4356, <i>L. plantarum</i> and <i>B. amyloliquifaciens</i> were inoculated in mMRS added with fructan extracted from banana (1% of stock solution at 10%) and in MRS with glucose and incubated at 37 °C/24 h.	Cell numbers obtained in mMRS with banana fructan was ↑ than in MRS with glucose; the highest (3.41 log CFU/mL) and the lowest (3.25 log CFU/mL) increases in mMRS with banana fructan after 24 h of incubation were recorded for <i>L. acidophilus</i> and <i>L. plantarum</i> , respectively.	Shalini, Abinaya, Saranya, & Antony (2017)
Glucan from <i>Crataegus pinnatifida</i>	<i>B. thetaiotaomicron</i> ATCC 29148, <i>B. ovatus</i> ATCC 8483 and <i>B. fragilis</i> ATCC 25285 were inoculated in minimal medium and <i>L. rhamnosus</i> GG and <i>B. animalis</i> subsp. <i>lactis</i> Bb-12 were inoculated in mMRS. Both media were added of purified polysaccharide from <i>Crataegus pinnatifida</i> (1.25 mg/mL and 2.5 mg/mL) and glucose; cultures were incubated at 37 °C/48 h.	Purified polysaccharide ↑ growth of three species of intestinal <i>Bacteroides</i> but ↓ the growth rate compared to minimal medium with glucose. Growth of <i>L. rhamnosus</i> GG and <i>B. animalis</i> subsp. <i>lactis</i> Bb-12 was not stimulated by the polysaccharide.	Zhang, Zhang, Li, Chen, & Ding (2019)
Konjac glucomannan oligosaccharides (KGOS)	<i>L. plantarum</i> CGMCC 19,087, <i>L. casei</i> CGMCC 1.575 and <i>L. brevis</i> YM 1301 were inoculated in mMRS broth containing 0.75% of Konjac glucomannan (positive control) and 0.75% of KGOS as the sole carbon source. Flasks were incubated at 37 °C/12 h.	All probiotic strains grew in the presence of KGOS whilst no significant growth was observed in mMRS supplemented with Konjac glucomannan.	Wan et al. (2022)

Poly and oligosaccharides in mucilage from <i>Opuntia ficus-indica</i> and <i>O. joconostle</i>	<i>L. rhamnosus</i> GG, <i>L. acidophilus</i> DSM 13241, <i>B. longum</i> ssp. <i>infantis</i> ATCC 15697 and <i>B. animalis</i> ssp. <i>lactis</i> Bb-12 were inoculated in mMRS broth with 1% of mucilage or glucose at 37 °C/48 h.	Fermentability by probiotics was relatively low, 11–27 % compared to glucose.	Cruz-Rubio, Mueller, Viernstein, Loeppert, & Praznik (2021)
Fermented goat milk with grape juice and/or grape pomace extract	Probiotic fermented goat milk (FGM) with grape juice (treatment 1, 200 mL/day), FGM with grape juice and grape pomace extract (6%) (treatment 2, 200 mL/day) were submitted to <i>in vitro</i> bacterial fermentation assays using SHIME® for 48 h.	Treatment 1: all bacterial populations analyzed ↓ in AC vessel, <i>Clostridium</i> spp. and total anaerobes population ↓ in TC vessel; <i>Lactobacillus</i> spp. and <i>Bifidobacterium</i> spp. populations ↑ while total anaerobes microorganisms ↓ in DC vessel. Treatment 2: ↑ <i>Lactobacillus</i> spp. and <i>Clostridium</i> spp. in AC vessel, <i>Lactobacillus</i> spp. in TC vessel, <i>Lactobacillus</i> spp. and <i>Bifidobacterium</i> spp. in DC vessel.	Freire et al. (2017)
Pineapple by-product (stems and peels)	Pineapple by-products were subjected to <i>in vitro</i> GIT simulation and the precipitate from this assay was added (2%) to basal medium along with fecal slurries. This mixture was incubated at 37 °C/48 h.	Pineapple steam ↑ <i>Lactobacillus</i> spp., <i>Bifidobacterium</i> spp. and Firmicutes and Bacteroidetes more markedly than pineapple peel. However, pineapple peel ↓ <i>Clostridium</i> .	Campos, Ribeiro, Teixeira, Pastrana, & Pintado (2020)
Acerola and guava by-products	Digested acerola and guava by-products were inoculated (20%) into human fecal slurry and fermentation medium. Fermentation was carried out at 37 °C/24 h.	Both by-products ↑ <i>Bifidobacterium</i> , <i>Eubacterium rectall</i> – <i>Clostridium coccoides</i> and <i>Bacteroides</i> – <i>Prevotella</i> ;	Menezes et al. (2021)
Fermented blueberry pomace (FBP)	Blueberry pomace was placed in sterile borosilicate glass bottles in triplicate and pasteurized (80 °C for 15min). Each bottle was inoculated with 5% (v/v) of <i>L. casei</i> (7.00 Log CFU/mL) and respectively fermented at 37 °C for 12 days without agitation.	acerola by-product ↑ <i>Lactobacillus</i> – <i>Enterococcus</i> FBP ↓ <i>Escherichia coli</i> , <i>Enterococcus</i> and F/B ratio, and ↑ <i>Bifidobacterium</i> , <i>Ruminococcus</i> , <i>Lactobacillus</i> , <i>Akkermansia</i> and butyrate-producing bacteria.	Cheng et al. (2020)

<b><i>In vitro</i> effects on gut microbiota</b>			
Polysaccharide (RTFP-3) from <i>Rosa roxburghii</i> Tratt fruit	An aliquot of 20 mL of RTFP-3 solution (5 mg/mL) was mixed with growth medium and human fecal inoculum and subjected to fermentation at 37 °C/48 h. A control treatment without polysaccharide solution was used.	RTFP-3 ↑ bacterial diversity. It also ↑ <i>Prevotella</i> , <i>Bifidobacteriaceae</i> , <i>Lactobacillaceae</i> , <i>Bacteroidaceae</i> , <i>Streptococcaceae</i> , <i>Bifidobacteriaceae</i> and <i>Lactobacillaceae</i> , but ↓ F/B ratio, <i>Lachnospiraceae</i> , <i>Veillonellaceae</i> , <i>Porphyromonadaceae</i> , <i>Coriobacteriaceae</i> , <i>Acidaminococcaceae</i> , <i>Christensenellaceae</i> , <i>Alcaligenaceae</i> , and <i>Peptostreptococcaceae</i> , all compared to control treatment.	Wang, Li, Huang, Fu, & Liu (2019)
Tamarind seed polysaccharide	Tamarind seed polysaccharide solution (20 mL of solution at 20 mg/mL) was mixed with 10 mL fecal inoculum and 20 mL fermentation culture. The mixture was incubated at 37 °C/48 h.	The tested polysaccharide ↓ F/B ratio, <i>Escherichia-Shigella</i> and <i>Dorea</i> and ↑ <i>Lactobacillus</i> , <i>Parabacteroides</i> , <i>Prevotella</i> and <i>Faecalibacterium</i> compared to control (without seed polysaccharide).	Li et al. (2020)
Alkali-extraction citrus pectin and high pressure-alkali-extraction citrus	<i>In vitro</i> fermentation was carried out by mixing 1.0 mL 10% faecal slurry and 9.0 mL nutrient growth medium containing pectin (5 mg/mL), followed by incubation at 37 °C/48 h.	Both types of pectin ↑ <i>Bifidobacterium</i> and <i>Phascolarctobacterium</i> and ↓ <i>Escherichia-Shigella</i> and <i>Klebsiella</i> . Moreover, both pectins showed ↑ community richness than commercial citrus pectin and inulin (controls).	Hou et al. (2022)
<b><i>In vivo</i> effects on gut microbiota</b>			
Blackcurrant and DF (from apple and broccoli)	Male Sprague-Dawley rats were assigned to eight groups (n = 16/group) and fed normal diet or high-fiber diet either with or without the blackcurrant extract (40%) and with or without fermentable fibers prepared from New Zealand grown apples and broccoli for six weeks.	Blackcurrant extract ↑ <i>Bacteroides-Prevotella-Porphyromonas</i> group and <i>Lactobacillus</i> spp.; ↓ <i>Bifidobacterium</i> spp. and <i>C. perfringens</i> ; ↔ <i>Enterococcus</i> spp., compared to diets without blackcurrant.	Paturi, Butts, Monro, & Hedderley (2018)
Cocoa fiber	Female Wistar rats (n=10/group) were fed for 3 weeks a normal diet, a normal diet + 10%-cocoa (C10), a normal	CF diet ↑ <i>Bifidobacterium</i> , <i>Lactobacillus</i> , <i>C. histolitycum</i> / <i>C. perfringens</i> , <i>Staphylococcus</i> and <i>Streptococcus</i> counts compared to group fed with standard	Massot-Cladera et al. (2015)

	diet + cocoa fiber (CF, 5.22% - with low amount of polyphenol) or a normal diet + inulin (I, 0.85%).	diet and C10 group. C10-fed animals had ↓ counts of <i>Bifidobacterium</i> , <i>Staphylococcus</i> and <i>Streptococcus</i> groups and ↑ of <i>Bacteroides</i> and <i>Clostridium coccooides/Eubacterium rectale</i> in comparison to the group fed with normal diet. Total counts were not affected by diets.	
Apple (from three varieties) peel powder, commercial apple polyphenol and commercial grape-seed powder	Male C57BL/6J mice were randomized in 11 groups (n=8/group) and fed a HFD for 4 weeks: control (only HFD), 10 or 20% apple peel (3 varieties), 1 or 2% apple extract, 40% whole RedDel apple, commercial extracts (apple and grape seed - positive controls).	Microbiota from mice fed with apple extract was similar to those fed with grape seed (positive control), whereas microbiota from mice fed with apple peel and whole apple was more similar to feces of mice on the HFD. Commercial extracts ↑ <i>Enterobacteriaceae</i> , <i>Turicibacter</i> and <i>Enterococcus</i> . Apple peel ↑ <i>Bacteroides</i> and whole apple ↑ <i>Akkermansia</i> .	Elkahoui, Levin, Bartley, Yokoyama, & Friedman (2019)
Dragon fruit oligosaccharides	Male Wistar rats (n=6/group) were fed a normal diet and received sterilized water (8 mL/day), or inulin (4 g/kg/day), or dragon fruit oligosaccharides at doses of 1, 2, and 4 g/kg/day for 28 days. Inulin and dragon fruit oligosaccharides were suspended in 8 mL of sterilized water prior to administration.	Dragon fruit oligosaccharides ↑ Bifidobacteria and lactobacilli (the highest increase was recorded for oligosaccharide at 2 g/kg/day) and ↓ <i>Bacteroides</i> and <i>Clostridia</i> (the highest decrease was recorded for oligosaccharide at 2 g/kg/day) compared to control group.	Pansai et al. (2020)
Jujube polysaccharides	Male C57BL/6 mice were induced to develop colitis-associated colon cancer and then were gavaged with 1000 mg/kg/day jujube polysaccharides or with sterile saline alone for 13 weeks.	Jujube polysaccharides ↓ microbial community diversity and pathogenic bacteria, F/B ratio, <i>Herpotrichiellaceae</i> , <i>Enterobacteriaceae</i> , <i>Aspergillaceae</i> , <i>Lachnospiraceae</i> , <i>Cyanobacteria</i> , <i>Deferribacteres</i> , <i>Spirochaetes</i> and <i>Mucoromycota</i> but ↑ <i>Lactobacillaceae</i> , <i>Bacteroidaceae</i> , <i>Debaryomycetaceae</i> , <i>Bacteroidetes</i> and <i>Actinobacteria</i> .	Ji et al. (2020)
Pectin polysaccharide from <i>Clausena lansium</i> (Lour.) Skeels fruit	Kunming mice received pectic polysaccharides (0.1 g/kg body weight) or daily for 30 days	There were no significant differences in gut microbiota diversity between the two groups, pectic polysaccharides ↑ F/B ratio and <i>Lactobacillus</i> whereas it ↓ <i>Muribaculaceae</i> abundance.	Song et al. (2021)

Longan polysaccharide C57BL/6 mice (n=15/group) were fed for 10 weeks a normal diet or a normal diet + longan polysaccharide (4 g/100 g solution of the longan polysaccharide, equivalent to 1 g crude longan polysaccharides per 100 g diet) which replaced starch and cellulose in the normal diet. *B. pseudolongum*, *L. reuteri* and *Parabacteroides merdae* ↑ in the longan polysaccharide group. Zhang et al. (2017)

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AC - ascending colon; ATCC - American Type Culture Collection; CFU - colony forming unit; DC - descending colon; DF – dietary fiber; DP - degree of polymerization; F/B - Firmicutes/Bacteroidetes; FOS - fructooligosaccharide; GAE - gallic acid equivalent; GIT – gastrointestinal tract; HFD - high-fat diet; HFHSD - high-fat high-sucrose diet; HPD - high-protein diet; LAB - lactic acid bacteria; LFD - low-fat diet; mMRS - modified de Man-Rogosa-Sharpe; MRS - de Man-Rogosa-Sharpe; MW - molecular weight; NFD - normal-fat diet; PBS -phosphate buffer saline; SHIME® - Simulator of the Human Intestinal Microbial Ecosystem; TC - transverse colon; ↔ - no effect; ↑ - increase; ↓ - decrease.

The fermentation of DF by the bacteria present in the gut microbiota can favor microbial composition, diversity and richness (Riccio & Rossano, 2017; Cronin, Joyce, O'Toole, & O'Connor, 2021). Whereas a human encodes only 17 enzymes responsible for the carbohydrates digestion (Cantarel, Lombard, & Henrissat, 2012), microorganisms from gut microbiota encodes several carbohydrate-active enzymes (CAZymes) (Louis, Solvang, Duncan, Walker, & Mukhopadhyaya, 2021), more specifically, about 130, 22 and 16 enzymes from glycoside hydrolase, polysaccharide lyase and carbohydrate esterase families, respectively. These enzymes allow the microbiota to switch between the different fiber energy sources available (Flint, Scott, Duncan, Louis, & Forano, 2012; Guan, Yu, & Feng, 2021). Members from the Bacteroidetes phylum encodes more CAZymes than members from Firmicutes phylum (Louis, Solvang, Duncan, Walker, & Mukhopadhyaya, 2021).

Considering that a specific type of DF may require several steps of enzymatic catalysis to be degraded to its final product, several bacteria are necessary for its metabolism. Some of these bacteria are called primary degraders because they are the main contributors to the breakdown of DF. In addition, other microorganisms have a less relevant role as they use the degradation products generated by the primary degraders, being known as secondary fermenters or cross-feeders (Cronin, Joyce, O'Toole, & O'Connor, 2021).

Some DF, besides being fermented by bacteria from the intestinal microbiota, stand out on account of their capability to positively change the gut bacterial community, showing a prebiotic activity. Prebiotic refers to a non-digestible food component which benefits the host by enhancing the growth or selective activity of a group or bacterial species in the colon (Gibson et al., 2017). A prebiotic property is the valuable physiological effect which arises from deeply modulation of the composition and activity of human intestinal microbiota through the degradation of a non-digestible compound (Bindels, Delzenne, Cani, & Walter, 2015).

Short-chain fatty acids (SCFA), especially butyrate, propionate, and acetate, are the main end products resulting from DF and prebiotic metabolization by gut microbiota. The production and concentration of each SCFA may depend on the type of fiber as well as on the abundance and species of microorganisms present in the colon. SCFA are also key metabolites related to the positive effects of DF and prebiotics consumption, since they are the main energy source for intestinal epithelial cells and have numerous health effects on the human host (Cerqueira, Photenhauer, Pollet, Brown, & Koropatkin, 2020;

Zheng et al., 2016). Most of the SCFA are absorbed by the colon and decrease the luminal pH, which helps to modulate the gut microbial composition into a healthier one, by raising the commensal bacteria population (favorable ratio of Firmicutes/Bacteroidetes), inhibiting the Gram-negative *Enterobacteriaceae*, *Salmonella* spp., and *Escherichia coli*, decreasing the duration of gut passage, and increasing the defecation frequency. Moreover, butyrate and other SCFAs have anti-inflammatory effects on the gut (Blaak et al., 2020; Gibson et al., 2017; Venegas et al., 2019). Lastly, SCFA helps the regulation of hormone secretion (Han et al., 2021), blood pressure (Bartolomaeus et al., 2019), and immune inflammation (Yao et al., 2020).

Besides the benefits associated with SCFA production, fiber also allows the formation of biofilms, and so beneficial and fermentative bacteria can bind to surface and persist for a long period in the gut. A high fiber diet also helps to keep the intestinal mucus layer intact because it inhibits mucus-degrading microorganisms (Thomson, Garcia, & Edwards, 2021). Moreover, high fiber intake is important to maintain the carbohydrate degrading metabolism prevalent in the gut. In a diet lacking DF, gut microbiota use other substrates, such as proteins and fats, leading to the accumulation of potentially harmful metabolites to intestinal mucosa, including ammonia, amines, N-nitroso compounds, and lipopolysaccharide (LPS), being the last associated with metabolic endotoxemia and gut dysbiosis (Guan, Yu, & Feng, 2021; Ojo, Ojo, Zand, & Wang, 2021; Windey, de Preter, & Verbeke, 2012).

In this regard, several studies have shown that people living in developing countries or in a traditional rural culture present higher fiber intake, which would lead to a more diverse gut microbiota, compared to people from Western countries (Das et al., 2018; De Filippo et al., 2010, 2017; Martínez et al., 2015; Oduaran et al., 2020). One study revealed that microbiota of children from a rural community, compared to microbiota of children from urban area, was characterized by a higher ratio of Bacteroidetes to Firmicutes, and a higher abundance of *Prevotella*, *Treponema*, and *Succinivibrio*, which are associated with fermentation of fiber and polysaccharides from vegetable (De Filippo et al., 2017).

Some studies regarding the effect of DF, fruit by-products and polysaccharides on LAB and gut microbiota are listed in Table 3. One of these studies examined the *in vitro* effects of a non-starch polysaccharide (RTFP-3) from *Rosa roxburghii* Tratt fruit (Wang, Li, Huang, Fu, & Liu, 2019) and showed that this DF increased bacterial diversity and the abundance of *Prevotella*, *Bifidobacteriaceae*, *Lactobacillaceae*, *Bacteroidaceae*,

*Streptococcaceae*, *Bifidobacteriaceae*, but decreased Firmicutes to Bacteroidetes ratio, *Lachnospiraceae*, *Veillonellaceae*, *Porphyromonadaceae*, *Coriobacteriaceae*, *Acidaminococcaceae*, *Christensenellaceae*, *Alcaligenaceae*, and *Peptostreptococcaceae*, compared to control treatment. The characterization of RTFP-3 revealed that it was mainly composed of arabinose, galactose, glucose, fucose and galacturonic acid, which might stimulate the growth of these specific gut bacteria.

Besides measuring how DF influence the intestinal microbiota, it is worthy of note the effect of ingredients with high DF content on LAB growth, such as fruit by-products. Recently, these substances have been extensively investigated regarding its effect on probiotic viability and on gut microbiota composition (Table 3). Vieira, Bedani, Albuquerque, Biscola and Saad (2017) evaluated the ability of 1% (w/v) fruit by-products (acerola, orange, passion fruit, and mango) which are great sources of DF on probiotic bacteria (*Lactobacillus* spp. and *Bifidobacterium* spp.) and gut microbiota (*Escherichia coli* and *Clostridium perfringens*) growth by using an *in vitro* fermentability test. Each fruit by-product showed a specific impact on the development of bacterial strains. Larger populations were observed after incubation in all tested by-products, for all *Lactobacillus* strains; however, *L. acidophilus* LA-5 and *L. rhamnosus* GG strains presented the lowest growth in the presence of the fruit substrates. In contrast, the greater selectivity for beneficial bacteria was observed in the acerola by-product since *E. coli* and *Cl. perfringens* populations were the lowest in its presence. This study showed that the ability to metabolize different substrates is strain-specific and may be influenced by the fruit by-products' biocompounds.

Ferreira-Lazarte, Kachrimanidou, Villamiel, Rastall and Moreno (2018) studied the *in vitro* modulatory effect of commercially citrus pectin compared to inulin and FOS. Similar growth of *Bifidobacterium*, *Lactobacillus* and *Bacteroides/Prevotella* among the tested compounds was observed. Di et al. (2017) compared citrus pectic samples (3 were POS and 2 were modified pectins) and observed that two POS and one modified pectin were fermented similarly by the human fecal cultures and showed bifidogenic effects. Both digestible and non-digestible carbohydrates are thought to stimulate *Bifidobacterium* and suppress *Clostridia* growth; on the other hand, *Lactobacillus*, *Eubacterium rectale*, *Ruminococcus* and *Roseburia* are favored only by non-digestible carbohydrates. Lastly, prebiotics enhance *Bifidobacterium* sp. and LAB, while reducing enteropathogenic *Clostridia* species (Singh et al., 2017).

Bianchi et al. (2019) screened the probiotic *Lactobacillus acidophilus* LA-5, *Lactobacillus paracasei* L-431, or *Bifidobacterium longum* BB-46 strains under simulated GI conditions and evaluated the impact of combining acerola (*Malpighia emarginata* D.C.) by-product and probiotic strains in the SHIME® approach. The acerola by-product increased the BB-46 growth during the gastric phase compared to L-431. *L. acidophilus* LA-5 presented the lowest resistance to GI environment. In order to know the best survival rate, the mix of BB-46 and acerola by-product was tested in the SHIME®. The acerola by-product presented low lipids, high proteins, fibers and total phenolic compounds, as well as high *in vitro* antioxidant activity. In general, during the SHIME® assay, the populations of *Lactobacillus* spp. and *Bifidobacterium* spp. were stable; however, there was a reduction in *Clostridium* spp. and total anaerobes. Additionally, in the ascending colon, a reduced amount of ammonium and an increased production of SCFAs were observed during treatment with BB-46 and the acerola by-product.

In summary, the studies have demonstrated that fruit components as fibers, oligosaccharides, polysaccharides, and pectin from the fruits, such as tropical fruits (lemon, jabuticaba, banana, pomegranate, tamarind, papaya, acerola, passion fruit, mango and coconut), berries (cranberry, blueberry), and apple can be metabolized as carbon source and stimulate the *in vitro* growth of LAB. These by-products modulate the gut microbiota, increasing the diversity of beneficial bacteria, such as *Lactobacillus* spp., *Bifidobacterium* spp., *Faecalibacterium* spp. and *Akkermansia* spp. as well as reduce harmful bacteria.

The physiological effect of fiber can vary depending on several factors, including the type of fiber, the composition of the host's diet and gut microbiota, as well as the food matrix in which the fiber is present (Thomson, Garcia, & Edwards, 2021). Therefore, although studies on the relationship between dietary fiber and intestinal microbiota have advanced in the last decade, there are still many gaps to be filled on this topic. As limitations to understand this relationship, we can mention the fact that *in vivo* experiments usually administer isolated and purified DF. When DF is present in food with a complex matrix, such as fruits, instead of its purified form, the physiological effects associated with its consumption can be modified because its viscosity or fermentability are affected (Louis et al., 2021; Guan, Yu, & Feng, 2021).

In addition, it is noteworthy that fiber-rich foods such as fruits contain other beneficial nutrients in addition to DF, including minerals and bioactive compounds, hindering to establish the accurate effects of isolated DF on the gut microbiota. DF

interacts with polyphenols in the fruit matrix and the interactions can be covalent (mostly irreversible) and noncovalent (reversible) (Rocchetti et al., 2022). When DF is fermented by the colonic microbiota, the polyphenols are released and become available to the gut bacterial community (Katsirma, Dimidi, Rodriguez-Mateos, & Whelan, 2021). It has already been demonstrated that gut microbes can metabolize these substances, as we are going to discuss in next items. In addition, DF can also be attached to several minerals, including calcium, magnesium, iron, copper, and zinc. Therefore, fermentation of DF lead to the release of these minerals in the colon, where they can inhibit pathogens and increase the diversity of the intestinal bacterial community, contributing to protect the gut from infection (Makki, Deehan, Walter, & Bäckhed, 2018).

Finally, current knowledge about the metabolism of insoluble DF, also found in fruits, by intestinal microorganisms is still limited. Thus, it is crucial to carry out additional studies in order to provide a clearer insight about the influence of food with high fiber content, such as fruits, on the gut microbiota.

#### *Phenolic compounds or polyphenols (PP)*

Phenolic compounds or polyphenols (PP) are generally found in plants and can be present in the peels, pulps, and seeds of fruits. They have one or more aromatic rings with hydroxyl groups and are classified as either flavonoids or non-flavonoids. Flavonoids may be flavonols, flavones, flavanones, flavanols, flavonones, and anthocyanins. Phenolic acids, lignans, tannins, and stilbenes compose a quite distinct group, known as non-flavonoid phenolics (Corrêa, Rogero, Hassimotto, & Lajolo, 2019). In foods, PP are generally found either in a conjugated form mainly with sugars or organic acids, or unconjugated, such as condensed tannin oligomers.

A great variety of fruits are rich in PP, such as açai (*Euterpe oleracea*), buriti (*Mauritia flexuosa* L.) (Neri-Numa et al., 2018), passion fruit (Pertuzatti et al., 2015), orange (Irkin et al., 2015), pitanga (*Eugenia uniflora*), jaboticaba (*Plinia peruviana*), camu-camu (*Myrciaria dubia*), red araçá (*Psidium cattleianum*), and chokeberry (*Aronia melanocarpa*) (Schmid et al., 2020); however, there is a great variation among plant tissues, such as leaves, bark, flowers, pulp, peel, seed, stem, or root (Domínguez-Avila et al., 2021). In plants, PP are responsible for the color and taste of fruits, such as anthocyanins, or are products of a secondary metabolism, which present the ability to modulate the growth of many microorganisms (Piekarska-Radzic & Klewicka, 2021).

The PP impart several bioactive properties; among them, antioxidant, anticancer, antimicrobial, anti-inflammatory, antiproliferative, neuroprotective, hypolipemic, immunomodulatory, and cardioprotective effects are distinguished (Ferreira, Martins, & Barros, 2017; Shen, Xu, & Sheng, 2017; Domínguez-Avila et al., 2021). In recent years, the impact of PP on intestinal microbiota modulation has been gaining relevance in several studies.

The intestinal microbiota may assimilate PP and interactions between PP and their metabolites may shape the intestinal microbiota by stimulating beneficial microbiota and inhibiting negative bacteria proliferation. This happens because gut microbiota converts bound and complex phenolic compounds into other bioactive compounds, which are more easily absorbed than the former compounds. Furthermore, the gut biotransformation results in compounds with better pharmacological effects if compared to the corresponding original compounds (Corrêa, Rogero, Hassimotto, & Lajolo, 2019; Anantharaju et al., 2016). This two-way interaction between phenolic compounds and the human intestinal microbiota may favor the host with many health benefits (Campos, Ribeiro, Teixeira, Pastrana, & Pintado 2020; Corrêa, Rogero, Hassimotto, & Lajolo, 2019; Pap et al., 2021). Among the intestinal microbiota, members from the *Lactobacillaceae* family present the highest ability to tolerate or degrade polyphenolic compounds, such as hydroxycinnamic acids. For instance, *Lactiplantibacillus plantarum* (formerly known as *Lactobacillus plantarum*) is undoubtedly the best species for enzymatic transformation of PP. Furthermore, some PP (and their metabolites) can present prebiotic activity by the stimulation of the growth of commensal bacteria populating the GI tract (Piekarska-Radzik & Klewicka, 2021). In the last few years, PP were included in the ‘three P’s for gut health’, along with probiotics and prebiotics, which means that PP are equivalent to prebiotic ingredients (Marchesi et al., 2016; Moorthy, Chaiyakunapruk, Jacob, & Palanisamy, 2020).

Although research on the interaction between bioactive compounds and the gut microbiota has largely concentrated on the effect against pathogenic bacteria, currently, many studies have been evidencing that the consumption of food containing PP, including fruits, may help preserve the gut microbial balance (ratio of Firmicutes/Bacteroidetes), through an enhancement of healthy bacteria growth, and suppression of the development of pathogenic bacteria, such as *Clostridium* spp., *Escherichia coli* and *Helicobacter pylori* (Cheng et al., 2020; Espín, González-Sarrías, & Tomás-Barberán, 2017; Henning et al., 2017; Piekarska-Radzik & Klewicka, 2021).

The recent findings (Table 4) have shown that some PP (antocyanins, catechin, quercetin, resveratrol, gallic acid, ferulic acid and hesperidin) present in fruits such as berries (cherry, blueberry, berry pomace, elderberry, black raspberry) and others (red grape, apple, limonin, avocado, pomegranate) can stimulate the growth of LAB (*L. casei*, *L. rhamnosus*, *L. paracasei*, *L. plantarum*, *L. acidophilus* and *Bifidobacterium lactis*) and modulate the gut microbiota increasing the beneficial bacteria (*Lactobacillus* spp., *Bifidobacterium* spp. and *Akkermansia* spp.) and decreasing harmful bacteria (*Clostridium* spp., *Escherichia* spp., *Klebsiella* spp. and *Citrobacter* spp.).

#### Fruit polyphenols and their metabolization by microbiota

Most PP are present in fruits as glycosides, whereas other PP are present in the form of polymerized molecules, which are poorly bioavailable; in order to be absorbed, they must be transformed into aglycones. Most PP are not metabolized in the small intestine; however, PP are highly hydrolyzed by the enzymatic activity of the colon microbiota (Henning et al., 2017; Marín et al., 2015; Xian et al., 2021).

From this vantage point, it is important to better know the steps of PP degradation (absorption, metabolization, and elimination) in the body in order to determine their *in vivo* action. Several authors have been researching the effect of food bioactive compounds (including PP) on the intestinal microbiota (Campos, Ribeiro, Teixeira, Pastrana, & Pintado 2020), while others are studying the effects of some specific PP on the microbiota modulation and on some specific strains present in the intestinal microbiota which are essential to human health (Kasprzak-Drozd, Oniszczuk, Stasiak, & Oniszczuk, 2021). These studies range from the apparently simple experimental view to complex experiments involving the entire fecal microbiota, by using batch cultures and dynamic simulators, or evaluating the samples of animal and human feces. The PP action on LAB and gut microbiota has been studied through *in vitro* and *in vivo* models (Table 4).

