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LARISSA RAMALHO BRANDÃO

EFEITOS DO *Lacticaseibacillus casei* 01 E SEU PARAPROBIÓTICO DERIVADO DE INATIVAÇÃO POR ULTRASSOM NOS PARÂMETROS CARDIOMETABÓLICOS E MICROBIOTA DE RATOS ALIMENTADOS COM DIETA HIPERLIPÍDICA

João Pessoa
2022

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RESUMO

O hábito alimentar, entre outros fatores ambientais, tem papel determinante para a ocorrência de diversas patologias, entre elas, hipertensão arterial, obesidade e hipercolesterolemia. O consumo excessivo de sódio e gordura saturada está relacionado a ocorrência dessas patologias na população. A ingestão de probióticos e paraprobióticos inativados por diferentes métodos tem sido associada a efeitos benéficos no metabolismo lipídico e glicêmico, bem como à modulação da microbiota. O presente estudo teve como objetivo avaliar os efeitos da administração de *Lacticaseibacillus casei* 01 (9 log UFC/mL) e seu paraprobiótico derivado de tratamento com ultrassom (20 kHz, 40 min) por 28 dias em ratos alimentados com dieta hiperlipídica. A tolerância à insulina e glicose, testes bioquímicos, parâmetros cardiovasculares e análise da ecologia microbiana fecal foram realizados ao final da intervença (28 dias). Vinte e quatro ratos *Wistar* foram divididos em quatro grupos de seis animais cada: grupo controle (CTL), dieta hiperlipídica (DHL), dieta hiperlipídica e *L. casei* vivo (DHL-LC) e dieta hiperlipídica e *L. casei* inativado (DHL-ILC). A administração de *L. casei* vivo e inativado por ultrassom reduziu ($p < 0,05$) os níveis de colesterol total e LDL e controlou a resistência à insulina nos ratos alimentados com dieta hiperlipídica. *L. casei* vivo e inativado promoveram aumento ($p < 0,05$) de *Lachnospiraceae* e *Ruminoccocaceae* e diminuição ($p < 0,05$) de *Clostridiaceae*, *Enterobacteriaceae* e *Helicobacteriacea*, atenuando os efeitos promovidos pela DHL. A administração de *L. casei* vivo preveniu o aumento da pressão arterial (PA) enquanto o *L. casei* inativado atenuou o aumento da PA. Os resultados mostram efeitos benéficos de *L. casei* 01 vivo e inativado e indicam a inativação por ultrassom como eficaz para a obtenção de um paraprobiótico com propriedades funcionais comparáveis ou melhores, a depender do efeito mensurado, que aquelas das células vivas.

Palavras-chave: dislipidemias; hipertensão; microbiota; probióticos; ultrassom.

ABSTRACT

Eating habits, among other environmental factors, play a determinant role in the occurrence of several pathologies, including arterial hypertension, obesity and hypercholesterolemia. Excessive consumption of sodium and saturated fat is related to the occurrence of these pathologies in the population. The ingestion of probiotics and paraprobiotics inactivated by different methods has been associated with beneficial effects on lipid and glycemic metabolism, as well as modulation of the microbiota. The present study aimed to evaluate the effects of administration of *Lacticaseibacillus casei* 01 (9 log CFU/mL) and its paraprobiotic derived from ultrasound treatment (20 kHz, 40 min) for 28 days in rats under consumption of hyperlipidic diet. Insulin and glucose tolerance, biochemical tests, cardiovascular parameters and fecal microbial ecology analysis were performed at the end of the intervention (28 days). Twenty-four male Wistar rats were divided into four groups of six animals each: group control (CTL), hyperlipidic diet (HLD), hyperlipidic diet and live *L. casei* (HLD-LC), and hyperlipidic diet and inactivated *L. casei* (DHL-ILC). Administration of live and ultrasonically inactivated *L. casei* reduced ($p < 0.05$) total and LDL cholesterol levels and controlled insulin resistance in the rats fed hyperlipidic diet. Live and inactivated *L. casei* promote increase ($p < 0.05$) of *Lachnospiraceae* and *Ruminoccocaceae* and decrease ($p < 0.05$) of *Clostridiaceae