**Table 4.** Effect of polyphenols (PP) on LAB and on gut microbiota.

	Study design	Effect	Reference
<b><i>In vitro</i> effects on LAB</b>			
Berry pomace phenolic extracts	<i>L. casei</i> ATCC 334 was inoculated in MRS broth supplemented with berry pomace phenolic extract (0.1 mg/mL GAE), followed by incubation at 37 °C/72 h.	At 48 h and 72 h, <i>L. casei</i> ↑ in the presence of berry pomace significantly by 0.71 and 0.19 log CFU/mL, respectively, compared to the growth in absence of extract.	Tabashsum et al. (2019)
Extracts from plum skins, Italian red grape skins, and elderberry (skin, seeds, and fruit)	<i>L. rhamnosus</i> IMC 501, <i>L. paracasei</i> IMC 502, their combination, and <i>L. plantarum</i> IMC 509 were individually inoculated in mMRS with five fruit extracts (1%) to evaluate the prebiotic potential of extracts, and incubated at 37 °C/72 h.	The viability of probiotics in the presence of the five fruit extracts was similar to the positive control (MRS with glucose).	Coman et al. (2018)
Apple peel extract rich in polyphenols	Apple peel polyphenol extract was added at 1%, 2%, 3%, 4% and 5% to yogurt ice cream formulated with probiotic cultures (Chr. Hansen). A control without the extract was prepared.	Apple peel extract led to ↑ <i>Lactobacillus acidophilus</i> and <i>Bifidobacterium lactis</i> at the end of storage (90 days); the highest counts were recorded for the product with 5% extract.	Ahmad et al. (2020)
Commercial catechin and gallic, vanillic, ferulic and protocatechuic acids, identified in different fruits	<i>L. rhamnosus</i> GG ATCC 53103 and <i>L. acidophilus</i> NRRLB 4495 were inoculated in mMRS with phenolic compounds fresh serial dilution (0, 15, 20, 25, 30 and 35 mmol/L) isolated or in combinations; cultures were incubated at 37 °C/24 h.	Catechin and gallic, protocatechuic and vanillic acids in mMRS broth allowed the growth of lactobacilli. Ferulic acid did not promote the growth of strains. Catechin combined with protocatechuic or vanillic acid promoted a slightly ↑ of both probiotics. Gallic acid combined with either protocatechuic acid or catechin, only allowed the growth of <i>L. rhamnosus</i> .	Pacheco-Ordaz et al. (2018)
Commercial limonin, widely found in citrus fruits	Cultures were grown in 96 well plates containing MRS medium supplemented with limonin (10 µM and 100 µM), at 37 °C/48 h.	Both limonin concentrations ↑ <i>B. longum</i> ATCC 15707 and <i>B. infantis</i> 272, ↔ <i>L. plantarum</i> ATCC BAA-793.	Gu et al. (2019)

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***In vitro* effects on gut microbiota**

Commercial tart and sweet cherry concentrates, two tart cherry genotypes, two apricot cultivars and concentrate tart cherry juice	Tart cherry and apricot (5 g) and tart cherry juices (5 mL) were submitted to <i>in vitro</i> bacterial fermentation assays using SHIME® for 48 h.	By comparing times 0 and 48 h: tart and sweet cherry products ↑ <i>Bacteroides</i> , <i>Veillonella</i> , <i>Bilophila</i> <i>Enterobacteriaceae</i> and <i>Escherichia</i> and <i>Clostridium</i> XIVa; tart cherry juice ↑ <i>Bacteroides</i> , <i>Clostridium</i> XIVa, <i>Lachnospiraceae</i> <i>Eubacteriaceae</i> , and <i>Bifidobacterium</i> ; and apricots ↑ Bacteroidetes, <i>Bacteroides</i> , and <i>Lactobacillus</i>	Mayta-Apaza et al. (2018)
Polyphenolic enriched extracts of Chilean currants ( <i>Ribes magellanicum</i> and <i>Ribes punctatum</i> )	Intestinal digested polyphenolic enriched extract was incubated at 40, 80 and 160 µg/mL in pre-reduced PBS medium (pH 7.0) and fecal slurry. FOS (1%, w/v) and fecal slurry without any supplementation were employed as a positive and negative control, respectively. The mixtures were incubated at 37 °C/24 h.	Compared to negative control, extract from <i>R. punctatum</i> (160 µg/mL) and extract from <i>R. magellanicum</i> at all concentrations ↑ <i>Clostridium</i> cluster XIVa. C after 1 h; extract from <i>R. punctatum</i> (160 µg/mL) ↑ <i>F. prausnitzii</i> after 24 h; both extracts ↔ on growth of <i>Lactobacillus</i> and <i>Bifidobacterium</i> genera, <i>A. muciniphila</i> and <i>E. coli</i> .	Burgos-Edwards et al. (2020)
Aronia polyphenols	Aronia juice (100 mL/day) was administered in SHIME® for 2 weeks.	Aronia juice ↑ Firmicutes and <i>Lactobacillus</i> in all vessels, Proteobacteria and <i>Megasphaera</i> in AC vessel, and <i>Akkermansia</i> , <i>Ruminococcaceae</i> , <i>Anaerostipes</i> and <i>Lachnospiraceae</i> in TC and DC vessels; but ↓ <i>Bifidobacterium</i> in all vessels, and <i>Lachnoclostridium</i> .	Wu et al. (2018)
Black raspberry (BRB) extract	Aliquot of 5.07 mL BRB extract in 1 L medium as feeding medium was tested in human colonic models (Macfarlane and SHIME® systems).	BRB intervention resulted in longitudinal and locational changes of gut microbial population. Proteobacteria was the most dominant phylum and bacteria from Firmicutes, Actinobacteria, Bacteroidetes, <i>Verrucomicrobia</i> , and <i>Chloroflexi</i> phyla were also detected. In the ascending, transverse, and descending colons, <i>Citrobacter</i> , <i>Escherichia</i> , <i>Klebsiella</i> , and <i>Phascolactobacterium</i> were the dominant genera.	Zhang et al. (2022)

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**In vivo effects on gut microbiota**

Limonic	Mice (n=10/group) were divided in control group (fed with normal diet) and limonic group (fed normal diet +0.05% limonic) for a 9-week intervention.	Treatment with limonic ↑ richness and diversity, Proteobacteria and Bacteroidetes phyla, <i>Oscillospira</i> ; ↓ Actinobacteria, Firmicutes, <i>Bifidobacterium</i> and <i>Lactobacillus</i> .	Gu et al. (2019)
Tart cherry juice	Ten healthy participants (5 = males, 5 = females) were instructed to consume 8 oz. of tart cherry concentrate juice daily for 5 days. For data analysis, individuals were divided into two groups: low (<10%; n=4; LB) and high (>20%; n=5; HB) <i>Bacteroides</i> in the initial gut microbiota. One individual's sample yielded low read counts and was excluded.	HB group ↓ <i>Bacteroides</i> and <i>Bifidobacterium</i> , and ↑ <i>Lachnospiraceae</i> , <i>Ruminococcus</i> and <i>Collinsella</i> as a result of tart cherry juice intake. LB group ↑ <i>Bacteroides</i> or <i>Prevotella</i> and <i>Bifidobacterium</i> , and ↓ <i>Lachnospiraceae</i> , <i>Ruminococcus</i> and <i>Collinsella</i> as a result of tart cherry juice intake.	Mayta-Apaza et al. (2018)
Commercial hesperidin	Male Lewis rats (n=6/group) were fed a normal diet + 0.5% carboxymethylcellulose (control), normal diet + hesperidin 3 times a day at 100 mg/kg or normal diet + hesperidin 3 times a day at 200 mg/kg, for 4 weeks.	Lower dose of hesperidin ↓ <i>Clostridium</i> <i>coccoides/Eubacterium rectale</i> ↑ <i>Staphylococcus</i> , whereas the highest dose ↑ total bacteria, <i>Streptococcus</i> , <i>Lactobacillus/Enterococcus</i> , <i>Staphylococcus</i> , <i>Bacteroides/Prevotella</i> , <i>Bifidobacterium</i> , and <i>Escherichia coli</i> groups.	Estruel-Amades et al. (2019)
Commercial catechin and quercetin	Wistar rats (n=6/group) were fed a normal diet (control group), or an HFD for 4 weeks without supplementation (HFD group) or individually supplemented with quercetin or catechin (both at 150 mg/kg).	Quercetin or catechin supplement did not change F/B ratio induced by HFD; however, the diversity of gut microbiota was significantly downregulated.	Huang et al. (2016)
Commercial quercetin and trans-resveratrol	Wistar rats were divided in four groups for a 6-week intervention: control group (n=5, fed an HFHSD), trans-resveratrol group (n=6, HFHSD + trans-resveratrol 15 mg/kg/day); quercetin group (n=6, HFHSD + quercetin 30 mg/kg/day); trans-resveratrol + quercetin group (n=6, HFHSD + trans-resveratrol at 15 mg/kg /day + quercetin 30 mg/kg /day).	When compared to control group, trans-resveratrol ↔ F/B ratio, ↓ <i>Graciibacteraceae</i> , <i>Erysipelotrichaceae</i> , <i>Parabacteroides</i> , <i>Clostridia</i> ; quercetin ↓ F/B ratio, <i>Erysipelotrichaceae</i> , <i>Bacillus</i> and <i>Eubacterium cylindroides</i> ; and trans-resveratrol + quercetin ↔ F/B ratio, ↑ <i>Clostridium methylpentosum</i> , <i>Clostridium</i>	Ettxeberria et al. (2015)

Anthocyanins from <i>Lycium ruthenicum</i> Murray	Male C57BL/6 mice divided into two groups (n=10/group) for a 12-week intervention: control group, which received normal water and diet; and treatment group, which received anthocyanin (200 mg/kg/day) in addition to normal water and diet.	<i>clariflavum</i> , <i>Akkermansia muciniphila</i> , and <i>Bacteroides</i> sp. ↓ <i>Bilophila wadsworthia</i> Anthocyanin ↑ richness, <i>Barnesiella</i> , <i>Alistipes</i> , <i>Eisenbergiella</i> , <i>Coprobacter</i> and <i>Odoribacter</i> compared to control. Anthocyanin and control had no difference in diversity.	Peng et al. (2020)
Proanthocyanidin-rich polyphenol extract from avocado	Male Wistar rats were assigned to one of the four following groups (n = 10): control (normal diet), normal diet + avocado extract (300 mg/kg), high-protein diet (HPD control), HPD + avocado extract (300 mg/kg). They received the different diets for 4 weeks.	Normal diet + extract ↑ Actinobacteria, <i>Paraprevotellaceae</i> in comparison to control. HPD + extract ↓ Actinobacteria, Firmicutes, <i>Lactobacillaceae</i> and <i>Lactobacillus</i> , ↑ <i>Paraprevotellaceae</i> and <i>Prevotella</i> than HPD control.	Cires et al. (2019)
Non-absorbable apple procyanidins	Male C57BL/6J mice were divided into four groups (n=10/group) for a 20-week intervention: normal diet, HFHSD, HFHSD + oligomeric procyanidins (OP) and HFHSD + polymeric procyanidins (PP, 0.5%).	PP treatment ↓ F/B ratio in comparison with normal diet group. In comparison to HFHSD, PP ↑ <i>Adlercreutzia</i> , <i>Roseburia</i> , <i>S24-7</i> , <i>Bacteroides</i> , <i>Anaerovorax</i> and eight times the proportion of <i>Akkermansia</i> , but ↓ <i>Clostridium</i> , <i>Lachnospiraceae</i> , and <i>Bifidobacterium</i> .	Masumoto et al. (2016)
Blueberry polyphenol extract	Male C57BL/6 J mice were divided into four groups and fed for 12 weeks: NFD group (negative control group); group receiving HFD; group receiving HFD + extract (200mg/kg/day); group receiving HFD + Orlistat (15.6 mg/kg/day - positive control group).	Blueberry extract ↓ diversity and richness, ↑ Proteobacteria, <i>Deferribacteres</i> , <i>Bifidobacterium</i> , <i>Desulfovibrio</i> , <i>Helicobacter</i> , and <i>Flexispira</i> and ↓ Actinobacteria, <i>Lactococcus</i> , <i>Coprobacillus</i> , <i>Bacillus</i> , <i>Clostridium</i> , <i>Odoribacter</i> and <i>Prevotella</i> compared to HFD group.	Jiao et al. (2019)
Whole blueberry polyphenol extract or its fractions	Male C57BL/6J mice were divided into six groups (n = 12/group): normal diet; HFHSD; HFHSD + whole blueberry polyphenol extract (WBE, 200 mg/kg); HFHSD + fraction rich in anthocyanins and phenolic acids (32 mg/kg); HFHSD + fraction rich in oligomeric proanthocyanidins (PACs), phenolic acids and flavonols	In mice treated with WBE and its fractions, the family <i>Coriobacteriaceae</i> , <i>Verrucomicrobia</i> and the order <i>Clostridiales</i> ↑ compared to HFHSD. WBE ↑ 2-fold <i>Adlercreutzia equolifaciens</i> whereas oligomeric PACs-rich F2 fraction ↑ 2.5-fold the proportion of <i>Akkermansia muciniphila</i> compared to HFHSD.	Rodríguez-Daza et al. (2020)

Pomegranate peel polyphenols	<p>(PACs DP &lt; 4, 53 mg/kg), HFHSD + fraction rich in PACs polymers (PACs DP &gt; 4, 37 mg/kg). They received the different diets for 8 weeks.</p> <p>Male SD rats were divided into four groups (n=12/group): rats fed a LFD, rats fed a HFD, rats fed a HFD + daily gavage of 150 mg/kg pomegranate peel polyphenols (PPP-L) and rats fed a HFD + daily gavage of 300 mg/kg pomegranate peel polyphenols (PPP-H). They received the different diets for 12 weeks.</p>	<p>F/B ratio ↓ in the PPP treatment compared to HFD group. PPP-L and PPP-H treatments altered genera abundance to the same direction in the control group for 8 and 4 genera, respectively. In comparison to HFD group, PPP-L treatment ↑ <i>Bacteroidales</i> S24-7 group_norank, <i>Paraprevotella</i>, <i>Lactobacillus</i>, Family XII AD3011 group, <i>Lachnospiraceae_uncultured</i>, <i>Ruminococcaceae_uncultured</i>, <i>Ruminococcaceae</i> UCG-009, and <i>Ruminococcus</i>. In comparison to HFD group, PPP-H treatment ↑ <i>Prevotellaceae</i> UCG-001, <i>Lactobacillus</i>, Family XII AD3011 group, and <i>Lachnospiraceae_uncultured</i>.</p>	Zhao et al. (2019)
Polyphenol-rich fraction obtained from table grapes	<p>Male C57BL/6J mice (n=10/group) were divided in eight groups and fed: LFD, HFD, HFD + whole table grape powder (5%), HFD + extractable, polyphenol-rich (HFD-EP) fraction, HFD + non-extractable, polyphenol-poor (HFD-NEP) fraction or HFD + equal combinations of both fractions (HFD-EP+NEP) from grape powder, for 16 weeks. EP and NEP fractions were added to the HFD in amounts equal to their relative in the 5% powdered whole grape diet.</p>	<p><i>Lachnospiraceae</i> ↓ in mice consuming the HFD but ↑ in the HFD-EP and in HFD-EP+NEP groups. <i>Coprococcus</i> ↑ in the HFD-NEP and HFD-EP+NEP groups. HFD-EP+NEP group ↓ <i>Ruminococcus</i> and <i>Mogibacteriaceae</i>. Grape powder restored microbiota diversity while EP or NEP partially restored the HFD-mediated reduction in diversity but not significantly.</p>	Collins et al. (2016)

Blueberry polyphenol extract	Male C57BL/6 J mice were divided into four groups and fed for 12 weeks: NFD group (negative control group); group receiving HFD; group receiving HFD + extract (200mg/kg/day); group receiving HFD + Orlistat (15.6 mg/kg/day - positive control group).	Blueberry extract ↓ diversity and richness, ↑ Proteobacteria, <i>Deferribacteres</i> , <i>Bifidobacterium</i> , <i>Desulfovibrio</i> , <i>Helicobacter</i> , and <i>Flexispira</i> and ↓ Actinobacteria, <i>Lactococcus</i> , <i>Coprobacillus</i> , <i>Bacillus</i> , <i>Clostridium</i> , <i>Odoribacter</i> and <i>Prevotella</i> compared to HFD group.	Jiao et al. (2019)
Flavonoid-rich Quzhou Fructus Aurantii extract	C57BL/6J mice were fed with either a normal diet or HFD with or without oral gavage of Quzhou fructus aurantii extract (TFQ, 300 mg/kg/day) for 12 weeks (n=12 mice/group).	TFQ treatment reversed HFD-induced gut dysbiosis: ↓ F/B ratio, ↑ <i>Akkermansia</i> and <i>Alistipes</i> , and ↓ <i>Dubosiella</i> , <i>Faecalibaculum</i> and <i>Lactobacillus</i> .	Bai et al. (2019)

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AC - ascending colon; ATCC - American Type Culture Collection; CFU - colony forming unit; DC - descending colon; DP - degree of polymerization; F/B - Firmicutes/Bacteroidetes; FOS - fructooligosaccharide; GAE - gallic acid equivalent; HFD - high-fat diet; HFHSD - high-fat high-sucrose diet; HPD - high-protein diet; LAB - lactic acid bacteria; LFD - low-fat diet; mMRS - modified de Man-Rogosa-Sharpe; MRS - de Man-Rogosa-Sharpe; MW - molecular weight; NFD - normal-fat diet; PBS -phosphate buffer saline; SHIME - Simulator of the Human Intestinal Microbial Ecosystem; TC - transverse colon; TFQ - Total flavonoids of Quzhou fructus aurantii extract ↔ - no effect; ↑ - increase; ↓ - decrease.

Studies involving the fecal metabolism of food PP, including flavonoids (isoflavones and anthocyanins), phenolic acids (hydroxycinnamic acids, ellagitannins, ellagic acid) and lignans, showed high variations in absorption, metabolism, and excretion among individuals, which was related to differences in the intestinal microbiota (Rowland et al., 2018; Tomás-Barberán et al., 2014).

The steps of PP catabolism by the gut microbiota involve the hydrolysis (deglycosylation and ester hydrolysis) of native PP, the cleavage of glycosidic linkages (carbon rings opening, C-C bonds disruption, and carbon removal from side chains), as well as the reduction (hydrogenation of a C=C double bond, reduction of carbonyl compounds, and specific dihydroxylation). The cleavage step converts non-absorbable low molecular weight compounds into bioavailable phenolic acid molecules, such as hydroxyphenyl-acetic acids and hydroxyphenyl-propionic acids, and the hydrolysis of hydroxyls removal depends on the bacterial species in the gut (Espín, González-Sarrías, & Tomás-Barberán, 2017; Rowland et al., 2018).

The microbial species involved in the PP hydrolysis include *L. plantarum*, *Clostridium* sp., some species of *Bacteroides*, such as *B. distasonis*, *Bacteroides uniformis*, and *Bacteroides ovatus*, some species of *Enterococcus*, such as *E. casseliflavus*, *Eubacterium cellulosolvens*, and *Eubacterium ramulus*, *Slackia equolifaciens*, *Slackia isoflavoniconvertens*, and other intestinal bacteria, such as *Lachnospiraceae* CG19-1, *Flavonifractor plautii*, *Adlercreutzia equolifaciens*, *Eggerthella lenta*, and *Bifidobacterium* spp. (Corrêa, Rogero, Hassimotto, & Lajolo, 2019; Marín et al., 2015; Braune, Engst, & Blaut, 2015; Rowland et al., 2018; Piekarska-Radzik & Klewicka, 2021).

Among the species aforementioned, *L. plantarum* is commonly used for vegetables fermentation (olives and sauerkraut), it is present in the human microbiota, and some strains show probiotic properties. *L. plantarum* have enzymatic activities for the degradation of PP and other compounds, such as reductase, tannase, gallate decarboxylase, phenolic acid decarboxylase, benzyl alcohol dehydrogenase,  $\beta$ -glucosidase,  $\alpha$ -glucosidase,  $\beta$ -galactosidase, esterase, aryl glycosidase, and feruloyl esterase activities (Esteban-Torres et al., 2013; Reverón et al., 2017; Plessas, 2022).

Some strains of *L. plantarum* present feruloyl esterase activity which is useful for the efficient hydrolysis of methyl ferulate, methyl caffeate, methyl p-coumarate, and methyl sinapinate (Esteban-Torres et al., 2013). Landete et al. (2014) showed that *L. plantarum* is responsible for the deglycosylation of specific aryl glycosides, which related

to an increase in their antioxidant activity, evaluated using the methods 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and superoxide dismutase. Therefore, the ability of *L. plantarum* strains to produce glycosidase increases the antioxidant activity of glycosylated phenolic compounds, besides improving PP bioavailability. Esteban-Torres et al. (2013) reported that the hydrolytic activity of some polyphenol substrates is a result of the esterase activity from *L. plantarum*. Other species, such as *Eubacterium ramulus*, *E. oxidoreducens*, *Flavonifractor plautii*, and *Clostridium* sp. strains, are responsible for the degradation of other PP, such as daidzein, quercetin, kaempferol, naringenin, xanthohumol, catechin and epicatechin (Corrêa, Rogero, Hassimotto, & Lajolo, 2019; Selma, Espín, & Tomás-Barberán, 2009). For example, gallic acid is degraded by *Gordonibacter urolithinifaciens* and *Gordonibacter pamelaiae* (Selma et al., 2014; Tomás-Barberán et al., 2014). It has been reported that chlorogenic, caffeic, and ferric acids can be degraded by *E. coli*, *Bifidobacterium lactis*, and *Lactobacillus gasseri* (Tomás-Barberán et al., 2014), while catechin can be degraded by *Clostridium coccooides* and *Bifidobacterium infantis* (Marín et al., 2015); *Slackia equolifaciens* and *S. isoflavoniconvertens* can convert isoflavones to O-desmethylangolensin and *Slackia equolifaciens*; and *Adlercreutzia equolifaciens* are responsible for the conversion of resveratrol into dihydroresveratrol and lunularin (Corrêa, Rogero, Hassimotto, & Lajolo, 2019).

Ellagitannins are resistant to degradation in the stomach; they are hydrolyzed into ellagic acid and absorbed in the small intestine. As a result, free ellagic acid and great amount of unabsorbed ellagitannins reach the colon and are metabolized into urolithins by the gut microbiota (Xian et al., 2021). Ellagitannin-rich fruits, such as pomegranate, blackberry, raspberry, açai berry, and strawberry are considered rich source of urolithins, which in turn will block the inflammatory response, thus improving muscle and mitochondrial functions (Corrêa, Rogero, Hassimotto, & Lajolo 2019; Piwowarski et al., 2016). Studies evidence that the consumption of extracts or plants rich in ellagitannins may increase the bioactivity of metabolites from the intestinal microbiota and inhibit the production of pro-inflammatory cytokines (tumor necrose factor  $\alpha$ , TNF- $\alpha$  and interleukin 6, IL-6) (Piwowarski et al., 2014). The ellagic acid is metabolized into urolithin by the intestinal microbiota and urolithin has shown strong inhibition of heme peroxidase enzymes (myeloperoxidase and lactoperoxidase), thereby decreasing cellular inflammation. Moreover, there was lower generation of superoxide radicals' with urolithin; in contrast, ellagic acid was inefficient to reduce the oxidative damage.

Therefore, it is clear that gut microbiota helps PP degradation, which in turn modulates gut bacterial community. As an example, in an animal intervention, mice were fed with different diets: low-fat diet (10% fat), high-fat diet (45% fat) and high-fat diet (45% fat) supplemented with 0.1%, 0.3% and 0.4% red raspberry seed PP. Red raspberry pulp and seed PP increased *Roseburia* abundance and decreased levels of an unclassified genus from *Mogibacteriaceae*. Additionally, the higher concentration of red raspberry seed (with 40.7% ellagic acid/total GAE) increased the population of *Bifidobacterium* compared to a high-fat diet not supplemented (Xian et al., 2021). In another study, the consumption of berries which are rich in quercetin (30 mg/kg/day) and resveratrol (15 mg/kg/day) for 10 weeks resulted in an increase in the Bacteroidetes/Firmicutes ratio associated with an expressive increase of *Parabacteroides*, *Bilophila*, *Akkermansia*, and a decrease of *Lachnospiraceae* (Zhao et al., 2017; Pap et al., 2021).

Although substantial number of human studies and meta-analyses have discussed the health properties associated with PP, including the modulation of the human microbiota, a well-established cause-and-effect relationship between PP and their possible beneficial human health remain unknown (Corrêa, Rogero, Hassimotto, & Lajolo, 2019; Iglesias-Aguirre et al., 2021; Tomás-Barberán et al., 2016).

The interplay between the intestinal microbiota and the different dietary PP is still not fully understood. Despite the studies showing promising and exciting results about the positive effects of PP on the intestinal microbiota, most of them disregarded the influence of the food composition and food processing conditions. This is a concern since food components interact with PP, significantly affecting their bioaccessibility and bioavailability and, consequently, their metabolism on the host (Aravind et al., 2021).

In this context, further research is crucial, especially the clinical trials, which allow to explain the mechanisms beyond the benefits of PP reported using *in vitro* or animal studies, to better understand the reciprocal interaction among PP, their metabolites and gut microbiota, as well as to assure a deep comprehension of PP metabolic pathways. Additionally, human interventions are complex and are influenced by individual characteristics (genetic, age, diet, life style, and clinical markers). Ultimately, the approaches using multi-omics techniques (genomic, proteomic and metabolomic) are vital as they provide substantial information about the impact of different types of PP and metabolites on gut community composition, and consequently, how PP exert health benefits on the host.

### *Fatty acids (FA)*

Fruits and nuts can be considered as important source of bioactive compounds, such as fatty acids (FA), dietary fiber, vitamins, minerals, antioxidants, and other phytochemicals. The FA are important for the human nutrition because of their multifunctional properties.

Dietary lipids can also alter the composition of the intestinal microbiota, acting as substrates for bacterial metabolic processes or decreasing bacterial growth due to their toxicity to certain bacteria (e.g *Bacteroides*, *Clostridium* and *Roseburia*) (Schoeler & Caesar, 2019; Agans et al., 2018).

It was reported that different dietary fat could have completely different effects on physiological indicators and on gut microbiome. The source of dietary fat as saturated FA (SFA), monounsaturated FA (MUFA), and polyunsaturated FA (PUFA) significantly affects the biochemical markers, intestinal microbial diversity, insulin sensitivity, and metabolic utilization of FA, and the composition of SFA in fat has the most significant effects on the gut microbiome (Patterson et al., 2014; López-Salazar et al., 2021; An et al., 2022). Among the MUFA, oleic acid ( $\omega$ -9, C18:1) is the most relevant one in terms of health benefits. Despite of that, few studies reported the impact of dietary FA from fruits on the intestinal microbiota, and their effects on the gut microbiota are poorly understood. Most of the studies focused on the  $\omega$ -3 and  $\omega$ -6 PUFA supplementation and modulation of the gut microbiota.

PUFA, such as  $\omega$ -3 FA, were detected in many fruit varieties, such as açai (*Euterpe oleracea*), buriti (*Mauritia flexuosa*), jenipapo (*Genipa Americana* L.), inajá (*Maximilianamaripa* D.), mucajá (*Coumarigida* M.), and pineapple (*Ananas comosus* L.) (Costa, Ballus, Teixeira Filho, & Godoy, 2011; Batista et al., 2016; Hong et al., 2021). PUFA were also detected in blackberry, chokeberry and raspberry seeds (Piasecka, Górska, & Ostrowska-Ligeza, 2021). These PUFA are beneficial to health due to their capacity to reduce the incidence of cardiovascular diseases (Costantini, Molinari, Farinon, & Merendino, 2017; Watanabe & Tatsuno, 2017) and inflammatory conditions (Baker et al., 2016). PUFA also assist adherence by the probiotic strains onto the mucosa of the distal portion of the intestines (Hiippala et al., 2018; Custer et al., 2022).

Kübeck et al. (2016) demonstrated that high-SFA diets are relatively unhealthy, since these fats significantly affect intestinal microbiota and body weight. Additionally, diets rich in SFA were associated with a decrease in bacteria from Bacteroidales and an increase in Clostridiales orders. Conversely, palmitic acid and stearic acid, which are

long-chain SFA, presented liver protective activity in chronic alcohol-fed Jinhua ham by increasing relative abundances of *Akkermansia muciniphila* and *Lactobacillus* in the gut, which were beneficial to regulating intestinal homeostasis (Nie et al., 2022).

Paturi, Butts and Bentley-Hewitt (2017) evaluated the impact of diets containing avocado (5, 10, or 15%) on the gut health of rats fed over a six-week intervention. Significantly, higher food intakes (10.8%) were observed for avocado-fed rats, while their body weights were similar to the control diet-fed rats. A significantly higher expression of  $\beta$ -defensin 1, mucin 3 or mucin 4 genes, as well as a greater number of mucin-producing goblet cells in the colon were detected in avocado-fed rats. In the study of Patrone et al. (2018), they administered two different diets: high in saturated fat-coconut oil (HFC) - or high in polyunsaturated fat-soy oil (HFS) - to C57BL/6 N mice. The body weight gain and fat storage of HFC diet was similar than HFS diet; however, HFC diet produced higher plasma cholesterol levels after 8 weeks of treatment. No variation in microbial diversity was observed between the two high-fat diets; nonetheless, an increased proportion of *Limosilactobacillus reuteri* and bacteria belonging to the genera *Allobaculum*, *Anaerofustis*, and *Deltaproteobacteria*, and a decreased proportion of *A. muciniphila* were reported for the cecal microbiota of mice fed with the HFC diet compared to the HFS diet. Additionally, the bacterial gene functions demonstrated that the cecal microbiota of mice fed with the HFC diet was reduced of pathways, which are responsible for the metabolism of many compounds (FA, amino acid, xenobiotic, terpenoids, and polyketides) compared to mice fed with the HFS diet. In summary, these findings show that HFS diet is more favorable in terms of its beneficial effects on gut microbiota.

In another study, Djurasevic et al. (2018) studied the influence of virgin coconut oil (VCO) on modulating the gut microbiota composition in both non-diabetic and alloxan-induced diabetic rats. The VCO positively impacted the fecal microbiome; a significant increase in the proportion of probiotic bacteria, such as *Lactobacillus*, *Bifidobacterium*, and *Allobaculum* species was observed.

The conjugated linoleic acid (CLA,  $\omega$ -6 PUFA) presented different biochemical effect on wild-type and obese-hyperglycemic mice (OB). The gut microbiota indicated a higher abundance of beneficial bacteria (e.g., *Lachnospirillum*, *Roseburia*, *Dubosiella*, *Oscillibacter*, and *Anaerostipes*) and a lower abundance of pro-inflammatory bacteria (e.g., *Tyzzarella* and *Alistipes*) in the OB mice treated with CLA group than those of the OB group (Gao et al., 2022).

Supplementation with  $\omega$ -3 PUFA in adults resulted in the gut microbiota modulation; there was a decrease in *Faecalibacterium* genera and an increase in the Bacteroidetes and butyrate-producing bacteria. Additionally, it was shown that  $\omega$ -3 PUFA have a positive effect on the microbiota composition of patients with inflammatory bowel disease (IBD), and increase the production of SCFA, which present anti-inflammatory activity (Costantini, Molinari, Farinon, & Merendino, 2017). Watson et al. (2018) studied the impact of  $\omega$ -3 PUFA supplements (4 g mixed EPA and DHA) on the fecal microbiome in healthy volunteers, by using the supplements in two formulations, for 8 weeks. No significant changes were observed in the bacterial diversity, or phyla composition related to  $\omega$ -3 PUFA supplementation. However, a reversible increased abundance of *Bifidobacterium*, *Lactobacillus*, and *Roseburia* was reported for one or both  $\omega$ -3 PUFA treatments.

Studies on the impact of  $\omega$ -3 PUFA on modulating the microbiota have mainly focused on the Bacteroidetes and Firmicutes phyla, by using animal and human models. The supplementation of  $\omega$ -3 PUFA decreases the proliferation of *Enterobacteriaceae*, thus reducing the population of pathogenic bacteria of the *Clostridium coccoides* group, which are the main bacteria that give rise to inflammatory bowel diseases (Ghosh et al., 2013, Custer et al., 2022). Also, supplementation with  $\omega$ -3 PUFA increases the Bacteroidetes phyla, and butyrate-producing bacteria belonging to the *Lachnospiraceae* family and decreases the *Faecalibacterium* genus (Ortega et al., 2022).

Such regulation of the gut microbiota decreases the inflammatory process, and may also improve insulin resistance, intestinal barrier functions (through increased regenerating islet-derived 3-gamma proteins), secretory immunoglobulin A concentrations and mucus protective properties and lipid profiles (Custer et al., 2022; Zapata et al., 2022).

Robertson et al. (2017) tested a  $\omega$ -3 supplementation in rats of different age groups and evaluated the composition of their fecal microbiota. Rats supplemented with  $\omega$ -3 presented considerably higher proportions of the *Bifidobacterium* and *Lactobacillus* genera among the adult group in comparison to the adolescent one. Additionally, the abundance of Bifidobacteria/Enterobacteria was significantly higher among the adult and adolescent  $\omega$ -3 supplemented groups. The conversion of  $\omega$ -3 into  $\omega$ -6 FA over the neural development contributed to the behavioral deficits observed in adult life.

Unlike mice, the impact of a diet rich in lipids on the human microbiota is more difficult to be measured, it is believed that this difficulty is associated with a wide variety

of microbiota composition as well as its individualization, which may not be influenced by dietary nutrition. The daily intake of  $\omega$ -3 PUFA, EPA and DHA provided an increase in several genera of bacteria considered beneficial, such as: *Bifidobacterium*, *Roseburia* and *Lactobacillus* (Watson et al., 2018). Conversely, it has been shown that SFA, MUFA and  $\omega$ -6 PUFA are associated with decreased populations of *A. muciniphila*, which are important microorganisms in preserving the integrity of the intestinal barrier (Rodriguez-Carrio et al., 2017). According to the aforementioned studies, the  $\omega$ -3 PUFA have demonstrated higher positive effect on modulating the microbiota compared to the other types of FA.

### *Carotenoids*

Carotenoids are lipophilic pigments accountable for the characteristic yellow, orange or red colors of several fruits; however, they are not synthesized by the human body and must be obtained through the diet (Lyu, Wu, Wang, Shen, & Lin, 2018), as fruit and vegetables, considered the main source of carotenoids.  $\beta$ -carotene,  $\alpha$ -carotene,  $\beta$ -cryptoxanthin, lycopene, lutein, and violaxanthin comprise most carotenoids found in food (Stephenson, Ross, & Stanton, 2021). These compounds have received considerable attention due to their unique physiological functions. Besides being related to the prevention of certain diseases and antioxidant, anti-inflammatory and provitamin A activities (Stephenson, Ross, & Stanton, 2021; Schmidt et al., 2021), carotenoids are linked to the activities of the gut microbiota (Bohn et al., 2015; Bohn et al., 2017).

In the colon, the fermentation by intestinal bacteria significantly alters the structure and profile of carotenoids, and only a small amount of them remains intact. However, in some cases, it is difficult to differentiate the origin of the metabolites - whether from the diet or produced *in vivo*. Therefore, the metabolism of carotenoids in the colon and other organs are poorly understood and the demonstration of the benefits has neither been properly studied nor available in the literature (Schmidt et al., 2021; Stephenson, Ross, & Stanton, 2021).

The bioaccessibility and the bioavailability of carotenoids depends on many factors, such as species of carotenoid, molecular linkage, amount consumed in a meal, effectors of absorption and bioconversion, nutrient status of the host, genetic factors, host-related factors and interactions, which can be responsible for the variation among individuals (Shilpa, Shwetha, Raju, & Lakshminarayana, 2020; Stephenson, Ross, & Stanton, 2021).

Recent findings showed that carotenoids would be beneficial for lowering the risk of diseases associated with gut dysbiosis (Lyu, Wu, Wang, Shen, & Lin, 2018). A dietary carotenoid supplementation can improve the immune system maturation. The intake of carotenoids results in an endogenous production of retinoic acid which is the oxidized form of vitamin A. Vitamin A in turn has remarkable actions on the mucosal barrier; it regulates the intestinal barrier function by modifying the expression of tight junction proteins besides modulating the innate and adaptive immune systems by activating B cell and producing immunoglobulin A (IgA), as well as modulates the gut microbiota (Farré, Fiorani, Abdu Rahiman, & Matteoli, 2020; Zhou et al., 2020).

IgA plays a critical role avoiding the microbiota passing from the intestinal lumen into the systemic circulation. Additionally, IgA is important to keep gut homeostasis and its proper function, as well as to prevent the development of dysbiosis (Lyu, Wu, Wang, Shen, & Lin, 2018). Gut microbiota impact the bile acid homeostasis and retinoid metabolism; it synthesizes secondary and tertiary bile salts by different biochemical reactions, e.g., deconjugation, oxidation, epimerization, esterification, and desulfation, which aid to absorb, metabolize, and transport carotenoids and retinoid acid (Srinivasan & Buys, 2019). Besides its effects on IgA production, carotenoids interact with the host's adaptive immune system towards the activation of macrophages, natural killer cells, Tregs cells, and/or Tregs cells which can also influence the gut microbiota composition (Lyu, Wu, Wang, Shen, & Lin, 2018).

Provitamin A and its retinoid metabolism pathway play an essential role in the adaptive intestinal immune response. Moreover, the retinoic acid signaling in B cells also contributes to create an adequate humoral response in the gut and to preserve a normal microbiota structure, with a small population of adherent bacteria from both the *Lachnospiraceae* and *Lactobacillus/Streptococcus* groups in stool samples, which has related to colorectal cancers (Pantazi et al., 2015). In contrast, diets which are deficient in vitamin A change the intestinal microbiota by decreasing the abundance of *Lactobacillus* spp. and the total bacteria in the GI tract; also, they increase intestinal leak and activate the nuclear factor- $\kappa$ B, a signaling pathway that leads to the expression of inflammatory cytokines in the gut (Zhou et al., 2020).

Considering the current scientific knowledge connecting gut dysbiosis and several health problems, different dietary strategies have been studied aiming to promote gut homeostasis. In this scenario, carotenoids have just started to be explored. Nevertheless, knowledge on the association of carotenoids and gut microbiome or and probiotic LAB

is poorly understood and, heretofore, derived products or bacterial metabolites generated from carotenoid degradation have not been identified yet (Bohn et al., 2015; Bohn, 2017; Bohn et al., 2017; Schmidt et al., 2021).

To the extent of our knowledge, there are few studies that used food rich in carotenoids instead of isolated compounds in order to evaluate its impact on the gut microbiota. As an example, tomato powder (containing 2.39 mg lycopene, 0.11 mg  $\beta$ -carotene, 0.29 mg ascorbic acid, and 0.32 mg  $\alpha$ -tocopherol per gram and administrated at 41.9 g/kg of diet) enhanced gut bacterial richness and diversity, as well as prevented the inflammation in mice fed with a high fat-diet for 24 weeks. The reduction in the relative abundance of *Clostridium* and *Mucispirillum* genera was associated with the lower inflammation, since *Clostridium* has been connected with increased hepatic lipogenesis and reduced FA oxidation whilst *Mucispirillum* has been associated with increased levels of inflammatory cytokines and serum leptin levels (Xia et al., 2018). In another study, pregnant women were enrolled in a two-arm study designed to determine associations among reported carotenoid intake, plasma carotenoid concentrations, and fecal bacterial communities. The  $\alpha$ -carotene (AC) and  $\beta$ -carotene (BC) concentrations were higher in women who recently consumed foods high in carotenoids, while cryptoxanthin (CR) concentrations were higher in women who consumed oranges/orange juice. Microbiota  $\alpha$ -diversity positively correlated with AC and BC and positively associated with dietary and plasma carotenoids, while microbiota  $\beta$ -diversity differed significantly between groups (Schmidt et al., 2021).

Even though these recent studies showed the effect of carotenoids on the human gut microbiota profile, further studies regarding the relation of carotenoids intake, healthy metabolites and modulation of gut microbiota, as well as carotenoids associations with NGP, such as *Akkermansia* and *Ruminococcus* are topics that deserve to be further investigated, especially those involving placebo-controlled double-blinded human trials.

### *Vitamins*

Fruits are rich in vitamins, mainly vitamins C and E. Emerging evidence show that vitamins can modulate the gut microbiota when administered in large amounts to avoid full absorption in the small intestine or when they are delivered through specific systems targeting the colon. Besides, vitamins seem to impact the gut microbiota indirectly via systemic circulation (Steinert, Lee, & Sybesma, 2020).

Vitamins should be ingested daily, in small amounts, since humans cannot

synthesize them. Another interesting option is to use vitamin-producing LAB for food production, which also represent a natural and consumer-friendly alternative to fortification compared to using chemically synthesized vitamins (Albuquerque et al., 2020). Vitamins play a systemic role in human health, being directly implicated in the proper functioning of metabolism and in a range of functions of the immune and physiological systems (Schmidt et al., 2019).

Fruits provide large amounts of pro-vitamin A carotenoids, vitamin C, folate, vitamin K-1, potassium, calcium, magnesium, iron, and several other trace elements. Therefore, health benefits attributed from fruit consumption are related to additive and synergistic effects of its components. Additionally, fruits are negatively associated with all-cause mortality and mortality from cardiovascular disease and cancer (Melse-Boonstra, 2020).

Vitamin C (ascorbic acid) is one of the main water-soluble vitamins which are naturally present in fruits. It is an essential vitamin which is involved in many biological processes of the immune response and antioxidant action. Citrus fruits, kiwifruit, pitanga and strawberry are widely acknowledged as good sources of vitamin C (Fenech, Amaya, Valpuesta, & Botella, 2019; Pereira et al., 2022).

Vitamin E is a fat-soluble nutrient which provides protection from oxidative stress and protects lipids from peroxidation, acting against aging and helping in the transport of nutrients (Choi et al., 2020; European Food Safety Authority, 2017; Grimm et al., 2015). Vitamin E can be found in fruits; however, the content is highly variable. Kiwifruit contains 2.81 µg/100 g and blackberry contains 1.17 µg/100 g vitamin E, while most of other fruits contain around 0.5 µg/100 g, such as peach, mango, apple, and orange. Buriti and açai berries stand out among other fruits and can be considered extremely healthy since they have a high content of vitamin E (α-tocopherol): 19.6 mg/100 g and 14.8 mg/100 g vitamin E, respectively (Combs & McClung, 2017; Tabela Brasileira de Composição de Alimentos, 2020).

The remarkable antioxidant activity of vitamins C and E justifies the evaluation of their efficacy for modulating the gut microbiota. The presence of oxygen in the gut has been pointed out as responsible for leading to gut dysbiosis by reducing beneficial bacterial groups, including *Lachnospiraceae*, *Ruminococcaceae*, and *F. prausnitzii*, which are strictly anaerobic. Moreover, the presence of oxygen has also been found to stimulate the growth of harmful microbes, such as certain *Enterobacteriaceae* (Steinert, Lee, & Sybesma, 2020). In this sense, a supplementation with vitamins C and E, or the

consumption of fruits which are rich in such vitamins, may help to improve the microbiota composition.

Some studies have shown the effect of fruit on the intestinal microbiota. For instance, Duque, Monteiro, Adorno, Sakamoto and Sivieri (2016) observed that fresh orange juice ( $43.13 \pm 0.44$  mg/100 mL ascorbic acid) increased *Lactobacillus* spp., *Enterococcus* spp., *Bifidobacterium* spp., and *Clostridium* spp., besides decreasing enterobacteria; on the other hand, the pasteurized orange juice ( $34.18 \pm 0.25$  mg/100 mL ascorbic acid) increased *Lactobacillus* spp. and reduced enterobacteria. Both orange juices increased SCFA amounts (butyric, acetic, and propionic) and presented high antioxidant activity, whereas ammonium production was reduced. In another study, an intervention trial period (12 weeks) of daily consumption of two SunGold kiwifruit resulted in significant improvement in the vitamin C status and decrease in both the blood pressure and anthropometric parameters. Additionally, an increase in the relative abundance of the *Coriobacteriaceae* family, mainly in the *Atopobium*, *Collinsella*, *Eggerthella*, *Gordonibacter*, *Senegalimassilia* genera, which metabolize plant polyphenols was reported, and consequently, it represents a desirable characteristic when aiming at human health promotion (Wilson et al., 2018).

Recently, ascorbic acid has been associated with a reduction of inflammatory cytokines (IL-17A, IL-4, and IFN- $\gamma$ ) in individuals with IBD and such effect was mainly caused by the ascorbate produced by gut microbiota, since dietary ascorbate is largely absorbed before reaching the colon. The authors tested which gut organisms can produce this vitamin and validated the production of ascorbate in *Pseudomonas aeruginosa*, which belongs to Crohn's disease-associated microbiota (Chang et al., 2018).

The study by Choi et al. (2020) evaluated the effect of a daily intake of DL- $\alpha$ -tocopherol at the concentration of 0.06 mg/20 g of body weight (low vitamin – LV) and 0.18 mg/20 g (high vitamin – HV) on the gut microbiota, over 34 days. The administration of the lower amount of vitamin E resulted in higher Firmicutes to Bacteroidetes ratio. The prevalence of Proteobacteria, which harbors various enteropathogens, such as *Escherichia coli* and *Salmonella*, in LV and HV groups was higher than in the control group, whereas the abundance of Verrucomicrobia which includes *A. muciniphila* was lower in LV than in HV and control groups.

The association among the visceral fat mass accumulation, gut microbiota composition, and vitamin E intake (among other nutrients) was evaluated in a cohort of elderly female twins (n = 1760). The intake of vitamin E influenced the association

between visceral fat mass accumulation and the *Bacteroides*, *Oxalobacter*, and *Acidaminococcus* genera, and the *Rikenellaceae* family. However, the authors suggest that further studies are needed in order to elucidate if the gut microbiota interfere with the effect of vitamin E on the human body (Le Roy et al., 2019).

As discussed above, questions on the impact of vitamins C and E on the gut microbiota community remain to be answered. Further investigations to explore vitamin-producing LAB are of crucial role to offer novel alternatives for food production, as well as to understand better the conditions to synthesize vitamins for therapeutic applications. Studies about the interaction of vitamins, LAB and the intestinal microbiota may also fulfill the knowledge gap regarding the effects of vitamins on the gut mucosal function and immune system.

### **Final remarks**

There is an increasing interest in fruits as potential sources of bioactive compounds in the diet, providing health benefits to the immune, cardiovascular, and GI systems. These beneficial compounds present in fruits can be explored by the food industry to develop novel functional food products, or to improve the nutritional value of food; they have the potential to enhance the consumers' health through the modulation of intestinal microbiota. Many studies have been focusing on the interactions of fibers and polyphenols with probiotic strains or intestinal microbiota; but concerning the other bioactive compounds, the knowledge regarding their effects still needs to be deeper investigated. So far, most of the studies have reported the direct effect of fruit, their extracts or by-products, which are complex matrices that contain a variety of nutrients and bioactive compounds, so the observed benefic effect cannot be attributed to a specific bioactive compound. For this reason, it is also important that studies in the future propose to evaluate how each isolated bioactive compound, detached from the complexity of fruit matrix, modulates gut microbiota. These studies will help to understand the pathways by which the bioactive compounds are absorbed, metabolized, and eliminated from the body, as well as how they interfere in the gut microbiota. Therefore, a thorough understanding of the effectiveness of bioactive compounds from fruit on health would help us establish a mode of action on the human intestinal balance with greater accuracy.

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## Declaration of interest statement

The authors have no interest to declare.

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## CAPÍTULO II – ARTIGO ORIGINAL

### **Buriti and passion fruit pulps are sources of bioactive compounds and stimulate the growth of potentially probiotic strains**

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#### **Abstract**

Fruits are known to be rich in vitamins, minerals, fatty acids, fibers, and bioactive compounds. They can be added to fermented products to enhance its healthy appeal and nutritional value although the effects of fruits on fermenting microorganisms needs to be previously analyzed. Therefore, this study aimed to evaluate the chemical characteristics and bioactive compounds of passion fruit and buriti pulp as well as their effect on lactic acid bacteria (LAB) growth. Buriti pulp stood out in relation to protein, °Brix and total fiber. Cellulose and hemicellulose were detected in both fruit pulps; however, buriti pulp stood out due to the larger amount of these compounds. Buriti pulp also showed higher fatty acid content (12 g/100 g), and approximately 76% of which was omega 9, while passion fruit pulp presented 0.5 g/100 g, around 37% was omega 9 and 29% palmitic acid. The major phenolic compounds present in buriti and passion fruit pulps were quercetin-3-rutinoside (90.76 mg/100 g pulp) and orientin-7-O-glucoside (1.45 mg/100 g pulp), respectively. Regarding carotenoids, higher amounts were found in buriti pulp (153.18

mg/100 g) than in passion fruit pulp (10.05 mg/100 g), and the major compound in both pulps was  $\beta$ -carotene. Both pulps did not inhibit the growth of the LAB, demonstrating that passion fruit and buriti pulps can be used together with these bacteria to produce functional fermented foods.

## 1. Introduction

Fruits are source of numerous bioactive compounds, such as fibers, vitamins, minerals, fatty acids, carotenoids, phenolic compounds including flavonoids, phytoestrogens, sulfur compounds, monoterpenes, and bioactive peptides. Some researchers have shown that there is a positive correlation between eating healthy foods, such as fruits and vegetables, and reducing the death rate from heart disease, cancer, and other diseases. This is mainly related to the presence of an efficient mixture of bioactive compounds, antioxidants, and dietary fibers present in these foods (MAQSOOD et al., 2020).

Bioactive compounds have been widely studied due to their contribution to human health through multiple biological effects, including antioxidant, anti-inflammatory, anti-mutagenic and anti-carcinogenic activities. They have several health benefits mainly towards chronic conditions, pro-inflammatory state and metabolic disorders, since they exert effects on energy intake and contribute to decrease the oxidative stress (SEPTEMBRE-MALATERRE; REMIZE and POUCHERET 2018; MAQSOOD et al., 2020; COMAN et al., 2018). Therefore, the increase of fruit consumption is encouraged in Western populations in order to reduce weight gain, cardiovascular diseases, diabetes, and hypertension.

Recently, Coman et al. (2018), Tabashsum et al. (2019) and Ahmad et al. (2020) studied how lactic acid bacteria (LAB) metabolize bioactive compounds, and Espín, González-Sarrías and Tomás-Barberán (2017) and Rowland et al. (2018) showed that bioactive compounds can initially be metabolized through hydrolysis (deglycosylation and ester hydrolysis), cleavage of glycosidic bonds (hydrogenation of a C=C double bond, reduction of carbonyl compounds and specific dihydroxylation) and reduction (hydrogenation of a C=C double bond, reduction of carbonyl compounds and specific dihydroxylation).

Increased consumption of fruits rich in polyphenols, may help preserve the ratio of Firmicutes/Bacteroidetes phyla in the by enhancing the healthy bacteria growth, and

decreasing the abundance of pathogenic bacteria (CHENG et al., 2020; ESPÍN, GONZÁLEZ-SARRÍAS and TOMÁS-BARBERÁN, 2017; HENNING et al., 2017; PIEKARSKA-RADZIK and KLEWICKA, 2021).

The effect of these compounds on bacterial development depends on the compound's structure and concentration (ESPÍN, GONZÁLEZ-SARRÍAS and TOMÁS-BARBERÁN, 2017), on the presence of probiotics and on the food matrix. Therefore, for the development of novel functional food, it is necessary to evaluate the interaction of probiotic LAB with bioactive compounds present in the food matrixes. It is worth mentioning that the consumer's living habits, the host's diet and, especially, the resident's intestinal microbiota (ESPÍN, GONZÁLEZ-SARRÍAS and TOMÁS-BARBERÁN, 2017) can also influence the degradation and absorption of bioactive compounds.

Unconventional tropical fruits such as buriti (*Mauritia flexuosa*) and passion fruit (*Passiflora* spp.) can be explored as sources of bioactive compounds. Passion fruit is native from tropical and subtropical zones of America, including southern areas in Brazil, through Paraguay to northern Argentina, and some hot area in United States, such as Florida and California. This species presents great economic importance for Brazilian's producers, which emerged as the world's largest producer and accounts for almost 70% of the global production. The fruit contains multiple seeds, surrounded by a gelatinous yellow to orange pulp, which has a pleasant sweet-acid taste and an intense aroma (AMARAL et al., 2016; CORRÊA et al., 2016).

Buriti is a native palm tree that abundantly grows in the Brazilian territory, especially in swamp areas of the Amazon and Cerrado biomes. It is also distributed for all South America (Bolivia, Peru, Ecuador, Colombia, Venezuela, Trinidad and Tobago, Guyana, Suriname, and French Guiana) and has an important social role for the population, mainly for the extractive communities as a source of income and employment generation (RIBEIRO et al., 2014; MOURA FILHO, 2017).

In view of the interest in increasing the consumption of bioactive compounds by the population, chemical characterization of unconventional fruits is necessary to evidence their functional potential. In addition, considering the tendency of adding fruits to products fermented by LAB, it is necessary to assess the interaction between them. As a result, the objectives of this study were to characterize the chemical characteristics and bioactive compounds of buriti and passion fruit pulps and to evaluate their effects on LAB growth.

## 2. Materials and methods

### 2.1 Passion fruit and buriti pulps preparation

Passion fruits (*Passiflora edulis f. Flavicarpa Deg.*), originating from Bahia state, were purchased in São José do Rio Preto-SP, Brazil. The fruits were washed and sanitized in running water and submerged for 10 minutes in 10% (w/v) sodium hypochlorite (NaClO) solution (Synth, Diadema-SP, Brazil). Subsequently, the pulp was manually separated from the seed using a sieve. The pulp was then stored in polyethylene plastic bags in the absence of light and frozen (-80 °C). Buriti fruits (*Mauritia flexuosa*) were harvested and processed in Teresina-PI, Brazil, typical region of buriti production. The fruits were washed and sanitized, as previously described, manually pulped using a stainless-steel knife and stored at 4 °C, as described by Moura Filho (2017). Subsequently, the pulp was sent to São José do Rio Preto-SP. After the reception, the pulp was frozen (-80 °C).

### 2.2 Physicochemical characterization

The chemical composition of fruit pulp was determined regarding moisture (CASE; BRADLEY JR and WILLIAMS, 1985), total protein (AOAC, 2010), ash, total fatty acids and titratable acidity (IAL, 2005), ascorbic acid (OTSUKA et al., 1981), °Brix and total soluble solids (AOAC, 2005), lipid profile (AOAC, 1997), total fibers and soluble and insoluble fibers (IAL, 2005). The available carbohydrates were determined by the difference, calculated as a percentage difference between 100 and the sum of moisture, protein, ash, and lipids. The pH value was determined using digital pH meter (IAL, 2005). All the analyses were performed in triplicate, except for the lipid profile, which was performed in duplicate.

### 2.3. Determination of lignin and carbohydrates in the fruit pulps

The total starch content of the fruit pulp samples was measured in duplicate using a Total Starch Assay Kit (AA/AMG) (Megazyme International Ltd., Wicklow, Ireland) following the protocol recommended by manufacturer. The total of cellulose, hemicellulose and lignin was performed according to Sluiter et al. (2012). In summary,

0.3 g (dry basis) of buriti and passion fruit pulp were shaken in 3.0 mL of sulphuric acid 72% (Synth) (150 rpm; 30 °C; 30 min). Afterwards, 84 mL of distilled water was added to the flask, the sample was autoclaved for 1 h (121 °C) and filtrated on paper. Soluble lignin from this filtrate was evaluated directly at  $\lambda=240$  nm, using gallic acid (Dinâmica, Indaiatuba-SP, Brazil) as the standard. The material retained on the filter paper was dried at 105 °C for dry weight determination and burned (500 °C; 4 h) for insoluble lignin quantification in duplicate. These data was used to calculate the amount of insoluble lignin (SLUITER et al., 2012). The supernatant was filtered with a 0.2  $\mu\text{m}$  syringe filter and the cellulose and hemicellulose were quantified by HPLC using an ICS 5000 Dionex High-Performance Anion-Exchange Chromatography/Pulsed Amperometric detection (HPAEC-PAD) ionic chromatograph, according to Moretti et al. (2014). Glucose, xylose, and arabinose were used as standards in the calibration curve.

## 2.4 Bioactive compounds and antioxidant activity

### 2.4.1 Total phenolic compounds and yellow flavonoids

The extraction of bioactive compounds was performed using an extractor solution containing methanol and acetone (both from Synth), according to Macoris et al. (2012), with modifications. Briefly, wet samples with the extractor solution (70% methanol + 50% acetone [v/v, 1:1]) were vortexed for 1 min and then subjected to ultrasound (135 W, 40 KHz) for 15 min. After this step, the samples were centrifuged at  $1935\times g$  for 20 min, and the supernatant was collected in a 50 mL flask (this process was repeated until the sample was completely exhausted) in the absence of light.

The total phenolic compounds were assessed from the extract obtained described above in triplicate according to Folin–Ciocalteu (WATERHOUSE, 2014) and the results were expressed as milligrams of gallic acid equivalents (mg GAE) per 100 g of sample. The yellow flavonoids content was determined according to Silva et al. (2014). Briefly, approximately 1.0 g of pulp was weighed and 30 mL of extracting solution (95% ethanol (Synth) + 1.5 N HCl (Synth) in a proportion of 85:15) was added. The mixture was homogenized in a dispersor ultra-turrax for 2 min, then transferred to a 50 mL flask and stored for 12 h in the dark. After this period, the mixture was filtered and the absorbance was read in a spectrophotometer (UV-mini 1240, Shimadzu, Kyoto, Japão) at 374 nm and the results were expressed in milligrams of yellow flavonoids per 100 g of sample.

#### 2.4.2 *Flavonoids profile*

To obtain the fruit pulp extracts, 10 g pulp (freeze dried sample) was homogenized with 100 mL methanol/water (70:30, v/v) for 1 minute using a disperser Ultra-Turrax (Polytron-Kinematica GmbH, Kriens-Luzern, Switzerland) and vacuum filtered on Buchner funnel. The supernatant was recovered and further re-extracted twice with 50 mL of the extraction solution. The extracts obtained were concentrated by rotary evaporator (Rotavapor® 120, Büchi, Flawil, Switzerland) at 40 °C until total methanol was removed and resuspended in water in a 50 mL flask prior to application to a solid-phase extraction column. Extractions were performed in duplicate.

The flavonoid profile was performed according to Bataglion et al. (2014). The extract (10 mL) obtained above was passed through a polyamide column (1 g) (CC 6, Macherey-Nagel, Germany) and preconditioned with methanol and ultrapure water. The sample was loaded onto the column and washed with ultrapure water and the phenolic compounds was eluted with ammonia in methanol. The obtained eluates were concentrated by removal of the methanol in a rotary evaporator at 40 °C under vacuum, resuspended in high-performance liquid chromatography (HPLC) grade methanol and filtered through polyvinylidene difluoride (PVDF) membrane (0.22 µm, Millipore Ltd., Bedford, MA, USA) for the quantification of flavonoids. HPLC with diode array detector (DAD) and Prominence liquid chromatograph (Shimadzu, Japan) coupled with an ion trap Esquires-LC mass spectrometer (Bruker Daltonics, Billerica, MA, USA) with an electrospray ionization interface (LC-ESI-MS/MS) were used.

Flavonoids were identified in triplicate using a LC-ESI-MS/MS. The column Poroshell 2.7 µm (100 x 3 mm) (Agilent Technologies) and a flow rate of 0.5 mL/min at 25 °C were used. Two solvents were used in the mobile phase; solvent A was composed of 0.5% formic acid in water and solvent B 0.5% formic acid in acetonitrile. For the analysis, the solvent gradient was applied as follows: 5-18% B at 0-7 min, 18-28% at 7-17 min, 90% at 17-20 min, and 5% at 20-26 min. ESI was used in negative mode. The MS was programmed to perform full scan between m/z 100-1000. The parameters required for MS operation were: 4000 V collision energy and 275 °C capillary temperature. To identify the compounds, the data obtained were compared with the retention times of authentic standards, when possible, as well as by absorption spectrum similarity, mass spectral characteristics, and comparison with literature data.

Flavonoids were quantified in triplicate using an Infinity 1260 Quaternary LC System (Agilent Technologies, USA) equipped with an automatic injector, quaternary

pump, coupled to a DAD, and controlled by Agilent software. The separation conditions were the same as those used for LC-ESI-MS/MS. Flavonoids were detected by monitoring the elution at 270 and 370 nm wavelengths. Luteolin-3-glucoside, vitexin, catechin and epicatechin (270 nm), quercetin-3-glucoside, rutin and isorhamnetin (370 nm) were used as standards (Extrasynthese; Lion, France and Sigma, Chemical Co., St. Louis, USA). Orientin concentration was expressed as luteoylin-3-glucoside, quercetin derivatives such as quercetin-3-glucoside; and isorhamnetin-rutinoside in isorhamnetin. Total flavonoids was determined from the sum of flavonoids detected.

#### 2.4.3 Carotenoids profile

The carotenoids profile in passion fruit pulp was determined in triplicate according to Reis et al. (2018). The extraction of pigments was carried out from freeze dried samples with acetone and the saponification in a KOH solution (10% in methanol) overnight. The extract obtained was concentrated by rotary evaporator (Fisatom, Model 801, Brazil) ( $T < 25\text{ }^{\circ}\text{C}$ ) and stored in a freezer ( $-18\text{ }^{\circ}\text{C}$ ) for quantification by HPLC. All these analyses were made in triplicate.

Samples stored in freezer were thawed and diluted in methyl tert-butyl ether (MTBE-JT Baker, CAS. Number 1634-04-4, purity 99.96%), sonicated for 1 min (Unique, Model USC 1400) and filtered (Millex LCR 0.45  $\mu\text{m}$ , 13 mm) for injection into the HPLC (Agilent 1100 Series, Santa Clara, CA, USA), using a UV-visible detector and with a quaternary system. A polymeric reversed phase column C30 was used (250 $\times$ 4.6mm ID, 3  $\mu\text{m}$ , YMC, model CT99SO3-2546WT). The mobile phase gradient (water: methanol: MTBE) (JT Baker, CAS Number 04.04.1634, 99.96% purity) started at 5:90:5, reaching 0:95:5 at 12 min, 0:89:11 at 25 min, 0:75:25 at 40 min, and finally 00:50:50 at 60 min. The column temperature was set at 33  $^{\circ}\text{C}$  and a flow rate of 1 mL/min (Spectra were obtained at a fixed wavelength of 450 nm for carotenoids). Compounds identification was performed by comparing retention times detected in controls. A standard curve was plotted for the carotenoids over the following ranges: lutein 1 to 65  $\mu\text{g/mL}$  ( $\geq 95\%$ , Sigma-Aldrich); zeaxanthin 1 to 40  $\mu\text{g/mL}$  ( $\geq 95\%$ , Sigma-Aldrich);  $\beta$ -cryptoxanthin 4 to 100  $\mu\text{g/mL}$  ( $\geq 97\%$ , Sigma-Aldrich);  $\alpha$ -carotene 2 to 25  $\mu\text{g/mL}$  ( $\geq 95\%$ , Sigma- Aldrich);  $\beta$ -carotene 5 to 50  $\mu\text{g/mL}$  ( $\geq 97\%$ , Sigma-Aldrich) and lycopene ( $\geq 85\%$ , Sigma-Aldrich). The limits of detection and quantification are described as follows: lutein:  $6.9\times 10^{-3}$  and  $1.15\times 10^{-2}$   $\mu\text{g/g}$ ; zeaxanthin:  $9.56\times 10^{-2}$  and  $1.59\times 10^{-2}$   $\mu\text{g/g}$ ;  $\beta$ -cryptoxanthin:  $2.11\times 10^{-2}$  and  $3.51\times 10^{-2}$   $\mu\text{g/g}$ ;  $\alpha$ -carotene:  $1.97\times 10^{-2}$  and  $3.28\times 10^{-2}$   $\mu\text{g/g}$ ;

$\beta$ - carotene:  $6.53 \times 10^{-2}$  and  $10.89 \times 10^{-2}$   $\mu\text{g/g}$  and lycopene:  $7 \times 10^{-3}$  and  $33 \times 10^{-3}$   $\mu\text{g/g}$ . Provitamin A was calculated using the bioconversion factor, yielding a value of 12 mg of  $\beta$ -carotene with 1 mg of Retinol Activity Equivalent (RAE) (REIS et al., 2018).

#### 2.4.4 Antioxidant activity

The antioxidant activity was determined in triplicate from the extract obtained from the fruit pulps, as described in item 2.3.1 using the DPPH method and the results were expressed as micromole Trolox equivalent ( $\mu\text{mol TE}$ ) per g sample (RUFINO et al., 2007). Trolox [( $\pm$ )-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, 97%] and DPPH (2,2-Diphenyl-1-picrylhydrazyl) were obtained from Sigma-Aldrich (St. Louis, USA) and Folin-Ciocalteu and gallic acid ( $\text{C}_7\text{H}_6\text{O}_5$ , P.A. ACS) were obtained from Dinâmica (Diadema, Brazil).

#### 2.5 Effect of fruit pulps on the growth of lactic acid bacteria (LAB)

*Lactiplantibacillus plantarum* ST8Sh, characterized as potentially probiotic and bacteriocinogenic strain (TODOROV; HOLZAPFEL and NERO, 2017), was tested and *Lacticaseibacillus rhamnosus* GG ATCC 53103 was evaluated as a reference probiotic LAB strain.

For the assay, media were added with 10 g/L of fruit pulp (buriti or passion fruit) without glucose. *Lacticaseibacillus* and *Lactiplantibacillus* strains were cultivated in MRS medium, prepared according to the following formulation: casein peptone (10 g/L), yeast extract (5 g/L), tween 80 (1 mL/L), bibasic ammonium citrate (2 g/L), sodium acetate (5 g/L), magnesium sulfate (0.1 g/L), manganese sulfate (0.05 g/L), dipotassium phosphate (2 g/L). MRS culture medium with glucose (10 g/L) was used for as positive controls. All media were sterilized at 121 °C for 20 min.

Before the tests, the LAB strains were cultivated twice in MRS at 37 °C for 18 h. Then, the strains were subjected to centrifugation (5000 x g, 5 °C, 5 min), washed twice with phosphate buffer, and resuspended (1%, v/v) in MRS for growth analysis. The fermentation processes were carried out in duplicate. The growth of the strains was evaluated by plate count (CFU/mL), at times 0, 6, 12 and 24 hours after inoculation at 37 °C.

For this, 100  $\mu\text{L}$  of MRS samples (MRS + fruit pulps and MRS + glucose) was mixed with 900  $\mu\text{L}$  of 0.85% saline solution. The mixture was stirred, submitted to serial

decimal dilutions up to  $10^{-8}$  using the same diluent, and then 10  $\mu\text{L}$  of each dilution was transferred to Petri dishes. MRS agar (Difco, Becton Dickinson Co., Sparks, MD, USA) was used for counting *Lactocaseibacillus* and *Lactiplantibacillus*. MRS plates were incubated under anaerobic conditions at 37 °C for 48 h (SOLIERI et al., 2014).

## 2.6 Statistical analysis

An Analysis of Variance (ANOVA) followed by a Tukey test was used to compare the effect of fruit pulp on the growth of LAB strains. A significance level of 5% probability and Minitab 16 software were used.

## 3. Results and discussion

### 3.1 Chemical characterization of fruit pulps

The composition of passion fruit pulp (Table 1) was similar to those found by Fischer, Melgarejo and Cutler (2018); however, for buriti pulp the values were slightly higher (protein, pH and °Brix) than those reported by Hamacek et al. (2018). These differences are probably related to the degree of fruit maturation, cultivation area, climate, among other factors. Buriti pulp has a higher total fiber content than passion fruit pulp (11.9% and 0.1%, respectively), then it can be used as a source of dietary fiber in foods (BECKER et al., 2014). Approximately 87% of the buriti pulp fibers present are classified as insoluble fibers, which has been extensively studied and recognized as healthy (SCHNEEMAN, 1987; YANG et al., 2020; WANG et al., 2021; LYU et al., 2022). Its beneficial effect on consumers includes modulation of the intestinal microbiota, stimulation of beneficial microorganisms, such as those from Bifidobacteriales and Lactobacillales families, and positive influence by the production of short-chain fatty acids (YANG et al., 2020; LYU et al., 2022).

Although soluble fibers are in small amount in buriti pulp (1.5 g/100 g soluble fiber), they can be fermented by colonic bacteria under anaerobic conditions and affecting the producing of SCFA, such as acetate, butyrate, and propionate. These acids may exert various beneficial effects on host health, including increase the absorption of some minerals, stimulate the diversity of the intestinal microbiota, may modulate the risks of

disease, for example, immunodeficiency, inflammation, diabetes, obesity, hypertension, and cancer (CUI et al., 2019).

**Table 1.** Centesimal composition, pH, acidity, °Brix, ascorbic acid, and fibers of buriti and passion fruit pulps.

<b>Parameters</b>	<b>Buriti</b>	<b>Passion fruit</b>
Moisture (g/100 g)	67.40 ± 0.25	88.10 ± 0.06
Ashes (g/100 g)	0.10 ± 0.01	0.71 ± 0.03
Proteins (g/100 g)	1.43 ± 0.05	0.90 ± 0.01
Total fatty acids (g/100 g)	12.0	0.5
Carbohydrates*	19.07	9.79
pH	3.62 ± 0.02	3.32 ± 0.06
Acidity (g citric Acid/100 g)	0.86 ± 0.01	3.11 ± 0.00
°Brix	13.3 ± 0.0	11.2 ± 0.26
Ascorbic acid (mg/100 g)	ND	12.76 ± 0.37
Soluble Fibers (g/100 g)	1.5	<0.1
Insoluble Fibers (g/100 g)	10.4	<0.1
Total fibers (g/100 g)	11.9	<0.1

ND = no detected; \*Carbohydrates was obtained from 100 – (moisture + ash + lipids + proteins).

Regarding the carbohydrate composition, the fruit pulps did not have enough starch and lignin that can be detected by HPAEC-PAD ionic chromatograph (Table 2). However, cellulose and hemicellulose were detected in both fruit pulps, and these compounds were more abundant in buriti pulp.

Buriti also presented high amount of total fatty acids compared to passion fruit pulp (12 g/100 g and 0.50 g/100 g lipids, respectively), as shown in Table 3. Approximately 81% fatty acids present in the buriti pulp, and 44% fatty acids present in the passion fruit pulp are monounsaturated (belonging to the omega 9 class, with a high concentration of oleic acid (Table 3). Considering the daily consumption of 2000 kcal, and the daily recommendation of oleic acid intake, which must be 10–15% total energy intake (LOPEZ-HUERTAS, 2010), the daily consumption of 100 g buriti pulp supplies more than 50% the daily need of this fatty acid.

**Table 2.** Carbohydrate composition of the buriti and passion fruit pulps.

<b>Parameters (db) (g/100 g)</b>	<b>Samples</b>	
	Buriti	Passion Fruit
Starch	ND	ND
Cellulose	12.3 ± 0.6	1.09 ± 0.7
Hemicellulose	16.8 ± 0.1	0.79 ± 0.0
Arabinose*	0.4 ± 0.00	ND
Xylose*	0.1 ± 0.00	ND
Glucose	0.2 ± 0.00	3.3 ± 0.02
Insoluble lignin	ND	ND
Soluble lignina	ND	ND

\*Values were quantified before the acid hydrolysis, heat treatment and filtration. ND = not detected. db = dry basis.

**Table 3.** Fatty acid profile (g/100 g lipids) of buriti and passion fruit pulps.

<b>Fatty acids (wd)</b>	<b>Buriti</b>	<b>Passion Fruit</b>
(C12:0) Lauric acid	<0.1	5.53
(C14:0) Myristic acid	0.11	1.14
(C16:0) Palmitic acid	17.25	29.5
(C16:1) Palmitoleic acid - omega 7	0.24	1.37
(C18:0) Stearic acid	1.42	12.36
(C18:1-cis) Oleic acid - omega 9	76.77	37.86
(C18:2-cis) Linoleic acid - omega 6	1.51	3.42
(C18:3) $\alpha$ -Linolenic acid - omega 3	1.37	1.38
(C20:0) Arachidic acid	0.13	ND
(C20:1) Cis-11-Eicosaenoic acid - omega 11	0.92	ND
(C22:0) Behenic acid	ND	1.37
(C24:0) Lignoceric acid	0.07	2.62
(C24:1) Nerve acid	-	1.19
Saturated	19.08	52.52
Monounsaturated	77.93	40.42
Polyunsaturated	2.88	4.80

ND- not detected. (wd - wet basis).

Fruits containing oleic acid may reduce the risk of reactive oxygen species-induced diseases and improve the immune system (RYDLEWSKI et al., 2017). This acid has beneficial effects on consumer health and is related to the prevention of

cardiovascular disease, cancer, inflammatory arthritis, and rheumatoid arthritis (BAKER et al., 2016; COSTANTINI et al., 2017; WATANABE and TATSUNO, 2017).

### 3.2 Characterization of bioactive compounds and antioxidant activity of fruit pulps

Buriti pulp showed  $172.60 \pm 0.39$  mg EAG/100 g total phenolic compounds and  $1.31 \pm 0.02$  mg/100 g yellow flavonoids, while passion fruit showed  $27.39 \pm 0.01$  mg EAG/100 g pulp total phenolic compounds and  $0.45 \pm 0.01$  mg/100 g yellow flavonoids (Table 4). Koolen et al. (2013) and Santos et al. (2015) found that buriti pulp had  $378.07 \pm 3.12$  mg EAG/100 g total phenolic compounds and 28 mg/100 g yellow flavonoids, respectively. For passion fruit, Ramaiya et al. (2013) reported 361.73 mg/L total phenolic compounds.

**Table 4.** Composition of phenolic compounds, yellow flavonoids, and antioxidant activity in the buriti and passion fruit pulps.

	Buriti	Passion fruit
Phenolic compounds (mg EAG/100 g pulp)	$172.60 \pm 0.39$	$27.39 \pm 0.01$
Yellow flavonoids (mg/100 g pulp)	$1.31 \pm 0.02$	$0.45 \pm 0.01$
Antioxidant activity ( $\mu$ mol Trolox/100 g pulp)	$523.00 \pm 0.37$	$87.00 \pm 0.08$

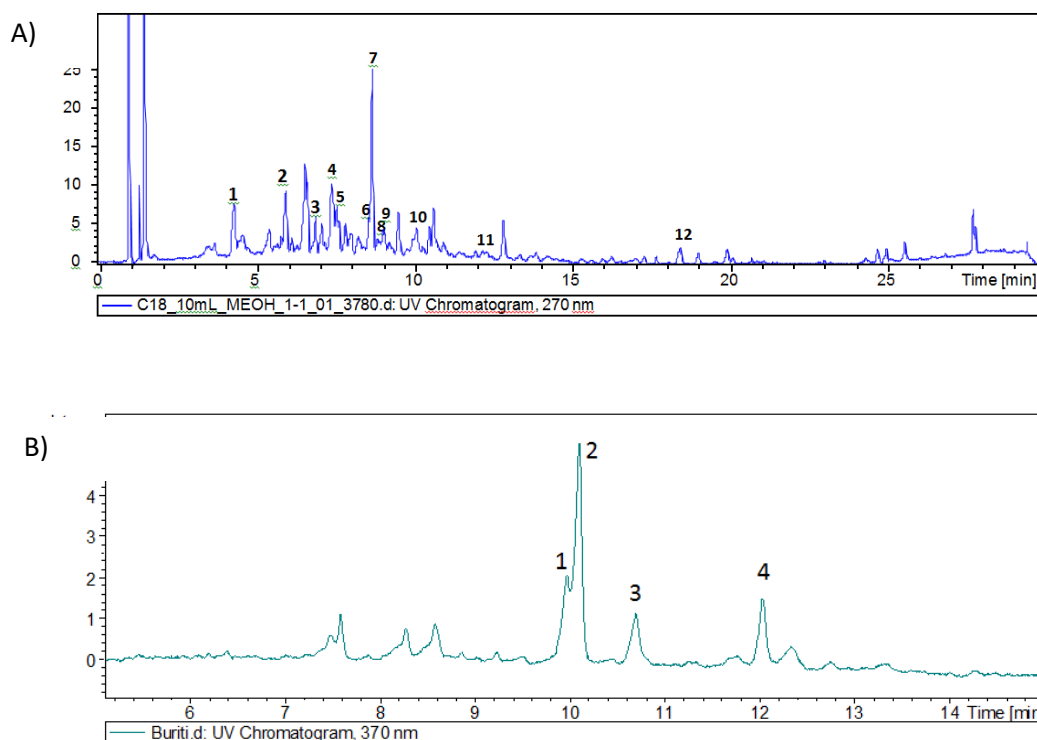
The buriti pulp stood out for presenting higher amounts of flavonoids than passion fruit pulp. Buriti pulp is rich in quercetin while the major compound of passion fruit pulp is orientin followed by vitexin (Table 5, Figure 1).

Quercetin is one of the most abundant polyphenols in the human diet, and its presence in fruit pulp is desirable, as it has a wide range of biological properties (anti-inflammatory, antibacterial, protective action against cardiovascular disease, diabetes, cancer), therefore, quercetin is one of the most studied phenolic compounds (BARBIERI et al., 2017; SHI et al., 2020).

**Table 5.** Flavonoid profile (mg/100 g dry pulp) in buriti and passion fruit pulps.

	Buriti	Passion fruit
Catechin	BLQ	BLQ
*Orientin-7-O-glucoside	ND	1.45 ± 0.09
*Orientin (Luteolin 8-C-Glucoside)	ND	0.10 ± 0.03
Vitexin	ND	0.19 ± 0.02
<sup>1</sup> Epicatechin	BLQ	ND
Glycosylated Quercetin	36.60 ± 4.89	ND
<sup>1</sup> Quercetin-3-rutinoside	90.76 ± 14.09	ND
<sup>1</sup> Quercetin-3-glucoside	23.97 ± 3.06	ND
Isorhamnetin-rutinoside	9.64 ± 1.34	ND
Total flavonoids**	160.97	1.74

\*Luteolin-3-glucoside equivalent. \*\* Total flavonoids were obtained by the amount of all flavonoids identified. ND - not detected. <sup>1</sup>Identity confirmed with commercial standard. Retention time according to the chromatogram shown in Figure 1. BLQ: Below the limit of quantification.



**Figure 1.** Chromatograms of passion fruit (A) and buriti (B) pulps. A) 2: orientin (luteolin 8-c-glucoside), 4: vitexin, 7: orientin-7-o-glucoside; B) 1: glycosylated quercetin; 2: quercetin-3-rutinoside; 3: isorhamnetin-rutinoside; 4: quercetin-3-glucoside.

Among its beneficial properties mentioned above, the antimicrobial property is undoubtedly one of the most studied (HOSSION et al., 2011; LEE et al., 2010; HOSSION and SASAKI, 2013; BARBIERI et al., 2017), due to its high potential to inhibit pathogenic bacteria. Chen and Huang (2011) demonstrated the bactericidal effect of quercetin against *Klebsiella pneumoniae*, an important Gram-negative pathogenic bacterium that can cause lung infections, urinary infections (evolving to pneumonia and pyelonephritis, respectively), with risk of death. Quercetin can also inhibit other pathogens that are equally important to human health, such as *Staphylococcus aureus* and *Staphylococcus haemolyticus* (BARBIERI et al., 2017), *Streptococcus pyogenes* (SIRIWONG et al., 2015) and *E. coli* (LEE et al., 2010).

An *in vivo* assay showed that quercetin supplementation in mice can have a prebiotic action, significantly improving the diversity of the intestinal bacterial community in antibiotic-treated mice, due to the recovery of the intestinal barrier, by decreasing the increase in intestinal villi and mucosal thickness, and increased the butyrate production (SHI et al., 2020). Moreover, quercetin may also act on intestinal dysbiosis in mice fed a high-fat diet by activating the lipoperoxidation-dependent TLR-4 pathway (PORRAS et al., 2017), may also reduce atherosclerosis lesions by decreasing the abundance of *Verrococcinia* and increasing the abundance of Actinobacteria, Cyanobacteria and Firmicutes in mice (ETXEBERRIA et al., 2015) in addition to attenuating symptoms of colitis caused by *Citrobacter rodentium* in mice (LIN, PIAO and SONG, 2019).

Orientin and vitexin are also known to modulate the gut microbiota (KIMURA et al., 2022). In addition to its modulating action, orientin has other medicinal effects, being able to act as anticancer, anti-inflammatory, antioxidant, antimicrobial, neuroprotective, vasodilator and cardioprotective agents (LEE, KU and BAE, 2014; LAM et al., 2016; KHALIL et al., 2022b), both have therapeutic effect in the treatment of sleep disorders and anxiety (SCHÄFER et al., 2021). These compounds are considered fundamental for the promotion of human health.

*In vivo* studies showed the anti-inflammatory (LEE, KU and BAE, 2014), anti-diabetic action (KU, KWAK and BAE, 2014), decrease in heart infarction size (FU et al., 2006) and protective effect on liver inflammation (KHALIL et al., 2022b) of orientin in mice. Orientin may also serve as a potential synergistic treatment for effective inflammation-mediated anticancer strategies (KHALIL et al., 2022a). In the same vein,

vitexin and isovitexin can also inhibit  $\alpha$ -glucosidase and  $\alpha$ -amylase activity related to postprandial hyperglycemia (YANG, HE and LU, 2014).

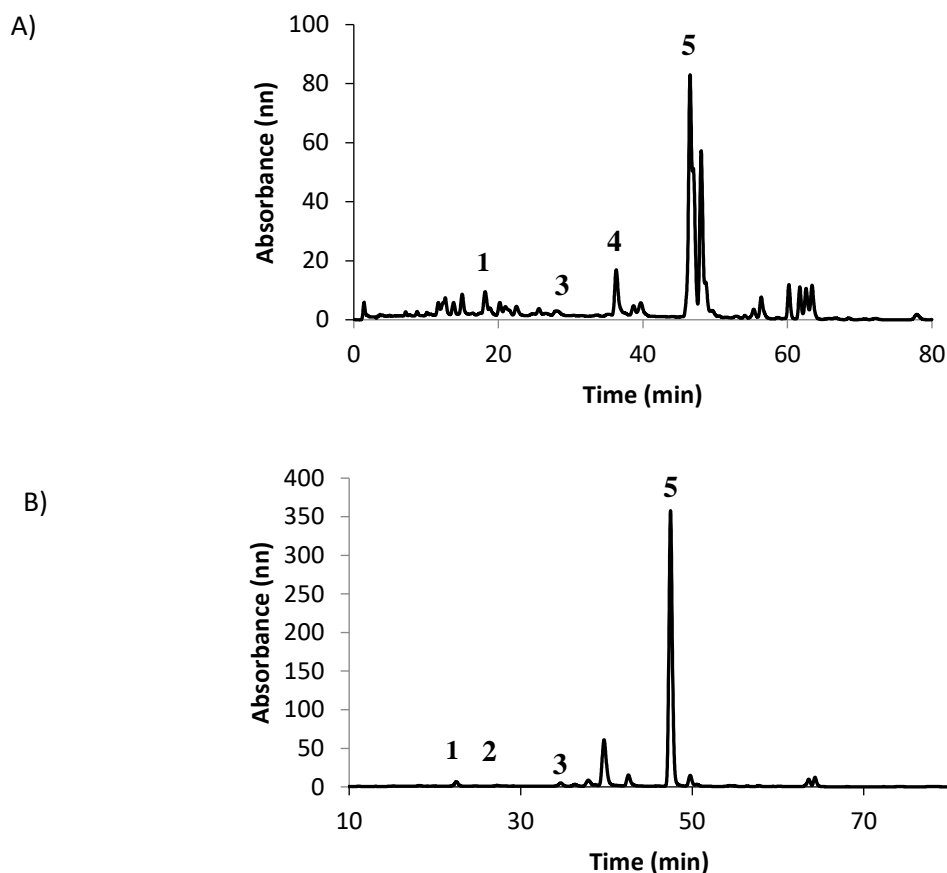
Fruit pulps, especially buriti and passion fruit pulp, are rich in carotenoids and  $\beta$ -carotene is the major carotenoid in both pulps (Table 6 and Figure 2). Manhães and Sabaa-Srur (2011) reported 1.48 mg  $\alpha$ -carotene/100 g pulp, 13.71 mg  $\beta$ -carotene/100 g pulp and 23.36 mg total carotenoids/100 g pulp. Hamacek et al. (2018) reported much lower  $\alpha$ -carotene and  $\beta$ -carotene contents in frozen buriti pulp ( $2.3 \pm 0.41$  and  $21.64 \pm 6.77$  mg/100 g, respectively), compared to this research. For passion fruit pulp, Silva and Mercadante (2002) observed 0.2 a 1.3 mg/100 g  $\beta$ -carotene in the *in natura* passion fruit pulp. This difference is justified since the concentration of bioactive compounds may vary among species and region of passion fruit cultivation.

**Table 6.** Carotenoid profile of buriti and passion fruit pulps.

Carotenoids (mg/100 g dry pulp)	Buriti	Passion fruit
Lutein	$1.21 \pm 0.03$	$0.17 \pm 0.00$
Zeaxanthin	ND	$0.04 \pm 0.01$
Cryptoxanthin	$18.65 \pm 0.01$	$0.22 \pm 0.10$
$\alpha$ -carotene	$3.75 \pm 0.06$	ND
$\beta$ -carotene	$127.46 \pm 2.94$	$10.07 \pm 0.91$
Lycopene	$3.67 \pm 0.29$	$0.50 \pm 0.02$
Total carotenoids	$153.18 \pm 3.54$	$10.05 \pm 0.93$
Retinol Equivalent (mg/RAE)	10.62	0.84

ND - not detected.

Buriti fruit is known for its nutritional value which can provide several benefits that are directly related to some compounds such as carotenoids, phenolic compounds, fatty acids and fibers (BECKER et al., 2014; BATAGLION et al., 2014). Romero et al. (2015) reported that buriti-supplemented diet had higher contents of phenolic compounds and carotenoids, as well as higher antioxidant activity compared with the standard feed. The buriti-supplemented diet consumed by young rats increased the *in vitro* and *in vivo* antioxidant activities, while lipid peroxidation in the plasma, kidneys and liver was not affected.



**Figure 2.** Chromatograms of buriti (A) and passion fruit (B) pulps. 1: Lutein; 2: Zeaxanthin; 3: Cryptoxanthin; 4:  $\alpha$ -carotene; 5:  $\beta$ -carotene.

The bioactive compounds such as phenolic compounds, flavonoids and carotenoids present in passion fruit and buriti pulps may have beneficial effects, such as antioxidant and anti-inflammatory capacities, decreasing the risk of cardiovascular diseases, such as arteriosclerosis and hypertension, and the symptoms of neurodegenerative diseases such as Alzheimer's; reducing the rate of glucose and cholesterol in blood; decreasing the inflammatory and infectious processes; acting as carcinogenesis and mutagenesis inhibitors, among others (PIERSON et al., 2014; REIS et al., 2018). In addition to the health-promoting characteristics, carotenoids from buriti can also be used as natural food colorings, are considered a great source of carotenoid ( $\beta$ -carotene) and one of the main sources of pro-vitamin A found in Brazilian biodiversity (SILVA et al., 2009; LIANG et al., 2020), increasing the immune response and reducing the incidence of diseases (LIANG et al., 2020). However, it is important to emphasize that although bioactive compounds present in the diet are directly related to the benefits

mentioned above, their beneficial health effects depend mainly on their bioavailability (KETNAWA; SUWANNACHOT and OGAWA, 2020).

Buriti pulp showed higher antioxidant activity than passion fruit pulp, which was in accordance to their differences regarding total phenolic compounds and carotenoids (Tables 4 and 6) (SEPTEMBRE-MALATERRE; REMIZE and POUCHERET, 2018; HUSSAINA; FAROOQC and SYEDE, 2020; LIANG et al., 2020). The antioxidant activity of buriti pulp has been studied using different methods, such as DPPH, FRAP and ABTS (KOOLEN et al., 2013; SANDRI et al., 2017; RUDKE et al., 2021). Although the data available in the literature were obtained by different methods, all reported values were lower than those found in the present study.

According to the literature, the antioxidant activity of passion fruit varies from 52.4  $\mu\text{mol Trolox}/100\text{ mL}$  passion fruit pulp (FISCHER; MELGAREJO and CUTLER, 2018) to 112.21  $\mu\text{mol Trolox}/100\text{ mL}$  passion fruit pulp (JANZANTTI et al., 2012), which include the values found in the present study.

Food with antioxidant properties is essential for health, since the excess of free radicals are strongly associated with many diseases, including cancer, heart disease, Alzheimer's and Parkinson's diseases (KIM; KIM and YOON, 2015; MAQSOOD et al., 2020), thus stimulating a huge consumers' interest towards high antioxidant food to avoid or reduce the incidence of such diseases.

These data emphasize the importance of buriti, a native fruit from Brazilian savanna, as well as passion fruit, which is one of the main produced and exported fruit from Brazil. These are relevant sources of natural antioxidants for healthy diets and for the food industry; however, additional studies on Brazilian native fruits should be performed to increase their productivity and market availability.

### 3.3 Effect of fruit pulp on the growth of LAB strains

The effect of fruit pulps on *L. plantarum* ST8Sh and *L. rhamnosus* GG (ATCC 53103) growth was evaluated. Both the buriti and passion fruit pulps did not inhibit the growth (Figure 3) of any of the tested strains. Similar results were observed by Borgonovi et al. (2021) in which the growth of *Lacticaseibacillus rhamnosus*, *Lacticaseibacillus casei* and *Streptococcus thermophilus* was not affected by the fruit pulps. Therefore, they can be used to produce fermented food with the addition of these fruit pulps. *L. plantarum* is a species widely used in the fermentation of vegetables, as it may produce some

enzymes capable of degrading different vegetable matrixes (PAUCEAN et al., 2013; AZIZ et al., 2022; PLESSAS, 2022). Additionally, *L. plantarum* has been studied and used in the production of dairy foods such as fermented milk (LANG et al., 2022). Furthermore, *L. plantarum* ST8Sh was characterized by Todorov, Holzapfel and Nero (2017) as a bacteriocin producer (pediocin-PA-1), with bactericidal action against pathogenic microorganisms of great interest in dairy products such as *Listeria monocytogenes*. The production of bacteriocin also enhance food preservation during the handling of the product and increase the product' shelf life. This biopreservation technology becomes a trend towards the possibility of reducing or eliminating the use of chemical preservatives for the elimination of pathogens with the advantage of clean label product.

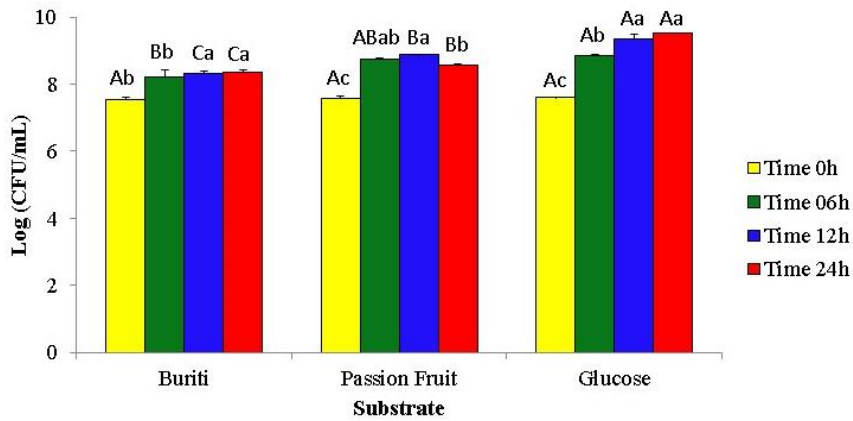
In recent years, several authors studied the effect of isolated bioactive compounds as: flavan-3-ols, proanthocyanidins, catechin and gallic, vanillic, ferulic and protocatechuic acids on the viability of various microorganisms (CUEVA et al., 2012; CARDONA et al., 2013; PACHECO-ORDAZ et al., 2017); however, few studies are found in the literature on the effect of fruit or fruit parts on the viability of LAB. Irkin et al. (2015) evaluated the effect of grapefruit peel, lemon peel, orange peel and orange juice on the viability of LAB strains and observed that these fruits components stimulated the growth of *Lactobacillus acidophilus* and *L. casei*. On the other hand, Costabile et al. (2015) observed that orange juice had an inhibitory effect on the growth of *Bifidobacteria* and *Lactobacilli*.

Bioactive compounds present in fruit pulps can stimulate or inhibit some microorganisms. The antimicrobial action of some fruits is due to bioactive compounds such as flavonoids, which could occur in function of their toxicity, and the mode of action includes inhibition of DNA replication, increase the cytoplasmic membrane permeability and inhibition bacterial energy metabolism (FERREIRA, MARTINS, and BARROS, 2017; BARBIERI et al., 2017). On the other hand, bioactive compounds can stimulate some microorganisms since they can use the bioactive compounds as an energy source. For instance, *L. plantarum* can improve the bioavailability of bioactive compounds such as polyphenols and increases the antioxidant activity due to its ability to produce glycosidase, esterase, and other enzymes (ESTEBAN-TORRES et al., 2013).

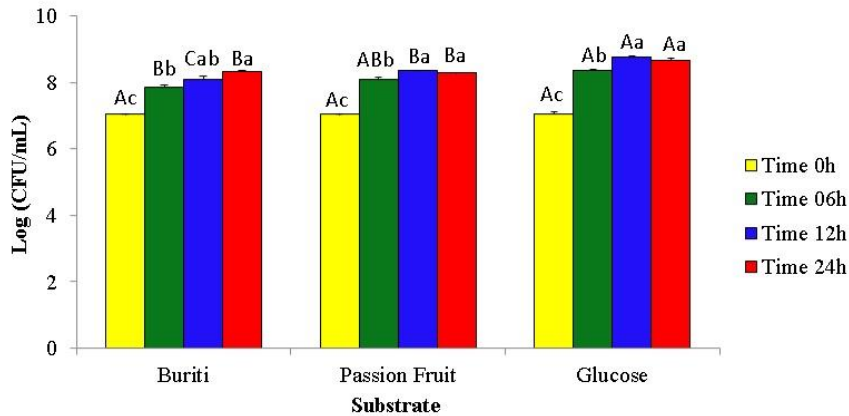
Some authors have shown that phenolic compounds can be metabolized by different microbial species (CORRÊA et al., 2019; SELMA, ESPÍN and TOMÁS-BARBERÁN, 2009), for example *Gordonibacter* ssp. can metabolize the gallic acid,

*Bifidobacterium lactis* and *Lactobacillus gasseri* can metabolize the ferric acids, *Clostridium* ssp. can metabolize the catechin and *Slackia* ssp. can metabolize the isoflavones and resveratrol (SELMA et al., 2014; TOMÁS-BARBERÁN et al., 2014; TOMÁS-BARBERÁN et al., 2014; MARÍN et al., 2015; CORRÊA et al., 2019).

A)



B)



**Figure 3.** Effect of fruit pulps on the growth of *L. plantarum* ST8Sh (A) and *Lacticaseibacillus rhamnosus* GG (B). Different capital letters in the same column denote a significant difference ( $p < 0.05$ ) among the pulps, for the same strain. Different lower-case letters in the same row denote a significant difference ( $p < 0.05$ ) over time for the same pulp and strain.

#### 4. Conclusion

Passion fruit pulp and especially buriti pulp are sources of essential nutrients for health. They are rich in bioactive compounds that can provide benefits to consumers, in addition to being able to enrich food products. The buriti and passion fruit can be associated with probiotic LAB to produce functional foods. However, additional studies focusing on the mechanisms of action and conversion of bioactive compounds by probiotic LAB still need to be explored.

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## CAPÍTULO III - ARTIGO ORIGINAL

### **Lactic acid bacteria and fruit pulps influence the kinetics of acidification and bacterial viability of potentially probiotic fermented milk**

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#### **Abstract**

Fermented milk has gained prominence among functional food due to its nutritional characteristics and for being a great vehicle for probiotics. Moreover, it can be enriched with fruit pulps which are source of bioactive compounds. This study aimed to evaluate the effect of *Lacticaseibacillus casei* SJRP38 (LC), *Lactiplantibacillus plantarum* ST8Sh (LP) and *Streptococcus thermophilus* TA 080 (SP), as pure culture or in co-culture, on the kinetics parameters of acidification of milk added of passion fruit (FMPF) or buriti pulp (FMPB) and on bacterial viability. Total phenolic compounds, antioxidant capacity, and carotenoids, flavonoids and fatty acids profiles of milk fermented by the best culture combination were also evaluated. Milk fermented by the combination of SP+LC+LP cultures stood out for the high bacterial viability and the shorter fermentation time (until to pH 4.6). Phenolic compounds and antioxidant activity were higher in FMPF ( $0.10 \pm 0.02$  GAE/100 g and  $0.33 \pm 0.13$   $\mu\text{mol}$  Trolox/100 g, respectively) and FMB treatments ( $0.11 \pm 0.00$  GAE/100 g and  $0.25 \pm 0.03$   $\mu\text{mol}$

Trolox/100 g, respectively) compared to the control ( $0.09 \pm 0.01$  GAE/100 g and  $0.11 \pm 0.02$   $\mu\text{mol}$  Trolox/100 g, respectively). Regarding carotenoids profile, these compounds were not detected in control treatment and FMB showed higher amounts of  $\alpha$ ,  $\beta$  and total carotenoids ( $28.97 \pm 0.08$ ;  $528.42 \pm 34.49$  and  $557.39 \pm 34.33$  mg/100 g, respectively) in comparison to FMPF ( $7.07 \pm 0.08$ ;  $45.72 \pm 0.11$  and  $52.79 \pm 0.20$  mg/100 g, respectively). Buriti and passion fruit pulps increased the contents of monounsaturated and polyunsaturated fatty acids, respectively. The main fatty acids found in FMPF and FMB were omega 9 ( $66.29 \pm 0.09$  and  $30.05 \pm 0.98$  mmol/L, respectively) and palmitic acid ( $19.96 \pm 0.16$  and  $27.90 \pm 0.08$  mmol/L, respectively). Therefore, buriti and passion fruit pulps can be considered a great option to produce probiotic fermented milk with functional characteristics.

## 1. Introduction

In recent years, changes in people's eating habits and lifestyle have resulted in the emergence of various diseases and health complications (POUWEKS et al., 2022; THATCHER et al., 2022). To reverse this situation, the demand for functional foods has been increasing, aiming to improve quality of life, well-being and reduce the incidence of diseases (GUR; MAWUNTU; MARTIROSYAN, 2018).

Among functional foods, probiotic fermented milk is pointed as one of the most important products. Probiotics are defined as living microorganisms that, when administered in an adequate amount, confer specific and beneficial effects on the consumer's health (HILL et al., 2014). Most probiotic microorganisms used in food products belong to the lactic acid bacteria (LAB) group. Although several strains of LAB are described as probiotics, relatively few meet the requirements necessary to produce therapeutic effects. The bacteria belonging to the family *Lactobacillaceae* and genus *Bifidobacterium* are the most used as probiotics. Some of the members of the genus *Enterococcus* (*E. faecium*) and yeasts of the genus *Saccharomyces* (*S. boulardii*) are other microorganisms used as probiotics (PENNA et al., 2015). These beneficial microorganisms have several health benefits and have been used to improve intestinal health for several decades. In the last few years, *Bacteroides*, *Clostridium*, *Faecalibacterium*, *Akkermansia*, *Eubacterium*, *Propionibacterium* and *Roseburia* have been also recognized for their beneficial health and are called the next generation of probiotics (NGP) (SANDERS et al., 2019; CARMO et al., 2020).

The main effects attributed to the consumption of probiotics are: increased absorption of nutrients, improved intestinal barrier function, increased mucus secretion, modulation of the immune system through anti-inflammatory action, risk reduction of colon cancer and cardiovascular disease, regulation of serum cholesterol level, modulation of intestinal microbiota and reduction of intestinal diseases, such as intestinal inflammation, diarrhea, colitis, irritable bowel syndrome and Crohn's disease (CHEN et al., 2020; JOHN et al., 2020; AGHAMOHAMMAD et al., 2022).

In addition to the therapeutic and nutritional effects, probiotics also play an essential role in the technological processing and nutritional value of fermented products due to their production of vitamins and contribution to the food product preservation, due to the low pH provided by the production of acids and antimicrobial compounds as bacteriocins (ALGBOORY; MUHIALDIN, 2021; SILVA et al., 2021). Additionally, the use of probiotic bacteria for fermentation is considered a good strategy for attending consumer demand for healthy food. Another trend in the development of functional dairy products is to add fruit pulps, which can improve sensory properties, such as taste, aroma, texture and color, diversifying the products available on the market; these are some of the reasons for its popularity (CASAROTTI et al., 2018; BARAT; OZCAN, 2018, BORGONOVI; CASAROTTI; PENNA, 2021). Moreover, processed fruits and/or fruit by-products added to fermented milk helps both the acceptance of the final product and the increase in nutritional value (BARAT; OZCAN, 2018; WANG; WANG; GUO, 2019).

Fruit increases the nutritional value and antioxidant activity of the product, and even, depending on the amount added, can be considered a source of dietary fiber, as well as contribute to increasing the shelf-life and the product's healthy appeal (COMAN et al., 2020; MAQSOOD et al., 2020; WANG; KRISTO; LAPOINTE, 2020).

Fruits are rich in bioactive compounds such as phenolic compounds, carotenoids, as well as vitamins and fatty acids, which are related to the prevention of various diseases (SEPTEMBRE-MALATERRE; REMIZE; POUCHERET, 2018). In fermented products, these bioactive compounds can also interact with the LAB present in the product (ESPÍN; GONZÁLEZ-SARRÍAS; TOMÁS-BARBERÁN, 2017) and modulate the intestinal microbiota of consumers (PAP et al., 2021). However, several factors can influence the interaction of bioactive compounds with LAB, directly influencing the kinetic parameters and viability of the bacteria, such as: strain from specific species, food matrix and pH (ESPÍN; GONZÁLEZ-SARRÍAS; TOMÁS-BARBERÁN, 2017; TOMÁS-BARBERÁN

and ESPÍN, 2019), concentration and structure of the compound (SEPTEMBRE-MALATERRE; REMIZE; POUCHERET, 2018).

Considering the commercial relevance of probiotic fermented milk added with fruit pulp, the amount of bioactive compounds of passion fruit and buriti pulps have been evaluated in a previous study and they were applied in fermented milk. The fermented product showed promising results, such as phenolic compounds stability, antioxidant activity and an increase in the *Lactobacillus* population during the shelf-life period (BORGONOVİ; CASAROTTI; PENNA, 2021), which encouraged further studies. In this context, the aim was to evaluate the effect of *Lacticaseibacillus casei* SJRP38 (LC), *Lactiplantibacillus plantarum* ST8Sh (LP) and *Streptococcus thermophilus* TA 080 (ST), as pure culture or in co-culture, on the kinetics parameters of acidification of milk added of passion fruit or buriti pulp and on bacteria viability. Total phenolic compounds, antioxidant capacity, and carotenoids, flavonoids and fatty acids profiles of milk fermented by the best culture combination were also evaluated.

## 2. Materials and Methods

### 2.1 Passion fruit and buriti pulps preparation

Passion fruits (*Passiflora edulis* f. *flavicarpa* Deg.), originating from Bahia, were purchased at local market in São José do Rio Preto-SP, Brazil and buriti fruits (*Mauritia flexuosa*) were harvested and processed in Teresina-PI, Brazil. Both fruits were sanitized and stored as described by Borgonovi, Casarotti and Penna (2021).

### 2.2 Lactic acid bacteria (LAB) cultures

*Lacticaseibacillus casei* SJRP38 (LC), a strain from Culture Collection of Lactic Acid Bacteria of São Paulo State University, UNESP (CCLAB-UNESP, WDCM 1182) was isolated from water buffalo mozzarella cheese, and *Lactiplantibacillus plantarum* ST8Sh (LP) isolated from Bulgarian salami, both characterized as potentially probiotic strains (SALOTTI-SOUZA et al., 2019; TODOROV; HOLZAPFEL; NERO, 2016) were used. *Streptococcus thermophilus* TA 080 (ST) (Sacco, Cadorago, Co, Italy) were used as a starter culture and *Lacticaseibacillus rhamnosus* GG ATCC 53103 as a probiotic reference strain.

### 2.3 Milk characterization

Skimmed-milk powder was reconstituted in distilled water to obtain 9% (w/v) total solids and milk was stirred until the powder was completely dissolved. For the characterization of reconstituted milk powder, the titratable acidity was quantified by acid-alkalimetric titration using the solution of 0.1 mol/L NaOH and phenolphthalein as an indicator (IAL, 2005). The results were expressed as % lactic acid. The levels of fat, protein, total solids (ST) and non-fat solids (SNG) were determined using Ekomilk-M equipment (Bulteh 2000 Ltda., Stara Zagora, Bulgaria).

### 2.4 Preparation of fermented milk

To evaluate the effect of the fruit pulps on the kinetics parameters of acidification and LAB viability, three treatments were prepared: fermented milk without pulp (control - FMWP), fermented milk with 1% passion fruit pulp (FMPF), fermented milk with 1% buriti pulp (FMB). The pH was adjusted before pasteurization to pH 6.8 in all treatments using lactic acid (Synth, Diadema-SP, Brazil) or a 10% sodium bicarbonate solution (Kinino, Mirassol-SP, Brazil).

The reconstituted milk with or without fruit pulp, according to the experiment, was distributed in sterile glass bottles, heated to 90 °C for 10 min., using a food processor (Thermomixer TM 31, Cloyes-sur-le-Loir, FR), cooled to the inoculation temperature (37 °C), and stored at 4 °C.

### 2.5 Preparation of cultures

The cultures LC and LP were activated 1% in MRS (Difco, Laboratories, Detroit, MI, USA) at 37 °C for 18 h. The freeze-dried ST was used directly in the product.

The cultures LC, LP and ST as pure culture, or in combinations: LC + ST, LP + ST, LC + LP, and LC + LP + ST were used to produce FMWP, FMPF and FMB, totalizing 21 experiments, performed in duplicates. For the fermentations, Lc and LP were inoculated in the proportion of 2% from the activated pre-inoculum and ST was inoculated in the proportion of 0.1%, according to the treatment, which allowed for initial counts of approximately 7 log CFU/mL after milk inoculation.

## 2.6 Fermentation conditions

For fermentation, bottles were incubated in a water bath (EVLAB, Londrina, Brazil) at 42 °C until reaching the pH 4.6. During fermentation, the pH was continuously measured from the culture inoculation to calculate the kinetic parameters ( $V_{\max}$ ,  $T_{V_{\max}}$ ,  $pH_{V_{\max}}$ ,  $T_{pH\ 5.5}$ ,  $T_{pH\ 5.0}$  and  $T_{pH\ 4.6}$ ) using the CINAC system (Cynetique d'acidificacion, Alliance Instruments, Frepillon, France) (CASAROTTI; CARNEIRO; PENNA, 2014). After the fermentation was completed, the product was initially cooled in water and ice bath to 20 °C, and the curd was manually broken by stirring for 2 min, in a standardized way for all treatments, followed by final cooling to 5 °C. The fermented products were distributed into sterile plastic containers and stored at 4 °C until the analysis of LAB viability and – 20 °C until the analysis of physicochemical, bioactive compounds and antioxidant activity.

## 2.7 LAB viability

After 1 day of fermentation, 100 µL aliquot of fermented milk from different treatments was individually transferred to a tube containing 900 µL of 0.85% saline solution. The mixture was stirred, and this suspension was submitted to serial decimal dilutions to  $10^{-8}$  using the same diluent. For counting, 10 µL of each dilution was transferred to Petri dishes (SOLIERI et al., 2014). LC and LP were counted using MRS agar (Difco), incubated in anaerobic conditions at 45 °C and 15 °C, respectively. *Streptococcus* spp. were counted using M17 agar (Difco), incubated in aerobic conditions at 50 °C, both for 48 h.

The LAB culture (pure culture, or in combinations) which present the best kinetic parameters of acidification and the highest LAB viability was selected for the evaluation of physicochemical characteristics, bioactive compounds and antioxidant activity.

## 2.8 Physicochemical characterization of milk fermented by selected culture

Total solids and total fat (CASE; BRADLEY JR; WILLIAMS, 1985), total protein (AOAC, 2010), ash (IAL, 2005) contents, and titratable acidity (IAL, 2005) and the pH value (IAL, 2005) of the fermented products were determined in triplicate at 1 day after fermentation.

## 2.9 Total phenolic compounds and antioxidant activity of milk fermented by selected culture

For the analyses of total phenolic compounds and antioxidant activity, the extracts were obtained by mixing the fermented milk samples with the extracting solution (70% methanol (Synth) + 50% acetone [v/v, 1: 1] (Synth), vortexed for 1 min and sonicated (135 W, 40 KHz) for 15 min. Following, the samples were centrifuged at  $1935\times g$  for 20 min, and the supernatants were collected in a 50 mL flask (this step was repeated until the sample was completely exhausted) in the absence of light, as described by Borgonovi, Casarotti and Penna (2021).

The phenolic compounds were assessed in triplicate, according to Folin–Ciocalteu (MACORIS et al., 2012; WATERHOUSE, 2014), and quantified at 1 day after fermentation; the results were expressed as milligrams of gallic acid equivalents (mg GAE) per 100 g of sample.

The antioxidant activity was determined 1 day after fermentation, in triplicate, using the DPPH method and the results were expressed as micromole Trolox equivalent ( $\mu\text{mol TE}$ ) per g sample (RUFINO et al., 2007). Trolox [(±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, 97%] and DPPH (2,2-Diphenyl-1-picrylhydrazyl) were obtained from Sigma-Aldrich (St. Louis, USA) and Folin-Ciocalteu and gallic acid ( $\text{C}_7\text{H}_6\text{O}_5$ , P.A. ACS) were obtained from Dinâmica (Diadema, Brazil).

## 2.10 Carotenoid profile of milk fermented by selected culture

The extraction of pigments was performed according to Xavier et al. (2012), using tetrahydrofuran and partitioning with petroleum ether and ethyl ether. The extract obtained were concentrated by rotary evaporator (Fisatom, Model 801, Brazil) ( $T < 25^\circ\text{C}$ ) and stored in a freezer ( $-18^\circ\text{C}$ ) for quantification by high-performance liquid chromatography (HPLC). The profile of carotenoids in fermented milk was determined 1 day after fermentation, according to Reis et al. (2018). All these analyses were made in triplicate.

## 2.11 Fatty acid profile of milk fermented by selected culture

For analysis of the fatty acid profile, lipids were extracted according to AOAC (2010, Official Method 996.06). The fatty acid esterification process was carried out according to AOCS (2014, Official Methods Ce 1a-13 and Ce 1h-05). Esterified fatty

acids were separated by gas chromatography, according to chain length, degree of establishment and geometry, and position of the double bonds.

## 2.12 Statistical analysis

The data analyses of acidification curves, viability of bacteria, chemical composition, pH, acidity in fermented milk as well as bioactive compounds and antioxidant activity were carried out by the Analysis of Variance (ANOVA) followed by a Tukey test to compare the treatments. A significance level of 5% probability and Minitab 16 software were adopted.

## 3. Results and Discussion

### 3.1 Milk characterization and preparation of probiotic fermented milk

The reconstituted milk powder presented  $8.88\% \pm 0.30$  g/100 g total solids, 0% fat,  $1.04 \pm 0.00$  g/mL density,  $3.21\% \pm 0.12$  g/100 g protein and  $0.14 \pm 0.01$  g/100 g lactic acid, which is a typical composition of skimmed-milk and is in accordance with the parameters established by the Brazilian law for skimmed-milk (BRASIL, 2018).

### 3.2 Fermentation kinetic parameters and LAB viability

Acidification and the viability of LAB are essential technological characteristics in the selection of a culture for the fermentation of dairy products. Therefore, these parameters were evaluated.

Fermentation time ranged from 3.7 h to > 48 hours (Table 1). When fruit pulp was added, the fermentation time was similar or lower than FMWP, depending on the culture used (Table 1). All treatments fermented by LC and LP alone did not present different final fermentation time ( $T_{pH\ 4.6}$ ), however, FMB fermented by LC presented a significant difference; higher  $V_{max}$  and lower  $T_{Vmax}$  were observed compared to FMWP and FMFP treatments. The addition of buriti pulp in the LC+LP decreased the fermentation time compared to the FMWP and FMFP treatments.

**Table 1.** Kinetics parameters of acidification for fermented milk without pulp (FMWP), fermented milk with passion fruit pulp (FMPF) and fermented milk with buriti pulp (FMB).

		$V_{\max}$ ( $10^{-3}$ pH units/min)	$T_{V_{\max}}$ (h)	$pH_{V_{\max}}$	$T_{pH5.5}$ (h)	$T_{pH5.0}$ (h)	$T_{pH4.6}$ (h)
<b>FMWP</b>	LC	3.66±1.36 Cb	5.11±0.37 Ba	6.72±0.05 Aa	17.77±0.00 Ba	26.5±0.11 Ba	34.24±0.30 Ba
	LP	22.60±1.93 Ba	9.25±2.16 Ab	5.79±0.05 Cb	9.48±2.10 Cb	10.19±1.25 Cb	11.68±2.19 C
	ST	21.55±0.04 Bb	1.15±0.01 Cb	5.79±0.05 Cb	1.39±0.05 Da	2.25±0.17 Da	4.84±0.30 Da
	LC+LP	2.48±0.13 Cb	1.69±0.01 Ca	6.78±0.03 Aa	22.14±0.47 Aa	35.34±0.27 Aa	45.83±0.15 Aa
	ST+LC	23.93±0.90 ABa	0.51±0.11 Cb	6.38±0.11 Ba	1.26±0.04 Db	1.89±0.01 Db	3.75±0.03 Da
	ST+LP	24.39±0.16 ABa	0.44±0.03 Cc	6.61±0.03 Aa	1.31±0.01 Db	1.97±0.06 Db	3.90±0.25 Da
	ST+LC+LP	26.14±0.65 Aa	1.01±0.05 Ca	6.25±0.07 Bab	1.61±0.06 Da	2.23±0.11 Da	4.16±0.25 Da
<b>FMPF</b>	LC	6.14±0.00 Cab	4.95±0.57 Ba	6.54±0.19 ABa	10.99±0.16 Bb	23.32±0.19 Bb	40.29±0.27 Aa
	LP	3.21±0.25 Cb	7.05±0.27 Ab	6.72±0.04 Aa	18.86±0.65 Aa	40.29±9.14 Aa	> 48 h
	ST	24.96±0.08 Aa	1.39±0.01 Ca	5.71±0.03 Cb	1.53±0.01 Da	2.22±0.02 Ca	4.64±0.01 Ca
	LC+LP	6.22±0.19 Ca	1.89±0.58 Ca	6.63±0.05 ABb	5.01±0.47 Cb	13.29±1.43 BCb	35.15±3.56 Bab
	ST+LC	18.63±0.13 Bb	1.37±0.05 Ca	5.43±0.03 Cb	1.30±0.07 Db	1.91±0.08 Cb	3.91±0.22 Ca
	ST+LP	18.38±0.20 Bc	1.41±0.01 Ca	5.44±0.01 Cc	1.34±0.01 Db	1.94±0.02 Cb	3.70±0.18 Ca
	ST+LC+LP	23.67±2.49 Aab	1.22±0.33 Ca	6.00±0.38 BCb	1.58±0.06 Da	2.24±0.05 Ca	4.56±0.25 Ca
<b>FMB</b>	LC	7.15±0.16 Ca	1.68±0.06 BCb	6.25±0.01 BCa	8.51±0.67 Bc	21.27±0.99 Bb	32.12±3.82 Aa
	LP	2.78±0.86 Db	20.45±0.78 Ac	5.79±0.06 Db	23.40±0.25 Aa	35.77±0.00 Aa	> 48 h
	ST	21.16±0.01 ABc	0.51±0.01 CDc	6.35±0.06 Ba	1.36±0.06 Da	2.33±0.11 Da	4.40±0.21 Ba
	LC+LP	8.19±1.32 Ca	2.18±0.67 Ba	6.19±0.02 Cc	6.29±0.40 Cb	15.12±2.28 Cb	25.89±6.69 Ab
	ST+LC	19.05±0.05 Bb	0.76±0.01 CDb	6.20±0.02 Ca	1.52±0.01 Da	2.36±0.08 Da	4.47±0.28 Ba
	ST+LP	20.90±0.91 ABb	0.77±0.04 CDb	6.14±0.04 Cb	1.45±0.03 Dc	2.24±0.05 Da	4.13±0.21 Ba
	ST+LC+LP	22.12±0.88 Ab	0.10±0.05 Dc	6.63±0.04 Aa	1.24±0.04 Db	1.99±0.05 Db	4.49±0.18 Ba

$V_{\max}$ : maximum acidification rate;  $T_{V_{\max}}$ : time required to reach  $V_{\max}$ ;  $pH_{V_{\max}}$ : pH in  $V_{\max}$ ;  $T_{pH5.5}$ : time required to reach pH 5.5;  $T_{pH5.0}$ : time required to reach pH 5.0;  $T_{pH4.6}$ : time required to reach pH 4.6 (end of fermentation). ST - *S. thermophilus* ST080, LC - *L. casei* SJRP38, LP - *L. plantarum* ST8Sh, LC+LP - *L. casei* SJRP38 + *L. plantarum* ST8Sh, ST+LC - *L. casei* SJRP38 + *S. thermophilus* ST080, ST+LP - *L. plantarum* ST8Sh + *S. thermophilus* ST080, ST+LC+LP - *L. casei* SJRP38 + *L. plantarum* ST8Sh + *S. thermophilus* ST080. Different capital letters in the same column denote a significant difference ( $p < 0.05$ ) among LAB cultures in fermented milk samples. Different lower-case letters in the same column denote a significant difference ( $p < 0.05$ ) among the fermented milk samples fermented by the same LAB culture.

This may be explained by the ability of both strains LC and LP to produce  $\alpha$ - and  $\beta$ -glucosidase enzymes to use some bioactive compounds, fibers, and fructose available in the pulps as substrates during fermentation (MENDONÇA et al., 2017). The assimilation of other carbohydrates also affects the fermentation parameters of LAB. Considering the composition of fruit pulps, buriti pulp presents higher fiber contents (data not shown).

Considering the dairy matrix, one of the most important enzymes is galactosidase, which is produced by all tested strains in the form of  $\beta$ -galactosidase, however, only LP produces  $\alpha$ -galactosidase (data not shown). Since lactose, a disaccharide composed of galactose and glucose, is the main carbohydrate in milk, this leads us to the hypothesis that there was greater hydrolysis of lactose in the treatments fermented by LP, reducing the fermentation time. During fermentation, the  $\alpha$  and  $\beta$ -galactosidase degrade galactose from milk and decrease the amount of lactose present in the fermented dairy product, making it consumable by lactose intolerant people (SOUZA-SALOTTI et al., 2019; OLDAK et al., 2020; KOCABAS et al., 2022).

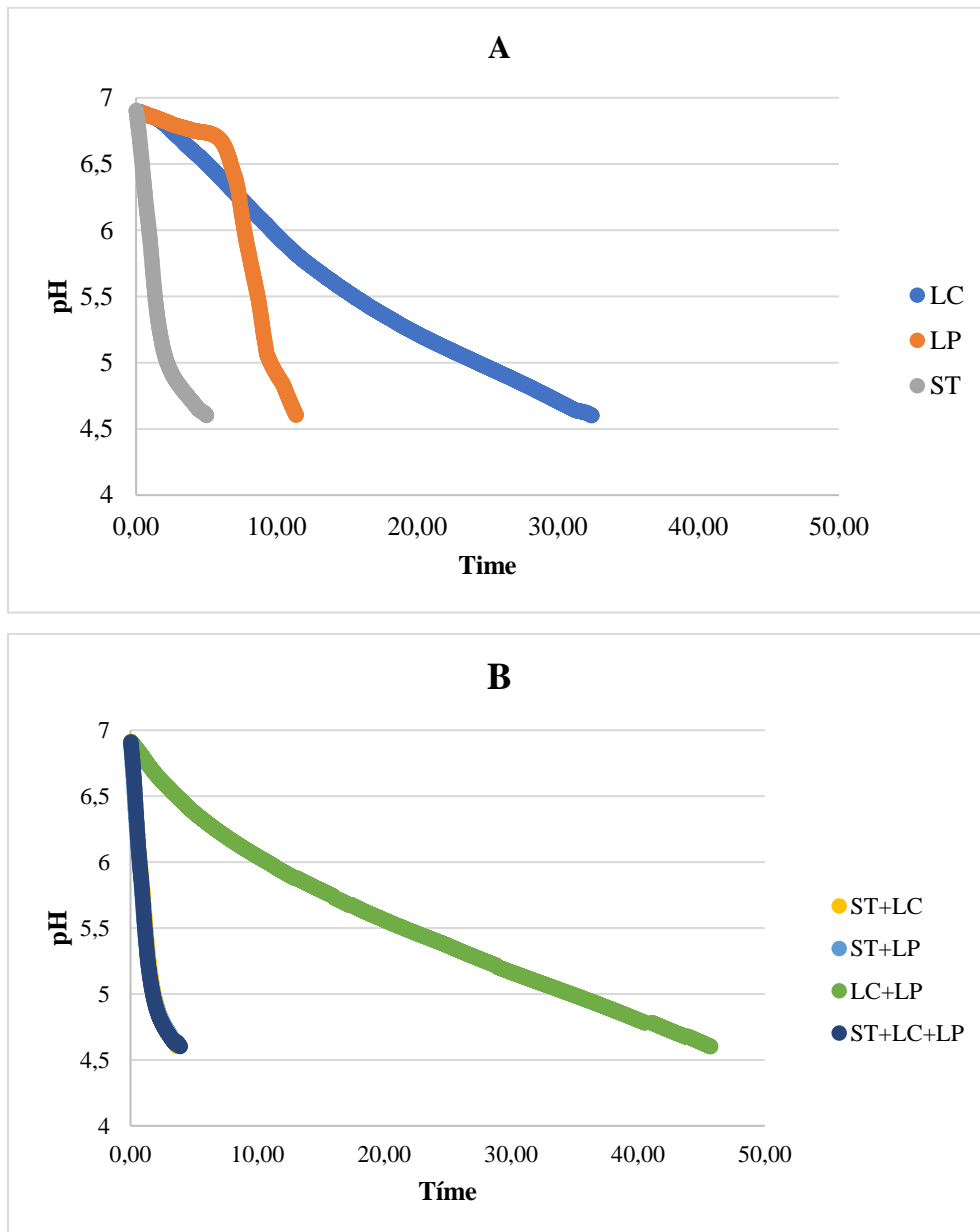
Data available in literature shows that *L. plantarum* ssp. has been used in the production of lactose-free milk for lactose intolerant consumers (ZEINAT et al., 2016; KHABIBULLAEV et al., 2020; LAUKOVÁ et al., 2022). The lactose hydrolysis also causes sensory changes in the product by increasing the sweetness level of the dairy product. In some cases, it can be an advantage, since reduced amount of sugar is added to the final product (HARJU et al., 2012; SCHMIDT et al., 2016; KOCABAS et al., 2022).

Another importance for using *L. plantarum* is due to the increased demand for LABs producing  $\alpha$ -galactosidase for various biotechnological purposes, such as the production of soy milk. For instance, the use of  $\alpha$ -galactosidase in soymilk production increases 27.3% of total phenols and 19.9% of flavonoids, which are considered bioactive compounds (ELSHAFEIET et al., 2022).

Casarotti et al. (2018) reported that fermented products (oat or rice beverages and goat milk) added of guava, orange and passion fruit by-products resulted in lower  $V_{max}$ , compared to the control (without fruit by-products). The fermentation time varied from  $2.35 \pm 0.32$  to  $6.75 \pm 0.58$  h, depending on the fruit by-product and food matrix.

LC, LP and LC+LP cultures presented longer fermentation time than other cultures in all types of fermented milk (Table 1, Figures 1-3). This fact can be explained

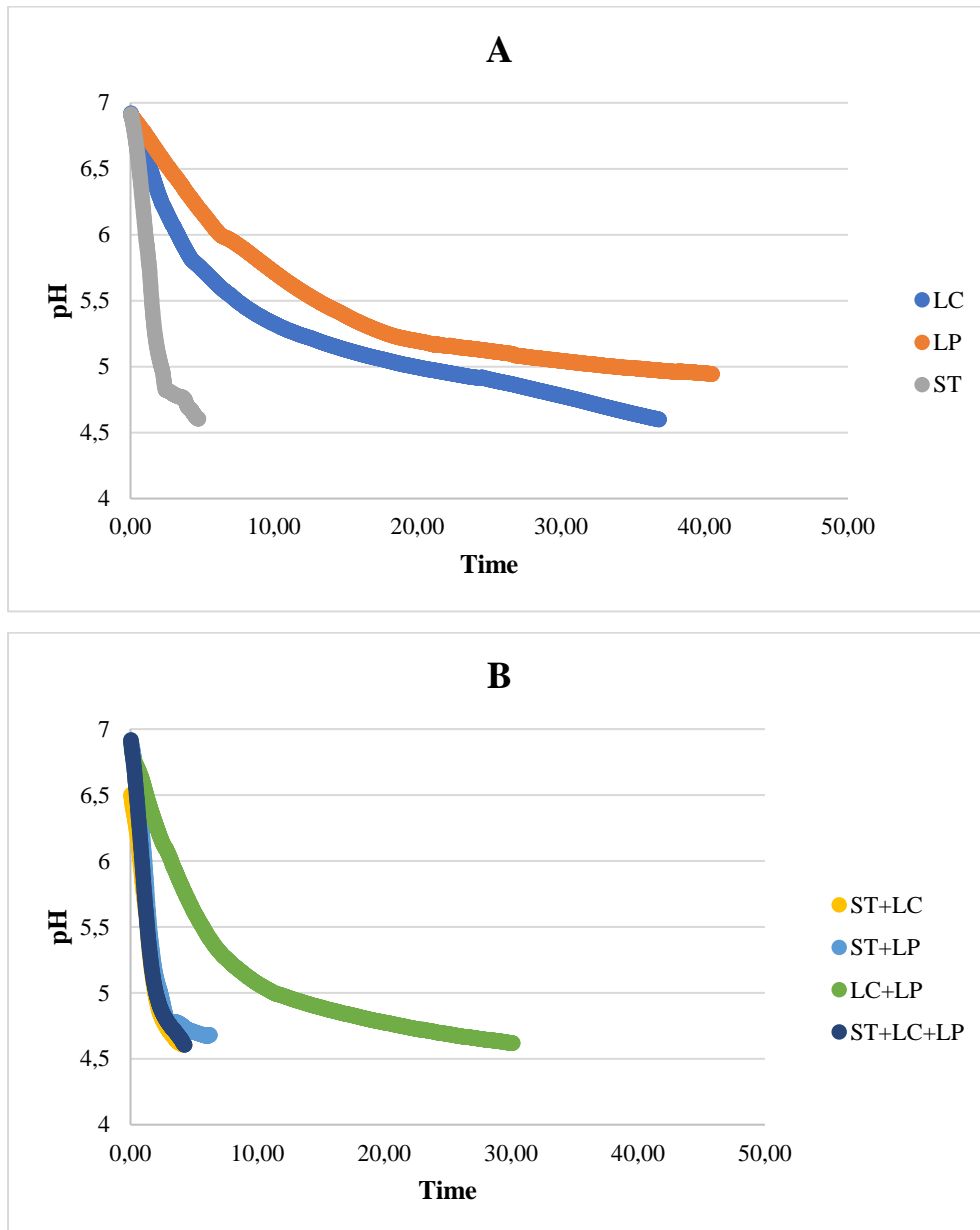
by the low capacity of these LAB to hydrolyze milk components, such as casein (MARKAKIOU et al., 2020).



**Figure 1.** Acidification curves of fermented milk without fruit. A) Milk fermented by pure cultures. B) Milk fermented by combination of cultures.

In general, these LAB are used in co-culture with starter cultures like *Streptococcus thermophilus*. *L. plantarum* is commonly used for vegetables fermentation and some strains show probiotic properties, have enzymatic activities for the degradation of polyphenols and other compounds, such as reductase, tannase, gallate decarboxylase, phenolic acid decarboxylase, benzyl alcohol dehydrogenase, aryl glycosidase, and

feruloyl esterase activities (ESTEBAN-TORRES et al., 2013; REVERÓN et al., 2017; PLESSAS, 2022), however, the LC and LP strains demonstrated to produce only  $\beta$ -glucosidase,  $\alpha$ -glucosidase,  $\beta$ -galactosidase, and esterase using API-ZYM test (data not shown). The slow growth of LC or LP as a pure culture is related to the lack of proteolytic enzymes, thus justifying the use of starter culture (*S. thermophilus*) to produce yoghurt and fermented milk (MARAFFON et al., 2011).

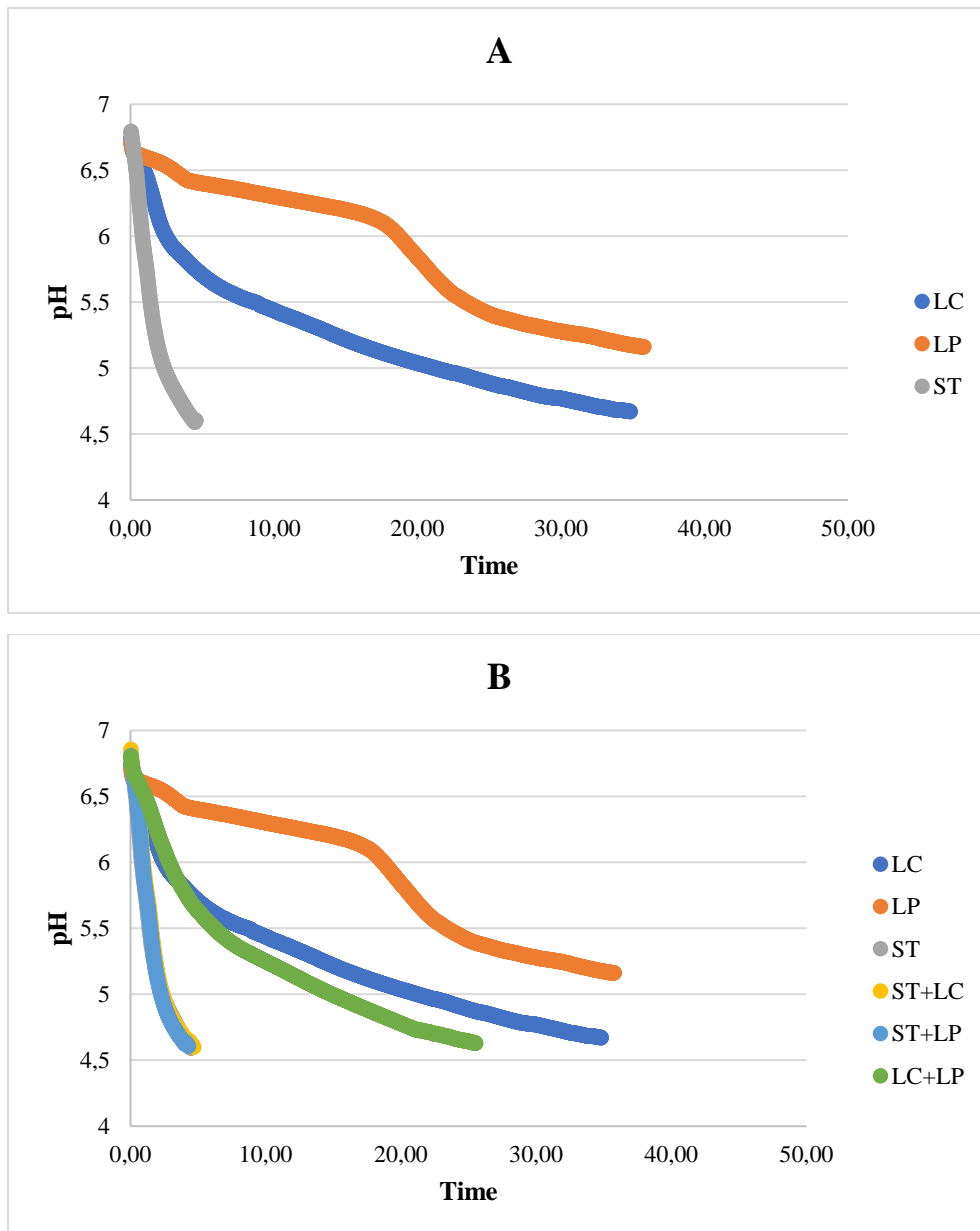


**Figure 2.** Acidification curves of fermented milk with passion fruit pulp. A) Milk fermented by pure cultures. B) Milk fermented by combination of cultures.

*S. thermophilus* have the enzymes necessary for the hydrolysis of casein, uptake of the resulting peptides and hydrolysis into free amino acids, they also have a unique lactose absorption system, after the uptake and cleavage of lactose by  $\beta$ -galactosidase, the glucose fraction is used through glycolysis, while galactose is excreted into the medium by most strains (MARKAKIOU et al., 2020). When *S. thermophilus* was used, as pure culture or combined with *L. casei* or *L. plantarum*, there was a significant reduction on the fermentation time.

From an industrial point of view, the proteolytic system of *S. thermophilus* is associated with faster growth and milk acidification, along with increased cell viability (MARKAKIOU et al., 2020). The treatments ST+LC, ST+LP and ST+LC+LP showed the lowest fermentation time (time to reach pH 4.6), about 4 to 5 h. This difference is due to cooperation that occurs between *S. thermophilus* and *L. plantarum* and *L. casei* cultures.

The cultures' acidification profile can be influenced not only by the combination of cultures, but also by enzymes produced and assimilable carbohydrates. The amount of fruit pulp added to the matrix, and the presence or absence of specific bioactive compounds in the fruit pulp can also influence the acidification profile. The influence of fiber and some bioactive compounds on the viability of LAB have been studied by several authors. Irkin et al. (2015) reported that *Lactobacillus* ssp. were significantly affected by fruit juices and fruit peel extracts that contained chlorogenic acid, hesperidin, naringin and caffeic acid compared to control samples. Mendonça et al., (2017) observed that addition of biomass, green banana flour and jabuticaba peel flour positively influenced the viability of the probiotic culture during the storage period. Morais et al. (2019) reported that *Lactobacillus acidophilus* LA-05 or *Bifidobacterium animalis* ssp. *lactis* BB-12 degraded some compounds present in red pitaya pulp after 48 h of cultivation. There was a decrease in pH and sugar levels, and an increase in organic acids and probiotics' viability.



**Figure 3.** Acidification curves of fermented milk with buriti pulp. A) Milk fermented by pure cultures. B) Milk fermented by combination of cultures.

**Table 2.** Viability of *Lactobacilli* and *Streptococcus thermophilus* (CFU/mL) in milk fermented without pulp (FMWP), fermented milk with passion fruit pulp (FMPF) and fermented milk with buriti pulp (FMB) by LAB cultures.

	ST	LC	LP	LC+LP	ST+LC	ST+LP	ST+LC+LP
<b><i>L. casei</i> SJRP38</b>							
<b>FMWP</b>		8.15±0.04 Ca		8.89±0.16 Ba	8.45±0.21 BCa		10.80±0.08 Aa
<b>FMPF</b>		8.14±0.13 Ba		8.69±0.30 Ba	8.45±0.21 Ba		10.65±0.02 Aa
<b>FMB</b>		8.19±0.02 Da		8.70±0.00 Ba	8.60±0.00 Ca		10.58±0.03 Aa
<b><i>L. plantarum</i> ST8Sh</b>							
<b>FMWP</b>			7.51±0.07 Ca	8.74±0.06 Ba		7.76±0.40 Ca	9.90±0.00 Aa
<b>FMPF</b>			7.65±0.03 Ca	8.69±0.12 Ba		7.81±0.05 Ca	9.84±0.09 Aa
<b>FMB</b>			7.56±0.17 Ca	8.65±0.07 ABa		7.89±0.27 BCa	9.54±0.34 Aa
<b><i>S. thermophilus</i> TA080</b>							
<b>FMWP</b>	8.87±0.04 Ba				8.95±0.00 Ba	8.80±0.14 Ba	9.77±0.10 Aa
<b>FMPF</b>	8.66±0.26 Ba				8.90±0.08 ABa	8.84±0.09 Ba	9.66±0.26 Aa
<b>FMB</b>	8.70±0.00 Ba				8.81±0.05 ABa	8.84±0.09 ABa	9.60±0.43 Aa

ST - *S. thermophilus* ST080, LC - *L. casei* SJRP38, LP - *L. plantarum* ST8Sh, LC+LP - *L. casei* SJRP38 + *L. plantarum* ST8Sh, ST+LC - *L. casei* SJRP38 + *S. thermophilus* ST080, ST+LP - *L. plantarum* ST8Sh + *S. thermophilus* ST080, ST+LC+LP - *L. casei* SJRP38 + *L. plantarum* ST8Sh + *S. thermophilus* ST080. Different capital letters in the same line denote a significant difference ( $p < 0.05$ ) among LAB cultures in the same fermented milk samples. Different lower-case letters in the same column denote a significant difference ( $p < 0.05$ ) among fermented milk samples fermented by the same LAB culture.

All samples showed population of *L. casei* SJRP38 (CFU/mL) and *S. thermophilus* above  $10^8$  CFU/mL, and *L. plantarum* ST8Sh above  $10^7$  CFU/mL (Table 2). There was no significant difference among fermented milk with or without fruit pulp using all LAB cultures (as pure culture, or in combinations), showing that fruit pulp in fermented products does not affect the viability of these LAB. On the other hand, there was a significant difference in LAB viability comparing the same fermented milk treatment fermented by different LAB cultures, and the combination of ST+LC+LP showed the highest viability.

*S. thermophilus* and *Lactobacillus* have different metabolic characteristics; as a result, a proto-cooperation occurs during fermentation, and a well-established result is described in the literature (MARKAKIOU et al., 2020; TRIMIGNO et al., 2020). The use of these microorganisms in co-cultures reduces the fermentation time through the rapid LAB growth and acidification of the medium, in addition to releasing aromatic compounds (URIOT et al., 2017; MAOLONI et al., 2020). This proto-cooperation takes place through changes of metabolites, in which free amino acids and peptides produced by the proteolytic system of *Lactobacilli* is released for *S. thermophilus* growth, while formic, folic, and pyruvic acids, as well as carbon dioxide, glutathione and long chain fatty acids produced by *S. thermophilus* improves *Lactobacilli* growth (WANG et al., 2016; MARKAKIOU et al., 2020; TRIMIGNO et al., 2020).

Another positive result about the presence of fruit pulp in probiotic fermented food is the protection of the probiotics under low pH values and adverse conditions as higher bile concentration found in the human gastrointestinal tract, and the pulp fruits may also present prebiotic potential, acting in synergy with probiotics in the human intestines after their ingestion (CASAROTTI et al., 2018).

The evaluation of the kinetic parameters of acidification and LAB viability demonstrated that the combination of ST+LC+LP resulted in lower fermentation time and higher LAB viability.

### 3.3 Centesimal composition, pH and acidity of milk fermented by selected culture

The composition of probiotic fermented milk showed small variations among treatments. The differences of moisture, ash and pH were low and acceptable for this kind of product and there was no significant difference between treatments in relation to protein and pH (Table 3). On the other hand, there was a significant difference in acidity among treatments, which was expected since during fermentation LAB produced lactic

acid using carbohydrates from the different matrices (CHUAH, MAO, 2020; GALLI et al., 2022), although this difference is not expressive at an industrial level.

**Table 3.** Composition, pH value and titratable acidity of milk fermented by selected culture.

	<b>FMWP</b>	<b>FMPF</b>	<b>FMBP</b>
Total solids (g/100 g)	10.46±0.14 <sup>c</sup>	11.54±0.09 <sup>a</sup>	11.13±0.14 <sup>b</sup>
Protein (g/100 g)	3.58±0.01 <sup>a</sup>	3.64±0.02 <sup>a</sup>	3.76±0.18 <sup>a</sup>
Ash (g/100 g)	1.20±0.02 <sup>b</sup>	1.45±0.01 <sup>a</sup>	1.22±0.02 <sup>b</sup>
Fat (g/100 g)	n.d.	n.d.	0.2
Carbohydrates(g/100 g)	84.76	83.59	83.46
Acidity (g/100 g lactic acid)	0.76±0.00 <sup>c</sup>	0.91±0.00 <sup>a</sup>	0.83±0.00 <sup>b</sup>
pH	4.52±0.02 <sup>a</sup>	4.53±0.03 <sup>a</sup>	4.56±0.01 <sup>a</sup>

FMWP - Fermented milk without pulp, FMPF - Fermented milk with passion fruit pulp, FMB - Fermented milk with buriti pulp. Different lowercase letters in the same row denote a significant difference ( $p < 0.05$ ) among fermented milk samples. n.d. – not detected.

### 3.4 Phenolic compounds, antioxidant activity and carotenoids profile of milk fermented by selected culture

The addition of fruit pulp in fermented milk increased the amount of total phenolic compounds of FMPF and FMB compared to FMWP (Table 4), which was expected since fruit pulps are rich in phenolic compounds such as flavonoids, phenolic acids, stilbenes, and lignans (SEPTEMBRE-MALATERRE; REMIZE; POUCHERET, 2018).

In contrast, the antioxidant activity of FMPF was only significantly higher than FMWP. These values are higher than that observed by Borgonovi, Casarotti and Penna (2021) for passion fruit and buriti fermented milk; probably the phenolic compounds and carotenoids are not the main responsible for this activity (Table 4).

The antioxidant activity of FMPF may be related to the presence of bioactive compounds such as phenolic compounds and carotenoids in passion fruit pulp. Ascorbic acid is an important vitamin with high antioxidant action, and well known as free radical scavenge (MUMTAZ et al., 2023). Another hypothesis for similar antioxidant activity between FMB and FMWP is that during fermentation, some microorganisms such as *Lactobacillus* sp. can metabolize phenolic compounds, carotenoids, and bioactive peptides with antioxidant activity, consequently decreasing the antioxidant action of the product

(MAQSOOD et al. 2020; BAO, WU, 2021; PIEKARSKA-RADZIK; KLEWICKA, 2021).

Buriti pulp presented higher content of total carotenoids compared to passion fruit pulp (BORGONOVİ; CASAROTTI; PENNA, 2021). As expected, when buriti pulp was added to fermented milk, it increased the total carotenoids content in the fermented product compared to fermented milk added of passion fruit pulp (Table 4). On the other hand, in FMWP carotenoids were not detected. The other carotenoids fractions as lutein, zeaxanthin,  $\beta$ -cryptoxanthin and lycopene were also not detected in any treatment.

**Table 4.** Phenolic compounds and antioxidant activity (DPPH $\cdot$ ) and carotenoids profile of milk fermented by selected culture.

	<b>FMWP</b>	<b>FMPF</b>	<b>FMB</b>
Phenolic compounds (mg GAE/100 g)	0.09 $\pm$ 0.01 <sup>b</sup>	0.10 $\pm$ 0.02 <sup>a</sup>	0.11 $\pm$ 0.00 <sup>a</sup>
Antioxidant activity ( $\mu$ mol Trolox/g)	0.11 $\pm$ 0.02 <sup>b</sup>	0.33 $\pm$ 0.13 <sup>a</sup>	0.25 $\pm$ 0.03 <sup>ab</sup>
$\alpha$ -Carotene ( $\mu$ g/100g)	n.d.	7.07 $\pm$ 0.08 <sup>a</sup>	28.97 $\pm$ 0.08 <sup>b</sup>
$\beta$ -carotene ( $\mu$ g/100g)	n.d.	45.72 $\pm$ 0.11 <sup>b</sup>	528.42 $\pm$ 34.49 <sup>a</sup>
Total carotenoids ( $\mu$ g/100g)	n.d.	52.79 $\pm$ 0.20 <sup>b</sup>	557.39 $\pm$ 34.44 <sup>a</sup>

FMWP - Fermented milk without pulp, FMPF - Fermented milk with passion fruit pulp, FMB - Fermented milk with buriti pulp. Different lowercase letters in the same line denote a significant difference ( $p < 0.05$ ) among fermented milk samples. n.d. – Not detected.

### 3.5 Fatty acid profile

When the buriti pulp was added to fermented milk, there was an increase in the content of unsaturated fatty acids, mainly oleic acid, when compared to FMPF and FMWP (Table 5). The passion fruit pulp in turn increased the content of myristic acid, palmitic acid, and unsaturated fatty acids, mainly linoleic acid,  $\gamma$ -linolenic acid.

Buriti has higher amount of total lipids (12 g/100 g) compared to passion fruit pulp (0.50 g/100 g) (data not shown). Approximately 81% of the fatty acids present in the buriti pulp and 44% of the fatty acids present in the passion fruit pulp are monounsaturated (belonging to the  $\omega$  3, 6, 7, 9 and 11 classes), with a high concentration of oleic acid ( $\omega$ -9). Fruits containing  $\omega$ -9 may have an action in reducing the incidence of diseases induced by reactive oxygen species and improvement of the immune system (RYDLEWSKI et al., 2017). This acid has beneficial effects on consumer health and is related to the prevention of cardiovascular diseases, since this fatty acid ( $> C16:0$ ) has the capacity to reduce platelet aggregation, and consequently, reduce the incidence of

cardiovascular diseases (COSTANTINI et al., 2017; WATANABE; TATSUNO, 2017). Polyunsaturated fatty acid (PUFA) can also improve visual and neurological development and have beneficial effects on inflammatory conditions, including arthritis and asthma (BAKER et al. 2016), in addition to decreasing the risk of cancer incidence (SARAVANAN; DAVIDSON, 2010; ASIF, 2011). In addition to the beneficial effects on human health, PUFA also assists adherence by the probiotic strains onto the mucosa of the distal portion of the intestines (DAS, 2002).

**Table 5.** Fatty acids profile of milk fermented by selected culture.

Fatty acids (g/100 g lipid)	FMWP	FMPF	FMB
(C12:0) Lauric acid	-	2.55±0.01	-
(C14:0) Myristic acid	6.9±0.20 <sup>B</sup>	8.66±0.04 <sup>A</sup>	1.66±0.11 <sup>C</sup>
(C14:1) Tetradecenoic acid	-	1.17±0.06	-
(C16:0) Palmitic acid	27.97±0.08 <sup>B</sup>	29.54±0.08 <sup>A</sup>	20.11±0.22 <sup>C</sup>
(C16:1) Palmitoleic acid-omega 7	-	0.72±0.04	-
(C18:0) Stearic acid	11.52±0.02 <sup>A</sup>	8.04±0.06 <sup>B</sup>	2.41±0.10 <sup>C</sup>
(C18:1-cis) Oleic acid - omega 9	31.02±0.98 <sup>B</sup>	25.89±0.10 <sup>C</sup>	66.38±0.13 <sup>A</sup>
(C18:2) Linoleic acid	15.28±0.17 <sup>B</sup>	16.69±0.36 <sup>A</sup>	4.30±0.02 <sup>C</sup>
(C18:3-n3) $\gamma$ -Linolenic acid	-	2.50±0.04 <sup>A</sup>	1.31±0.02 <sup>B</sup>
(C20:0) Arachidic acid	-	1.04±0.03	-
Saturated	49.93±0.00 <sup>A</sup>	50.47±0.27 <sup>A</sup>	25.15±0.21 <sup>B</sup>
Total unsaturated	50.07±1.14 <sup>B</sup>	48.53±0.54 <sup>B</sup>	74.85±0.15 <sup>A</sup>
Monounsaturated	33.54±0.98 <sup>B</sup>	28.7±0.18 <sup>C</sup>	69.02±0.13 <sup>A</sup>
Polyunsaturated	16.53±1.16 <sup>A</sup>	19.83±0.36 <sup>A</sup>	5.84±0.02 <sup>B</sup>
Trans	<0.1±0.0	<0.1±0.0	<0.1±0.0

FMWP - Fermented milk without pulp, FMPF - Fermented milk with passion fruit pulp, FMB - Fermented milk with buriti pulp. Different capital letters in the same row denote a significant difference ( $p < 0.05$ ) among fermented milk samples.

#### 4. Conclusion

The kinetic parameters were influenced by the combination of culture and the presence of fruit pulp, while viability of probiotic cultures in fermented milk was influenced only by the type of culture used. The addition of fruit pulp enriched the final product in terms of bioactive compounds and antioxidant activity. Therefore, buriti and

passion fruit pulps can be considered a great alternative to produce innovative probiotic fermented milk with functional characteristics.

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## CAPITULO IV – ARTIGO ORIGINAL

### **Functional fermented milk with fruit pulps modulates the *in vitro* intestinal microbiota**

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#### **Abstract**

Probiotic fermented milk enriched with fruit pulp has gained space in the market, not only for its pleasant sensory characteristics but also for the beneficial effects it can provide to consumers' health. Therefore, the aim of this work was to evaluate the effect of probiotic fermented milk with buriti (*Mauritia flexuosa*) pulp – FMB or passion fruit (*Passiflora edulis*) pulp – FMPF or without fruit pulp - FMC on the microbiota of healthy human using SHIME<sup>®</sup>, which simulates human gastrointestinal conditions. Fermented milk treatments were administered for 5 days, followed by 5 days of washout. Samples were collected in triplicate from fermented milk and ascending colon vessels on each experimental period (day 4 and day 5) to evaluate the viability of LAB, viability of indicator microorganisms, microbiota composition through 16S rRNA sequencing, short chain fatty acids (SCFA) and ammonium ions. The viability of probiotic LAB in fermented milk was affected by the addition of fruit pulps; however, it meets the minimum required value of  $\geq 6 \log$  CFU/mL for consumption. Regarding the viability of indicator microorganisms in the colon, there was a significant difference only in the genus of *Streptococcus* sp. ( $7.35 \pm 0.18$ ,  $7.28 \pm 0.19$  and  $6.69 \pm 0.03$  CFU/mL) for FMC, FMPF and FMB, respectively. On the other hand, fermented milk added of fruit pulp modulated

the intestinal microbiota, changed the balance of Firmicutes/Bacteroidetes phyla, increasing Actinobacteria and decreasing Proteobacteria phyla. At the genus level, in all treatments the abundance of *Bifidobacterium* sp. increased, and only FMPF and FMB decreased *Enterobacter* and stimulated *Veillonella* genera and *Ruminococcaceae* family in relation to FMC and their respective washout-control. After treatment, the production of acetic acid increased after the administration of all types of fermented milk and it decreased during the washout periods. Additionally, a significant and remarkably high amounts of propionic and butyric acids were produced during the treatment of fermented milk with buriti pulp. All fermented milk treatments decreased the ammonium ions compared to control (stabilization) ( $557.22 \pm 9.74$  mmol/L), however fermented milk with fruit pulps promoted a greater decrease ( $284.89 \pm 6.04$  FMPF;  $378.67 \pm 6.15$  FMB mmol/L). Thus, probiotic fermented milk with fruit pulp can stimulate the growth of beneficial bacteria present in the microbiota of healthy humans, as well as the production of short-chain fatty acids in SHIME<sup>®</sup>.

## 1. Introduction

The functional food property is related to the metabolic or physiological role that the nutrient or non-nutrient has in the growth, development, maintenance, and other normal functions of the human organism (BANWO et al., 2021). For the development of functional foods, milk and dairy products are the main used materials, since they are consumed regularly, have high nutritional value and many health benefits. They present high quality proteins and essential amino acids, are rich in calcium which can prevent osteoporosis, and lipids rich in essential fatty acids, and are also sources of vitamins, among other nutrients (TEIXEIRA et al., 2022).

Among functional dairy products, fermented products are identified as the most important type. The use of probiotic bacteria for fermentation is a strategy to meet consumers' demand for healthy foods. Moreover, probiotic fermented milk (FM) can also modulate the gut microbiota (CHA et al., 2018; VEIGA et al., 2014; CASAROTTI et al., 2021).

Another trend in the development of functional dairy products is to add fruit pulps, which can improve sensory properties, such as taste, aroma, texture, and color, diversifying the products available on the market (CASAROTTI et al., 2018; BARAT and OZCAN, 2018; BORGONOV I et al, 2021). Tropical fruits pulps, especially from the

unconventional ones, contribute to the healthy appeal and, at the same time, increase the nutritional value of the product by the bioactive compounds naturally present in it. Many of these bioactive compounds have antioxidant properties, which can contribute to increasing the shelf-life of products and are related to the low incidence of certain diseases (ALMEIDA et al., 2011; MAQSOOD et al., 2020; COMAN et al., 2019), through the modulation of the intestinal microbiota.

Initially, studies focused on the effect of biocompounds on pathogenic bacteria present in the microbiota, however, in recent years research has focused on evaluating the effect of polyphenols (PP), including fruits rich in bioactive compounds, on the modulation of the intestinal microbiota (COMAN et al. 2018; TABASHUM et al., 2019; AHMAD et al., 2020; ZHANG et al., 2022). The balance that these biocompounds provide (stimulating beneficial bacteria and decreasing pathogenic ones) on Firmicutes/Bacteroidetes ratio has been mainly evaluated (CHENG et al., 2020; ESPÍN, GONZÁLEZ-SARRÍAS and TOMÁS-BARBERÁN, 2017; HENNING et al., 2017; PIEKARSKA-RADZIK and KLEWICKA, 2021). Probiotics are also known to have a modulating effect on the intestinal microbiota (ALKUSHI et al., 2022; GEBRAYEL et al., 2022) and it is also known that dairy products, mainly probiotic FM, can act as coadjuvants to improve the function of the gastrointestinal barrier and as immunomodulators to promote gastrointestinal health (ANDRADE et al. al., 2015; HO et al., 2022; REN et al., 2022). However, research to evaluate the modulating effect of probiotic FM on the intestinal microbiota has gained prominence in recent years (CASAROTTI et al., 2021; HO et al., 2022; ROY et al., 2022), although studies aimed at better understand the modulation are still necessary, especially for FM added with fruit pulp.

Therefore, the aim of this study was to evaluate the effect of probiotic FM added with fruit pulp on the intestinal microbiota by means of an *in vitro* test using Simulator of Human Intestinal Microbial Ecosystem (SHIME®).

## **2. Materials and Methods**

### **2.1 Preparation of fermented milk**

To evaluate the effect of the fruit pulps on gut microbiota, 3 treatments were prepared: FM without pulp (control - FMC), FM with 1% passion fruit pulp (FMPF) and FM with 1% buriti pulp (FMB). The reconstituted milk with or without fruit pulp,

according to the experiment, was heated to 90 °C for 10 min., using a food processor (Thermomixer TM 31, Cloyes-sur-le-Loir, FR) and cooled to the inoculation temperature (37 °C) and distributed into sterilized bottles. The cultures were activated and inoculated in the treatment samples, in the proportion of 2% for both strains *Lacticaseibacillus casei* SJRP38 and *Lacticaseibacillus plantarum* ST8Sh and 0.1% for *Streptococcus thermophilus* TA 080. For fermentation, the bottles were incubated in a water bath (EVLAB, Londrina, Brazil) at 42 °C until reaching the pH 4.6. After the fermentation was completed, the product was cooled and the curd was manually broken, in a standardized way for all treatments, followed by final cooling to 4 °C and later stored, as described by Borgonovi et al. (2021).

## 2.2 Lactic acid bacteria viability in fermented milk

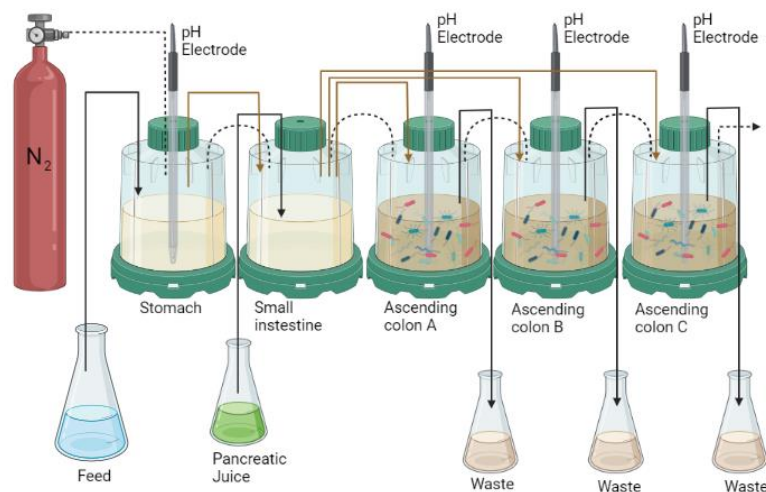
For the analysis of lactic acid bacteria (LAB) viability in FM, a 100 µL aliquot of milk fermented was transferred to a tube containing 900 µL of 0.85% saline solution. The mixture was stirred, and this suspension was submitted to serial decimal dilutions to 10<sup>-8</sup> using the same diluent. For counting, 10 µL of each dilution was transferred to Petri dishes according to Solieri et al. (2014) with modifications. *Streptococcus* spp. were counted using M17 agar (Difco, Laboratories, Detroit, MI, USA) in aerobic conditions at 50 °C, *L. casei* was counted in MRS agar (Difco) at 45 °C in anaerobiose conditions and *L. plantarum* was counted in MRS agar (Difco) at 15 °C in anaerobiose conditions, both after incubation for 48 h. The incubation temperature of *L. casei* and *L. plantarum* were defined after preliminary tests in which different incubation temperatures (15 °C, 25 °C, 30 °C, 37 °C, 45 °C, 50 °C, 55 °C) were evaluated.

## 2.3 Simulator of Human Intestinal Microbial Ecosystem (SHIME®)

The SHIME® model was used to simulate the human digestion process. It is a computer-controlled system and consists of 5 compartments representing the stomach, small intestine, and ascending colon; a schematic description of SHIME® system is presented in Figure 1 (MOLLY et al., 1994). In this experiment, SHIME® was adapted for this study and the transverse and descending colon were replaced by the triplicate of ascending colon, according to Salgaço et al. (2021).

### 2.3.1 Composition of the SHIME® feed medium

The feed medium used in SHIME<sup>®</sup> was prepared in distilled water, consisting of 3 g/L starch (Maizena<sup>®</sup>, Unilever Brazil, SP, BR), 2 g/L pectin (Sigma-Aldrich, MO, US), 4 g/L of gastric mucin type II swine (Sigma-Aldrich), 1 g/L xylan (Megazyme, Bray, IE), 1 g/L peptone (Kasvi, PR, BR), 1 g/L of arabinogalactan (Sigma-Aldrich), 0.4 g/L glucose (Synth, SP, BR), 3 g/L yeast extract (Kasvi, PR, BR) and 0.5 g/L of L-cysteine (Sigma-Aldrich) (POSSEMIERS et al., 2004). In order to reach the duodenum conditions, in the second reactor was add an artificial pancreatic juice composed of 12.5 g/L sodium bicarbonate (LS Chemicals, Maharashtra, India), 6 g/L ox-bile (Sigma-Aldrich) and 0.9 g/L pancreatin (Sigma-Aldrich) (POSSEMIERS et al., 2004). This carbohydrate-based medium presents an important role on the environmental adaptation, and inoculum growth with the formation of a stable and representative community (MOLLY et al., 1994).



**Figure 1.** SHIME<sup>®</sup> system and conditions of each reactor. R1: stomach - pH 2.0-2.5; R2: small intestine - pH 4.3-4.8; R3.1: ascending colon - pH 5.6-5.9; R3.2: ascending colon - pH 5.6-5.9; R3.3: ascending colon - pH 5.6-5.9.

### 2.3.2 Microbiota colonization

The SHIME<sup>®</sup> reactors were colonized with feces from healthy male volunteers, with ages between 18 and 22 years old. All donors had not taken antibiotics within a period of six months prior to the study and had not consumed probiotic products over the past 3 to 6 months. The stool inoculum was prepared according to the procedures described by Duque et al. (2016), adapted from Possemiers et al. (2010). The samples (40 g) were collected on 3 different days, homogenized, and diluted in 200 mL of phosphate buffer (pH 6.5), composed of 7.08 g/L of monosodium phosphate (Synth, Diadema-SP,

Brazil), 5.98 g/L of disodium phosphate (Synth) and 1 g/L of sodium thioglycolate (Merck, DE). This mixture was homogenized in stomacher and centrifuged at 3,000 x g for 15 minutes. The supernatant was collected, and 10 mL added to the last three compartments (Figure 1) along with 500 mL of sterile feed medium. For stabilization, the three compartments were added of the feed medium (240 mL) and pancreatic juice (60 mL) in SHIME<sup>®</sup> system for 14 days before the experiments, according to Possemiers et al. (2004) and Van de Wiele et al. (2004).

### 2.3.3 Experimental protocol

After 14 days of stabilization of human microbiota, the FM treatments (FMC or FMPFP or FMBP) were administered for 5 days, followed by 5 days washout, as described by Duque et al. (2016). The treatments were consisted of 80 g of FM and 240 mL of feed medium. During washout period only the feed medium (240 mL) and pancreatic juice (60 mL) were added. After the treatment and washout of FMC, the other treatments (FMPF and FMB) were added separately, under the same conditions. The volumetric capacity, pH, temperature (37 °C) and retention time (24 hours) was controlled in the last three compartments (POSSEMIERS et al., 2004) and stirred with a magnetic stirrer throughout the whole process. The anaerobiosis of the system was achieved with the addition of nitrogen and the pH corrected in each vessel with hydrochloric acid or sodium hydroxide, reaching the pH range of 5.6-5.9 (MOLLY et al., 1994; POSSEMIERS et al., 2004). Samples were collected in triplicate from the ascending colon (last 3 reactors) on two different days. In the stabilization period, samples were collected on 13th and 14th day. During treatment administration (FMC, FMPF and FMB) and washout period samples were collected on 4th and 5th day.

### 2.4 Production of metabolites in SHIME<sup>®</sup>

The short-chain fatty acid (SCFA) levels and ammonium ions were determined from samples collected from reactors representing the ascending colon and frozen at -20 °C. This analysis was performed according to Espírito Santo et al. (2012) and Adorno, Hirasawa and Varesche (2014). Briefly, the fatty acids were extracted with diethyl ether and analyzed in a gas chromatograph equipped with a flame ionization detector. For ammonium ions ( $NH_4^+$ ), after the digestion process in the SHIME<sup>®</sup> simulator, the samples were collected and quantified using a specific ion meter (Model 710A, Orion) coupled to

a selective ammonia ion electrode (Model 95-12, Orion), according to Bianchi et al. (2014).

## 2.5 Viability of indicator microorganisms

The counts of colony forming units (log CFU/mL) of *Lactobacilli*, *Streptococcus* spp., *Clostridium* spp., *Bifidobacterium* spp. and total anaerobic bacteria were determined at the beginning and end of each treatment, using pour plate method and serial dilutions of samples (2 g) in sterile 0.85% saline solution on selective culture media, as described by Bianchi et al. (2014).

Numbers of total anaerobic counts was determined by plating on Standard Methods agar (Acumedia, Indaiatuba, SP, Brazil) and incubation at 37 °C/48 h. MRS agar (Difco) and incubation at 37 °C/48 h, anaerobically, was used to determine the number of *Lactobacilli*, *Streptococcus* spp. were enumerated aerobically using M17 agar (Difco) and incubation at 37 °C/48 h. *Bifidobacterium* spp. was enumerated anaerobically using formulated BIM-25 medium (RCA agar, nalidixic acid, polymyxin B sulfate, kanamycin sulfate, iodoacetic acid and 2,3,5 – triphenyl tetrazolium chloride) and incubation at 37 °C/72 h. RCA and incubation at 37 °C/48 h, anaerobically, was used to determine the population of *Clostridium* spp. Anaerobic incubation of plates was performed in jars with an atmosphere adjusted by Anaerobac (Probac, São Paulo, Brazil).

## 2.6 Microbial DNA extraction and 16S rRNA gene-based Illumina MiSeq sequencing

The determination of the composition of the intestinal microbiota collected from SHIME® was performed using Next-generation Sequencing by Neopropecta Microbiome Technologies (Florianópolis, SC, Brazil). The V3–V4 hypervariable region of the 16S rRNA gene was amplified using specific primers. The data was organized by microbiota phyla, families, genus and species, according to Casarotti et al. (2020).

Initially, the DNA was extracted from 2 mL of samples collected (described in item 2.3.3) in the reactors which represent the ascending colon in duplicate (42 samples), using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol, and visualized by agarose gel electrophoresis 1% stained with SYBR® Safe (Invitrogen). Following, the DNA quantification was made (Qubit® 3.0 Fluorometer - Life Technologies) based on the ratio 260/280 and verification of the quality of the DNAs of the samples, using the Nanodrop ND-1000 spectrophotometer

(Nanodrop Technologies, Wilmington, USA). The libraries were sequenced using the equipment MiSeq Sequencing System (Illumina Inc., USA),

## 2.7 Bioinformatics and Statistical analysis

The analyses of viability of bacteria in the FM as well as ammonium, SCFA and viability of bacteria in the reactors representing the ascending colon in the SHIME® were carried out by analysis of Variance (ANOVA) followed by a Tukey test was used compare the treatments. A significance level of 5% probability and Minitab 16 software were adopted.

For the microbiota composition analysis using 16S rRNA gene sequencing data, the sequencing libraries were prepared according to Neoprosecta® Microbiome Technologies, and the 16S rRNA gene sequencing analyses, relative abundance and beta diversity indices were performed using the R program (v. 3.4.4). Beta diversity was estimated after normalization by centered log-ratio using the DESeq2 R package (v. 1.18.1). After normalization, a principal coordinate analysis (PCoA), based on distance matrices Unifrac and Bray-Curtis was performed using Phyloseq software (v. 1.22.3). Alpha diversity and relative abundance were tested using the Kruskal–Wallis test. For the comparison among treatments (FMC, FMPF and FMB), the test Wilcoxon was used.

## 3. Results and Discussion

### 3.1 LAB viability in fermented milk

A positive effect of fruit pulps towards LAB in FM was not observed. The population of *L. casei* in the FM with pulp fruits was lower than in FMC (Table 1). Similar result was observed by Borgonovi et al. (2021), in which synbiotic relation between fruit pulps and the LAB in FM with pulp fruit was not observed. However, Espírito-Santo et al. (2010) showed the synergistic effect between probiotic strains (*L. acidophilus* L10, *B. animalis* ssp. *lactis* B104 and *B. longum* B105) and açai pulp. The beneficial effect of fruit pulps on the viability of LAB has been ascribed to the presence of bioactive compounds such as phenolic compounds, carotenoids and organic acids, fibers, such as fructooligosaccharides (FOS) found in fruits (OZCAN et al., 2015; PERRICONE et al., 2015; BARAT and OZCAN, 2018).

The viability of *L. plantarum* ST8Sh in FM was not affected by the type of fruit and the difference was little compared to the control, while the viability of *S. thermophilus*

TA080 was not affected by the addition of pulp fruit in FM. These differences in the growth of *L. plantarum* ST8Sh and *S. thermophilus* TA080 in FM, can be explained due to compounds present in milk that can stimulate or inhibit the viability of these strains. Additionally, the total solids present in the FM can alter the viability of the LAB strains.

Table 1. Viability (Log CFU/mL) of FM applied in SHIME®.

Log CFU/mL	<i>L. casei</i>	<i>L. plantarum</i>	<i>S. thermophilus</i>
FMC	9.95±0.00 A	9.87±0.04 A	3.45±0.21 A
FMPF	9.59±0.02 B	9.43±0.09 B	3.74±0.06 A
FMB	8.69±0.01 C	9.56±0.03 B	3.78±0.00 A

FMC - Fermented milk control, without pulp, FMPF - Fermented milk with passion fruit pulp, FMB - Fermented milk with buriti pulp. Different capital letters in the same column denote a significant difference ( $p < 0.05$ ) among fermented milk samples.

Despite the lower viability of LAB in FMPF and FMB, the total count of probiotic in all FM treatments meets the minimum required value of  $\geq 6$  log CFU/mL for consumption (BARAT and OZCAN, 2018). The fruit pulp present in probiotic fermented food is desirable since they may protect the probiotics from adverse conditions found in the human GI tract and may also present prebiotic potential, acting in synergy with probiotics in the human intestines after their ingestion (CASAROTTI et al., 2018).

### 3.2 Viability of indicator microorganisms in the SHIME®

The viability of the main genera present in the colon was not affected by the treatments (FMC, FMPF and FMB) of the experimental protocol; however, there was a small reduction only in *Streptococcus* sp. in the FMB (Table 2) which is a common bacterium present in the human microbiota (as we can see in the stabilization period). Even though the product has a low *Streptococcus* spp. count, there was greater viability in the intestinal microbiota. In addition, FMB reduced the counts of *Clostridium* spp. Koolen et al. (2013) reported antimicrobial activity of buriti towards pathogens species. There are no reports in the literature that some compounds present in buriti pulp can specifically inhibit *Streptococcus* spp.

Table 2. The counts of colony forming units (log CFU/mL) of *Lactobacilli*, *Streptococcus* spp., *Bifidobacterium* spp., *Clostridium* spp. and total anaerobic bacteria.

	<i>Lactobacilli</i>	<i>Streptococcus</i> spp.	<i>Bifidobacterium</i> spp.	<i>Clostridium</i> spp.	Total anaerobic bacteria
Stabilization	7.13±0.49 <sup>a</sup>	7.02±0.13 <sup>a</sup>	7.24±0.31 <sup>a</sup>	8.03±0.16 <sup>a</sup>	9.07±0.65 <sup>a</sup>
FMC	7.38±0.36 <sup>Aa</sup>	7.35±0.18 <sup>Aa</sup>	8.07±0.16 <sup>Aa</sup>	8.21±0.18 <sup>Aa</sup>	8.94±1.15 <sup>Aa</sup>
Washout FMC	7.02±0.61 <sup>a</sup>	7.28±0.46 <sup>a</sup>	7.53±0.56 <sup>a</sup>	7.75±0.44 <sup>a</sup>	8.80±0.92 <sup>a</sup>
FMPF	7.12±0.37 <sup>Aa</sup>	7.28±0.19 <sup>Aa</sup>	8.00±0.04 <sup>Aa</sup>	7.63±0.76 <sup>Aa</sup>	8.29±0.56 <sup>Aa</sup>
Washout FMPF	7.51±0.05 <sup>a</sup>	6.46±0.85 <sup>a</sup>	7.40±1.00 <sup>a</sup>	7.76±0.54 <sup>a</sup>	8.52±0.94 <sup>a</sup>
FMB	7.38±0.75 <sup>Aa</sup>	6.69±0.03 <sup>Ba</sup>	7.79±0.41 <sup>Aa</sup>	6.94±0.74 <sup>Aa</sup>	8.17±0.72 <sup>Aa</sup>
Washout FMB	7.00±0.18 <sup>a</sup>	7.21±0.40 <sup>a</sup>	7.32±0.31 <sup>a</sup>	7.12±0.80 <sup>a</sup>	8.07±0.60 <sup>a</sup>

Different capital letters in the same column denote a significant difference ( $p < 0.05$ ) among ferment milk samples.

Different lower-case letters in the same column denote a significant difference ( $p < 0.05$ ) during the experimental periods.

In contrast, according to Borgonovi et al., (2021) LAB such as *Lactobacillus* spp. and *S. thermophilus* are not inhibited in the presence of buriti pulp. Therefore, further studies aiming at elucidating which compounds are present in buriti pulp may have a bactericidal effect and which mechanisms of inhibition of these compounds are necessary to decipher possible causes of inhibition of the *Streptococcus* spp.

### 3.3 Microbiota composition evaluation by gene sequencing

SHIME® has already been proven to be a useful model for nutrition studies and has been used for long period since it can predict the in vivo response of the dietary modulation on microbial community.

The microbiota composition at phylum level in the present study showed differences among the FM samples. The abundance of Firmicutes and Bacteroidetes were 45.7 % in FMC, 71.1% in FMPF and 60.1 % in FMB (Figure 2). Treatments FMPF and FMB increased Firmicutes (F) and Bacteroidetes (B), compared to its respective washout-control and all treatments increased Actinobacteria and decrease Proteobacteria phyla (Figure 2). The microbiota modulation and F/B ratio can be influenced due to the administration of prebiotics, the ability of bacteria to metabolize non-digestible carbohydrates such as cellulose, hemicelluloses, resistant starch, pectin, oligosaccharides, and lignin into SCFAs, such as acetic, propionic and butyric acids (GOMAA, 2020) that can be used as an energy source by some specific microorganisms. Additionally, the administration of probiotics can reduce the lumen pH or produce bacteriocin like substances. *L. plantarum* ST8Sh used in this study is producer of bacteriocin like substance (TODOROV et al., 2017). Moreover, competition for nutrients can occur, which may favor the growth of specific microorganisms (RODRIGUES et al., 2020).

Similar results were obtained by Rodrigues et al. (2020) who administered a food supplement or ice cream containing probiotics (*L. acidophilus* and *B. animalis*) for 14 days on SHIME®. Both matrices modulated the intestinal microbiota, increasing beneficial microorganisms such as *Bifidobacterium* spp., *Bacteroides* spp., *Faecalibacterium* spp. and *Lactobacillus* spp. However, the ice cream administration resulted in better effects on the microbiota due to the greater amount of acetate, propionate, butyrate ions produced.

The increase in Firmicutes phylum observed in the present study is desirable because this increase has been associated with increased fiber consumption (BEISNER

et al., 2020) and decrease the occurrence of inflammatory diseases (YAO et al., 2020). Firmicutes and Bacteroidetes, when present in an adequate proportion, may be related to the greater ability of these phyla to degrade carbohydrates in the colon (FLINT et al., 2012). It has already been reported that Bacteroidetes species, such as *Bacteroides fragilis*, can be used as a probiotic to treat behavioral symptoms of autism (HSIAO et al., 2013). Some *Bacteroides* spp. are considered promising probiotics known as next-generation probiotics aimed at decreasing the risk of occurrence of diseases such as cancer, intestinal inflammation, and heart disease, among others (O'TOOLE, MARCHESI and HILL, 2017).

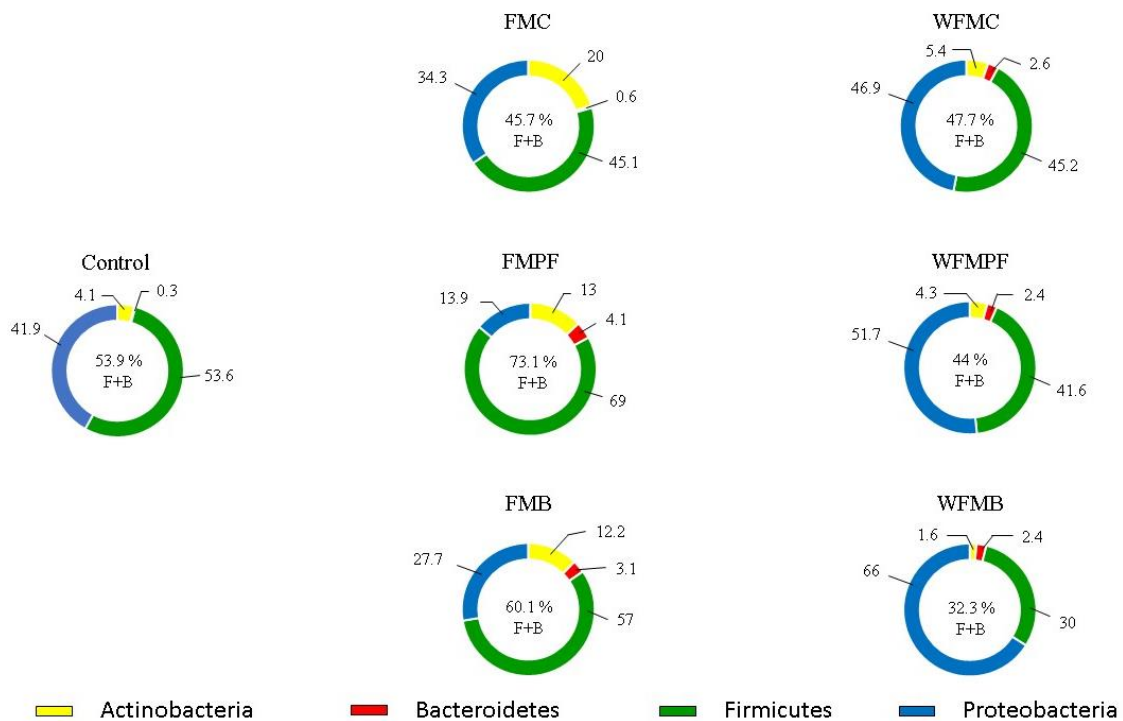


Figure 2. Microbiota composition in the reactors representing the ascending colon of SHIME® at phylum level. Control – microbiota control; FMC – Fermented milk control (without fruit pulp); FMPF – Fermented milk with passion fruit; FMB – Fermented milk with buriti; WPMC – Washout Fermented milk control (without fruit pulp); WFMPF – Washout Fermented milk with passion fruit; WFMB – Washout Fermented milk with buriti.

The increase in the Actinobacteria phylum is another positive outcome observed during the administration of FM. This phylum is related to a healthy microbiota, since it harbors the main bacteria that play a protective role in maintaining intestinal barrier homeostasis (PURCHIARONI et al., 2013), such as acetate-producing *Bifidobacterium* (BINDA et al., 2018). The human intestine does not have many enzymes necessary for the biotransformation of nutrients ingested daily. Most of these enzymes are produced by commensal bacteria located in the colon and belonging to the phylum Actinobacteria such as *Bifidobacterium* and others genus such as *Bacteroides*, *Ruminococcus* and *Roseburia* (BINDA et al., 2018). Actinobacteria are also involved in the biodegradation of resistant starch, in particular the *Bifidobacteria* that hydrolyze the glycosidic bond between two or more sugars and cooperate in the breakdown of carbohydrates derived from starch and polysaccharides, such as fructooligosaccharides, galactooligosaccharides (GOS) and xylooligosaccharides, inulin or arabinoxylan, as well as transforming linoleic acid into conjugated linoleic acids, which can promote health, preventing cancer, atherosclerosis, diabetes, obesity and aids in immune functions (KIM et al., 2016; BINDA et al., 2018).

On the other hand, the decrease in the Proteobacteria phylum is desirable, since this phylum is related to the incidence of inflammatory diseases (AZIMIRAD et al., 2022) and are known for their proteolytic activity which includes the genus *Clostridium* and *Bacillus* (RETTEDAL et al., 2019). In the present study, the decrease in Proteobacteria may be related to the lower availability of amino acids that may have been metabolized by LAB that have proteolytic activity during the fermentation process of FM. Oddi et al. (2020) also reported modulation of the intestinal microbiota with reduction of Proteobacteria, mainly the bacteria from the family *Enterobacteriaceae*, and from genera *Escherichia*, *Shigella*, and *Clostridium\_sensu\_stricto\_1*, when probiotic strains *L. plantarum* 73a alone or in combination with *B. animalis* subsp. *lactis* INL1 were administered in SHIME®.

Considering the increase in Firmicutes and Actinobacteria phyla related to eubiotic microbiota and decrease in the Proteobacteria phylum, we consider that this microbiota remained in homeostasis during the administration of FM. In addition, the initial microbiota (control) was composed of Firmicutes (53.6 %), Proteobacteria (41.9 %), and minimal proportion of Actinobacteria (4.1 %) while is desirable that Firmicutes and Bacteroidetes compose 85-90% of the total microbiota and, in lower abundance, Actinobacteria, Proteobacteria and Verrucomicrobia (CANI, MOENS DE HASE, and VAN HUL, 2021), different from the microbiota observed during and after the

administration of FM. We know that intestinal microbiota homeostasis is important for the host's immune system. The intestinal microbiota plays an important role in the maintenance of intestinal barrier functions, acting in the expression of tight junctions, regulating mucin biosynthesis and catabolism, providing energy for the proliferation of epithelial cells (BINDA et al., 2018).

The composition of the microbiota at the genus level showed that the abundance of *Bifidobacterium* genus increased in all treatments of FM and was higher in FMC when compared to FMPF and FMB (Figure 3). This increased can be related to the ability of *Bifidobacteria* to metabolize carbohydrates such as GOS produced from lactose through the transgalactosylation activity of the  $\beta$ -galactosidase enzyme. GOS degradation occurs more effective if associated with probiotic cultures, forming a synergistic combination with some species of *Bifidobacteria* and *Lactobacillus* (MARTINS and BURKET, 2009).

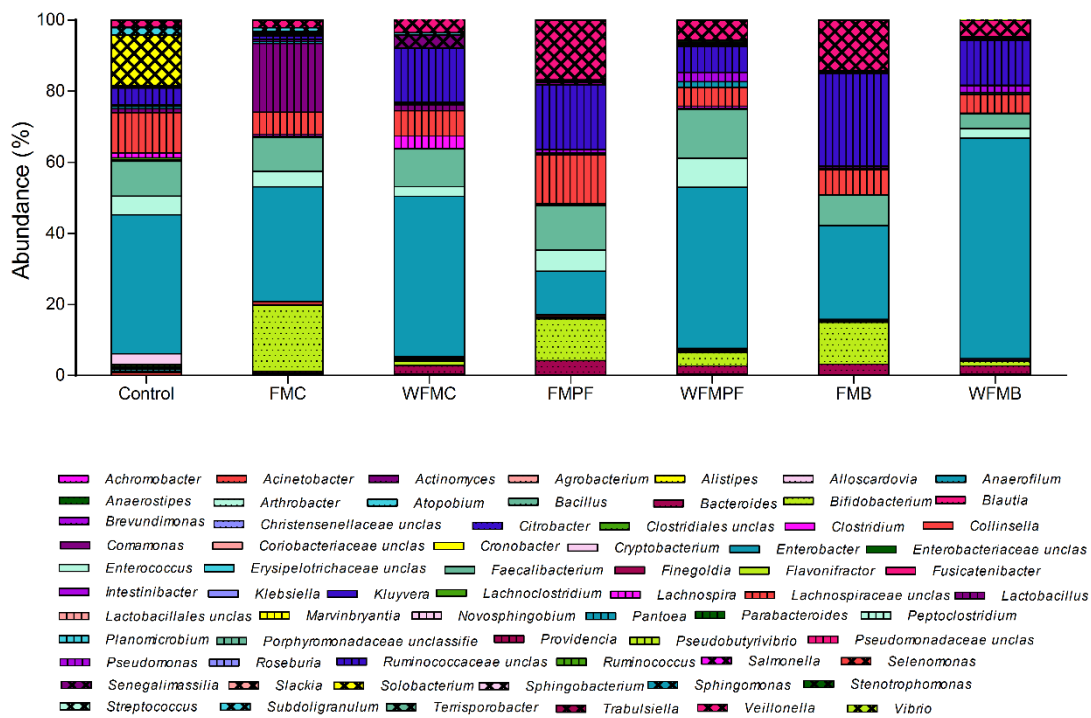


Figure 3 – Microbiota composition in the reactors representing the ascending colon of SHIME<sup>®</sup> at genus level. Control – microbiota control; FMC – Fermented milk control (without fruit pulp); WFMC – Washout Fermented milk control (without fruit pulp); FMPF – Fermented milk with passion fruit; WFMPF – Washout Fermented milk with passion fruit; FMB – Fermented milk with buriti; WFMB – Washout Fermented milk with buriti.

The increase of *Bifidobacterium* in the gut microbiota is desired not only for its probiotic characteristics, but also for its production of health-promoting SCFA (WANG et al., 2021). *Bifidobacterium* (*B. longum* NCC2705) produces acetate that serves as a co-substrate for other bacteria to produce butyrate (BINDA et al., 2018) or may have an effect against enteropathogenic infections such as enterohemorrhagic *Escherichia coli* and *Shigella* (FUKUDA et al., 2012); it also has the ability to produce lactate which can be used by “lactate-using” bacteria for the production of butyrate (BINDA et al., 2018). In addition, *Bifidobacterium* may have the ability to co and self-aggregate, resistance to pH and bile salts, adherence to hydrocarbons, susceptibility to antibiotics and the ability to produce exopolysaccharides (NAMI et al., 2019). Some strains have antimicrobial activity against pathogens such as *E. coli*, helping in intestinal dysbiosis, in addition to having properties for preventing diarrhea, anticancer and antitumor and modulating the immune system. (BINDA et al., 2018; AZAD et al., 2018).

FMF and FMB decreased *Enterobacter* and stimulated *Veillonella* genus and *Ruminococcaceae* family in relation to FMC and their respective washout-control (Figure 3). The *Faecalibacterium* increased in all washout periods after treatments administration (Figure 3).

*Enterobacter* genus comprises commensal bacteria species of the human intestine, which can live in a symbiotic state in the microbiota. However, the reduction of the abundance of this genus is desired, since it can cause damage mainly in immunosuppressed people (ECKBURG et al., 2005). Although *Enterobacter* decreased in all treatments during the period of FM administration, there was an increase of them when the treatments ended, showing that it is desirable the daily consumption of FM in order to have a beneficial effect on the modulation of the intestinal microbiota.

The increase of abundance of *Veillonella* genus and *Ruminococcaceae* family, which belong to the *Firmicutes* phylum, is considered a good result since there is evidence that bacteria from *Ruminococcaceae* family are abundant in the fecal bacteria of healthy adults and *Veillonella* genus is effective in preventing the development of asthma (ARRIETA et al., 2015, TAP et al., 2017). *Veillonella* spp. are also associated with the production of carbohydrate metabolizing enzymes (PEDROGO et al., 2018) and consumer of lactate, that can be form a trophic chain with *Streptococcus* spp. capable of converting lactate to propionate (BRASILI et al., 2019).

*Veillonella* and *Ruminococcaceae* have several beneficial functions to the human body, including the reduction of intestinal inflammatory pathologies, as these microorganisms as well as the *Lachnospiraceae* family have a common characteristic, which is the ability to generate SCFA, mainly butyrate, from the fermentation of non-digestible plant fibers (BRASIL I et al., 2019).

The increase in *Faecalibacterium* genus during washout periods is considered an important output showing that after ingestion of FM for 5 days, it may still have a positive residual effect on *Faecalibacterium*. This genus is a butyrate producer, which have anti-inflammatory properties (LOPEZ-SILES et al., 2017), and due to its beneficial properties, some studies focused on confirming the use of *F. prausnitzii* as a probiotic (LOPEZ-SILES et al., 2017). In contrast, the decrease in this genus may be related to some diseases such as: inflammatory bowel disease, Crohn's disease, colorectal colitis and irritable bowel syndrome (ZHANG et al., 2014).

We believe that the introduction of a probiotic dairy products with beneficial characteristics of intestinal microbiota modulation such as our product can add value to the products on the market, as the gut dysbiosis may influence host physiology, metabolism, immunity, and inflammation (HUL et al., 2020). Several factors can influence the microbiota composition, such as individual's diet, physicochemical gut properties, host age, antibiotic use, physical and psychological stress, host genetic factors and immunosenescence can also affect the microbiota (COMAN and VODNAR, 2020; CANI, MOENS DE HASE, and VAN HUL, 2021). Furthermore, the gut microbiota diversity and density distribution evolve complex mechanisms to restrain pathogen growth (KHAN et al., 2021).

Probiotic microorganisms are mainly carried by dairy products such as FM and cheese, although plant-based probiotic products are becoming more popular as dairy alternatives (RASIKA et al., 2021). The consumption of probiotics, prebiotics and symbiotics has been potentially related to improvements in intestinal microbiota. Such modulation may occur through the activation of nuclear receptor proteins, regulation of cytokines activity, or production of SCFAs, which are indicative of intestinal health (SILVA et al., 2020). Moreover, the mechanisms of action of probiotics includes modulation of the immune system, interaction of gut microbiota, production of organic acids, colonization resistance, improvement of barrier function, interaction with host mediated by cell surface structures, production of small molecules and enzymes (SANDERS et al., 2019). The modulation of the intestinal microbiota by the consumption

of probiotics, mainly *Lactobacillus*, is already demonstrated in the literature (ZHAO et al., 2020; ZHOU et al., 2022).

It has been suggested that fermentation can alter the availability of nutrients and bioactive compounds, including the formation of bioactive peptides, which can modulate the microbiota (RETTEDAL et al., 2019) and nowadays the consumption of probiotic fermented foods can also be associated with improvements in mood, cognitive function, anxiety, and depression (TREMBLAY et al., 2021). A range of bioactive compounds can potentially be produced during LAB fermentation processes, with potential health benefits, such as angiotensin converting enzyme (ACE)-inhibitory peptides, which display anti-hypertensive characteristics or peptides with immunomodulatory effects by the increased production of the anti-inflammatory cytokine IL-10, among others (MATHUR et al., 2020). For instance, bioactive peptides from simulated gastrointestinal digestion of Parmigiano Reggiano cheese supported the growth of most lactobacilli and bifidobacteria present in human colonic microbiota (BOTTARI et al., 2017). Other milk peptides, such as lactoferrin, are known to exert anti-inflammatory and immunostimulatory effects and whey proteins also increased the counts of lactic acid bacteria and bifidobacteria (BIELECKA et al., 2022).

Rettedal et al. (2019) evaluated the effect of different types of milk (cow's milk, fermented cow's milk, sheep's milk, fermented sheep's milk) *in vivo* (mice) on the intestinal microbiota. The fermented or non-FM of the two species can modulate the intestinal microbiota in different ways. The authors observed an increase in *Anaerostipes* and *Eubacterium* or a decrease in *Enterobacteriaceae* in fermented milk compared to unfermented milk and Proteobacteria, Bacteroidetes and Parabacteroides was stimulated in mice fed with fermented milk.

The *Lactobacillaceae* family has the ability to adhere, competitive adhesion, self and co-aggregation in the intestine, and can prevent the colonization and elimination of pathogenic bacteria in the gastrointestinal tract (ZAWISTOWSKA-ROJEK et al., 2022), consequently increasing relative abundance of beneficial bacteria and protection against intestinal infection (VEMURI et al., 2018) through the metabolism of dietary fiber, polysaccharides, oligosaccharides and phenolic compounds (LEAL et al., 2017). *Lactobacillus* can metabolize sugars and other carbohydrates (SONG et al., 2021), with production of lactic acid as the primary end product (HANCHI et al., 2018).

Administration for 3 weeks of probiotic FM (*L. acidophilus* CSG, *Lactobacillus brevis* HY7401, *Bifidobacterium longum* HY8001, *L. casei* HY2782 e *S. thermophilus* +

dietary fiber + lactulose) increased the population of Bacteroidetes, due to a possible reduction in lumen pH, bacteriocin production and nutrient competition (UNNO et al., 2015).

The fruit pulps used in this study are rich in phenolic compounds, as shown by Borgonovi et al. (2021). Therefore, we can hypothesize that these compounds may be stimulating the beneficial microorganisms of the microbiota, since they are considered prebiotics. The prebiotics are non-digestible ingredients that can be fermented in the colon, stimulating the growth of beneficial bacteria and altering the gut microbiota in favor of a healthier composition (GIBSON, ROBERFROID, 1995). In addition, studies indicate that polyphenols and their metabolites can modulate the intestinal microbiota by stimulating beneficial microbiota and inhibiting pathogenic bacteria proliferation (CORRÊA, ROGERO, HASSIMOTTO, and LAJOLO, 2019, PAP et al., 2021).

In addition to stimulating probiotics, some studies available in the literature have shown that polyphenols present in fruits can be metabolized by the resident microbiota, leading to the production of biologically active metabolites (VAN DUYNHOVEN et al., 2011). These metabolites can stimulate the growth of LAB and shape the gut microbiota increasing the beneficial bacteria and decreasing the pathogenic ones (AHMAD et al., 2020; BURGOS-EDWARDS et al., 2020; ZHANG et al., 2022). As example, Ma and Chen (2020) showed that polyphenol supplementation increases the growth of *Lactobacillus* and *Bifidobacterium*, while reducing the number of pathogenic species of *Clostridium*, like *Clostridium perfringens*.

Digestion of biocompounds begins in the mouth, where the flavonoids are converted into aglycones and in later into other bioactive compounds to be absolved. In the stomach, oligomeric polyphenols are transformed into monomeric units (METERE and GIACOMELLI, 2017; SORRENTI et al., 2020) and the remaining glycosides, esters, and polymers (inactive polyphenols) are hydrolyzed by small intestinal enzymes or reach the colon and are metabolized by the intestinal microbiota (KAY et al., 2004; D'ARCHIVIO et al., 2010) to be transformed into active compounds after sugar removal by the gut microbiota (MARIN et al., 2015; ADAK and KHAN, 2019). Polyphenols can also detoxify intestinal metabolites and suppress the growth of pathogenic bacteria such as *Clostridium perfringens*, *Clostridium difficile* and *Bacteroides* spp. (NICHOLSON et al., 2012; ADAK and KHAN, 2019) which may explain the decrease in *Enterococcus* in FMB (Figure 3). Among the intestinal microbiota, bacteria from the *Lactobacillaceae* family present the highest ability to tolerate or degrade polyphenolic compounds,

especially *Lactiplantibacillus plantarum*, due to its enzymatic machinery to hydrolyze the polyphenols (PIEKARSKA-RADZIK and KLEWICKA, 2021).

The prebiotic effects and selective antimicrobial action against pathogenic gut microbes of these compounds on the intestinal microbiota are related to the bioavailability of polyphenols, chemical structures and concentration, host-related factors, interaction with other nutrients and food matrix (SINGH et al., 2019; SORRENTI et al., 2020).

Buriti and passion fruit are rich in flavonoids such as orientin and quercetin. These compounds are also present in other fruits such as cocoa. Sorrenti et al. (2020) showed that the ingestion of the cocoa fruit which contains flavanols, flavones like orientin, flavanones, flavonols like quercetin and anthocyanidins and their metabolites shape the intestinal microbial population, stimulating the *Lactobacillus* and *Bifidobacterium* and reduce the abundance of pathogenic microorganisms, such as certain species of the genus *Clostridium*. Therefore, microbiota modulation may be related to three factors present in probiotic FM added to fruit pulp: matrix, probiotic and biocompounds present in the pulps.

Unifrac and Bray-Curtis index presented similar results. The administration of FMC or FM with fruit pulp altered the microbiota community in relation to the initial microbiota (stabilization) (Figure 4). The PCoA graphic analysis of the Unifrac and Bray-Curtis dissimilarity between treatments show that all FM modulated the microbiota (Figure 4): the FMC samples were significantly displaced from FMPF and FMB samples. On the other hand, the FMPF and FMB are grouped, showing no difference in the modulation of the microbiota.

The FMC washout group showed less modulation, as they are clustered close to the stabilization (initial microbiota). On the other hand, the FMPF and FMB washout groups modulated the initial microbiota in a more prolonged way, since they are grouped farther from the control group. Casarotti et al. (2020) showed that probiotic goat milk added with passion fruit by-products modulate the microbiota of obese adults, due to a balance in abundance between bacterial genus that may or may not produce SCFA, and between bacterial genus with high or low proteolytic activity.

On the other hand, Rettedal et al. (2019) did not observe a notable separation among the treatment groups (cow's milk, fermented cow's milk, sheep's milk, fermented sheep's milk) in the Bray-Curtis principal component analysis (PCA) plot, but a gradient-like distribution in the treatment with cow's milk.

Rodrigues et al. (2020) demonstrated isolation of samples corresponding to the treatment with ice cream in relation to the other experimental periods (control, food supplement and washout), demonstrating a different behavior of the microbiota when administered ice cream samples using Unifrac. The modulation of the microbiota with the addition of ice cream increased the Firmicutes phylum, such as *Dialister* spp., *Roseburia inulinivorans*, *Veillonella* spp. and *Ruminococcus obeum*. Similar to the results of the present study where the genus *Veillonella* spp. and propionate-producing *Ruminococcaceae* were also stimulated in the presence of probiotic FM.

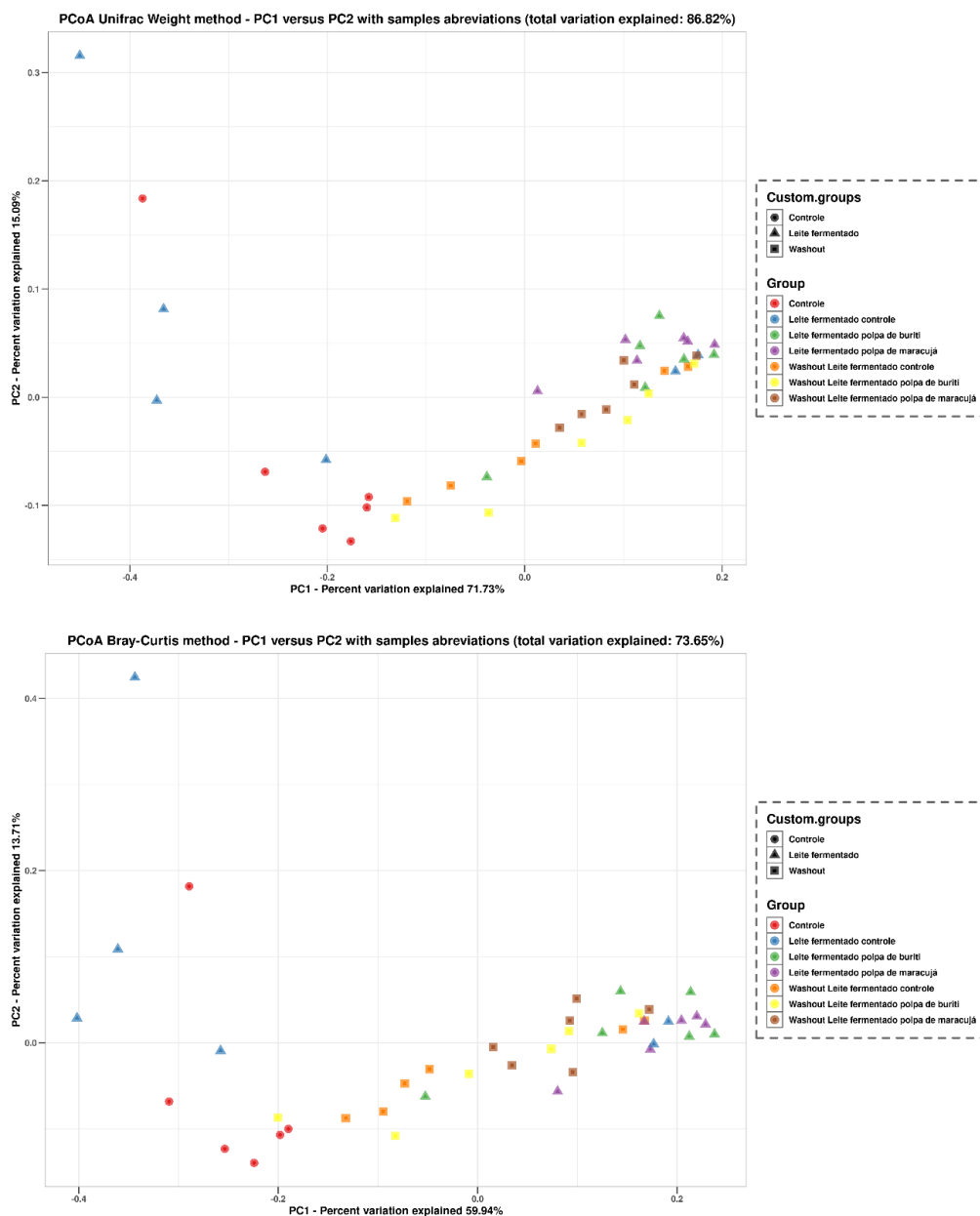


Figure 4 - Main component analysis.

A possible explanation for the low production of SCFAs during washout periods is that these fatty acids are rapidly consumed as a source of energy by the resident microbiota or colonocytes during their formation, not allowing an accumulation (BIANCHI et al., 2014). This demonstrates that probiotic FM has a beneficial effect during the administration period and can modulate the intestinal microbiota, however this effect is not prolonged after consumption, indicating that, the product must be consumed daily to obtain the desired beneficial effect.

The modulation of microbiota by FM was reported by other authors (VEIGA et al., 2014; UNNO et al., 2015; RETTEDAL et al., 2019; CASAROTTI et al., 2020; LIU et al., 2022). However, further studies are still needed to elucidate in detail the mechanisms of action after the consumption of fermented dairy products, to demonstrate the mechanism of action of compounds present in milk, probiotic bacteria and bioactive compounds present in fruits on the intestinal microbiota.

#### 3.4 Short-chain fatty acids and ammonium ions

There was a significant increase of acetic acid during the treatment periods in all types of FM and a decrease in its levels during washout. Feng et al. (2018) reported that the main genera of acetate-producing bacteria are *Bifidobacterium* spp., *Prevotella* spp., *Akkermansia* ssp., *Blautia hydrogenotrophica*, *Lactobacillus* spp. and *Bacteroides* spp. This explains the high production of acetate in all FM treatments, as there was an increase in *Bifidobacterium* that produces acetate. This SCFA is produced mainly by the pathways Wood-Ljungdahl and acetyl-CoA, and it is an important regulator of blood pH and nourishes butyrate-producing bacteria which has anti-inflammatory action (LOUIS et al., 2014; FERNÁNDEZ et al., 2016).

A significant and remarkably high amount of propionate was produced with the addition of FMB. The high production of propionate in the FMB may be beneficial to the host as it helps in the regulation of serum cholesterol and may provide a greater feeling of satiety (ILLMAN et al., 1988; RUIJSCHOP, BOELRIJK and TE GIFFEL, 2008). This higher production of propionate can be explained by the increase in the number of Bacteroidetes, *Ruminococcaceae* and *Veillonella*, propionate producers.

The highest amount of butyric acids was produced when FMC or FMB was added to SHIME® (Table 3). Butyrate is an important SCFA as it induces the production of

MUC, an important glycoprotein that makes up the mucosal layer of the intestinal barrier (BINDA et al., 2018). Butyrate is produced by bacteria from Firmicutes, as *Faecalibacterium* spp., that were stimulated during the addition of treatments in SHIME<sup>®</sup>. Other microorganisms such as *Roseburia* spp., *Eubacterium rectale*, *Clostridium leptum* and *Coprococcus catuse* are also butyrate producers through the phosphotransbutyrylase/butyrate kinase and butyryl-CoA:acetate CoA-transferase pathways. Propionate is produced by bacteria from the phyla *Bacteroidetes* and Firmicutes, such as *Dialister* spp., *Phascolarctobacterium succinatutens*, *Roseburia inulinivorans*, *Salmonella* spp., *Veillonella* spp. and *Ruminococcus obeum*, through the succinate, propanediol and acrylate pathways (FENG et al., 2018).

For ammonia, the treatments of FM with the addition of fruit pulp had lower production compared to FMC, and the FMPF stood out for presenting lower amounts of ammonia in the medium (Table 2). Authors suggested that the production of ammonia ions is related to the hydrolysis of urea and deamination of amino acids by the bacteria present in the intestine, correlating the increased production of ammonia with diets rich in protein (NYANGALE, MOTTRAM and GIBSON, 2012; BIANCHI et al., 2018; MEDEIROS et al., 2022). The production of ammonia ions also can be influenced by the treatment time and the substrate administered (CASAROTTI et al., 2020).

Oddi et al. (2020) also observed a decrease in ammonia production and a reduction in the Proteobacteria phylum, a similar result observed in the present study (Figure 2). Moreover, a significant increase in the Firmicutes phylum in all FM treatments and a small increase in Bacteroidetes phylum in the FMPF and FMB treatments may be related to a lower ammonia production. Bacteria belonging to the Bacteroidetes phylum can alter their metabolism favoring saccharolytic over proteolytic fermentation pathways depending on nutrient availability (RODRIGUES et al., 2020), leading to an increase in the production of SCFA and a reduction in ammonia levels as observed in this study.

Table 3. Short chain fatty acid levels and ammonium ion (mmol/L) in the vessels corresponding to the ascending colon during the experimental period.

Periods	Acetic acid	Propionic acid	Butyric acid	NH <sub>4</sub> <sup>+</sup>
Control	22.35±2.26 <sup>D</sup>	3.21±0.24 <sup>B</sup>	14.03±1.66 <sup>B</sup>	557.22±9.74 <sup>A</sup>
FMC	110.67±16.44 <sup>A</sup>	1.74±0.79 <sup>B</sup>	24.56±0.00 <sup>A</sup>	492.11±34.36 <sup>B</sup>
Washout FMC	47.05±3.31 <sup>CD</sup>	1.01±0.30 <sup>B</sup>	11.24±0.81 <sup>BC</sup>	506.56±29.43 <sup>AB</sup>
FMPF	125.66±10.07 <sup>A</sup>	1.15±0.00 <sup>B</sup>	15.96±0.16 <sup>B</sup>	284.89±6.04 <sup>D</sup>
Washout FMPF	52.65±2.79 <sup>CD</sup>	3.79±1.33 <sup>B</sup>	7.72±0.08 <sup>C</sup>	496.57±7.98 <sup>B</sup>
FMB	108.90±5.06 <sup>AB</sup>	18.47±3.32 <sup>A</sup>	25.80±1.87 <sup>A</sup>	378.67±6.15 <sup>C</sup>
Washout FMB	71.04±2.37 <sup>BC</sup>	3.13±0.98 <sup>B</sup>	16.97±0.79 <sup>B</sup>	472.11±6.27 <sup>B</sup>

FMC - Fermented milk control, without pulp, FMPF - Fermented milk with passion fruit pulp, FMB - Fermented milk with buriti pulp. Different capital letters in the same column denote a significant difference ( $p < 0.05$ ) during the experimental periods.

Cow's milk used in this study is mainly composed of lactose, which is known to be the main carbohydrate, with approximately an 80:20 ratio of total casein and whey proteins (50%  $\alpha$ -casein, 40%  $\alpha_{S1}$ -casein) (BALTHAZAR et al., 2017; RETTEDAL et al., 2019). Proteins are one of the main precursors of ammonia production by bacteria present in the intestinal microbiota (NYANGALE, MOTTRAM and GIBSON, 2012; ODDI et al., 2020; RODRIGUES et al., 2020), Therefore, factors such as the type and amount of protein present in the matrix (RETTEDAL et al., 2019), as well as the use of probiotic strains in the fermentation of fermented products, may have had an important effect on preventing ammonia production.

The decrease in ammonia production during the administration of FM observed in the present study is considered beneficial, since when present in high concentrations, in addition to being toxic to the organism and altering the cellular morphology of colonocytes, high concentrations of ammonia have been related to promote carcinogenesis in intestinal tissue, increasing the likelihood of cancer development (MONTALTO et al., 2009; DAVILA et al., 2013; HE et al., 2019) and when it is in the bloodstream, it can be linked to hepatic encephalopathy as well as neurotoxic effects (RODRIGUES et al., 2020).

*Lactobacillus acidophilus* (La-5) reduced ammonia production in the SHIME® using the gut microbiota of medium-age healthy individuals but had no effect on the production of SCFA, according to Medeiros et al. (2022). *Lactobacillus plantarum* 73a

also reduced the production of ammonia in the SHIME<sup>®</sup> using the fecal microbiota of an obese child. In both cases the reduction in ammonia production is correlated with the decrease mainly in the genus *Bacteroides* and *Clostridium* which are the main genus responsible for proteolytic activity in the intestine.

The production of SCFA and ammonia are interrelated, as both are metabolites produced from the metabolism of bacteria present in the gastrointestinal tract (WANG et al., 2021; ODDI et al., 2020). The main SCFA and ammonia produced through bacterial fermentation will vary according to the carbohydrate type, presence of inorganic terminal electron acceptors, microbiota diversity, composition, activity, individual preference of each species and the microorganism's fermentation strategy, and gut transit time. Additionally, the concentrations of fecal SCFA (acetate, butyrate, and propionate, in a molar ratio of approximately 60:20:20) and lower concentrations of ammonia are indicative of intestinal health. Furthermore, a beneficial effect on host metabolism have been reported (SPILJAR, MERKLER and TRAJKOVSKI, 2017; SILVA et al., 2020). Aiming at increasing carbohydrate fermentation and decreasing amino acid and peptide fermentation the reduction of ions ammonia is desired, as they can alter the intermediary metabolism and morphology of intestinal cells, avoid promotion development tumor, and increase DNA synthesis (BIANCHI et al., 2018; MEDEIROS et al., 2022). Conversely, SCFA can inhibit pathogenic microorganisms and increase mineral absorption due to decreased luminal pH, stimulate cell proliferation in epithelial tissue, increase mucin production, modulate metabolic activity (intestinal homeostasis), as well as prevent metabolic syndrome, cancers, and bowel disorders (HU et al., 2010; RÍOS-COVIÁN et al., 2016).

Administration of functional products containing probiotics and prebiotics and resulting in increased SCFA and decreased ammonia has been demonstrated in the literature over the years (WINDEY, PRETER and VERBEKE, 2011; TERPEND et al., 2013; FREIRE et al., 2017; WANG et al., 2021). It is known that dairy products can influence SCFA and ammonia production through microbiota modulation (CASAROTTI et al., 2020). In some cases, some proteins can bind to other nutrients, such as sugar, making them less digestible, disfavoring the bacteria present in the intestinal microbiota that use proteolytic pathways (DOMINIKA et al., 2011). These proteins can even be used due to the proteolysis process carried out by starter LAB such as *S. thermophilus*, directly influencing the availability of types of substrates in fermented products, such as peptides,

which can influence microbial abundance, consequently production of SCFA and ammonia (RAVESCHOT et al., 2018).

Freire et al. (2017) showed that fermented goat milk, with or without grape pomace extract, had a positive effect on the metabolism of the intestinal microbiota, increasing the production of SCFA, and decreasing the concentration of ammonium. Rodrigues et al., 2020 reported an increase in SCFA in SHIME<sup>®</sup> using healthy human microbiota after administration of ice cream containing *Lactobacillus acidophilus* and *Bifidobacterium animalis* and reduction of ammonia after administration of a dietary supplement containing *Lactobacillus acidophilus* and *Bifidobacterium animalis* (both after 7 days of treatment).

#### **4. Conclusion**

In general, probiotic FM with or without fruit pulp influenced beneficially the intestinal health; they increased the production of short chain fatty acids, especially acetic and butyric acids, and decreased ammonia production, and changed the balance of Firmicutes/Bacteroidetes phyla, increasing Actinobacteria and decreasing Proteobacteria phyla (mainly the Enterobacteriaceae family). The addition of pulp fruits to FM boosted the beneficial effects observed in intestinal microbiota which could be related by the presence of bioactive compounds.

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## CAPÍTULO V - CONCLUSÕES FINAIS

A polpa de maracujá e principalmente a polpa de buriti são fontes de nutrientes essenciais para a saúde. São ricos em compostos bioativos que podem proporcionar benefícios aos consumidores. As polpas de buriti e de maracujá podem ser associadas às LAB probióticas para a produção de alimentos funcionais.

As duas polpas de frutas influenciaram os parâmetros cinéticos, enquanto a viabilidade das culturas probióticas em leite fermentado foi influenciada apenas pelo tipo de cultura utilizada. A adição de polpa de fruta enriqueceu o produto final em termos de compostos bioativos e atividade antioxidante. Portanto, as polpas de buriti e maracujá podem ser consideradas ótimas alternativas para produzir leite fermentado probiótico inovador com características funcionais.

Os tratamentos de leite fermentado probiótico, com ou sem adição de polpa de frutas, influenciaram benéficamente a saúde intestinal; eles promoveram aumento da produção de ácidos graxos de cadeia curta, especialmente ácidos acético e butírico e reduziram a produção de amônia, e alteraram a proporção entre os filos Firmicutes e Bacteroidetes, aumentaram bactérias do filo Actinobacteria e reduziram do filo Proteobacteria (principalmente da família Enterobacteriaceae). A adição de polpas de frutas ao leite fermentados aumentaram os efeitos benéficos observados na microbiota intestinal, que pode estar relacionado com a presença de compostos bioativos.

No entanto, estudos adicionais para melhor elucidar os mecanismos de ação e conversão de compostos bioativos pelas bactérias ácido lácticas probióticas e pelas bactérias da microbiota intestinal ainda precisam ser realizados.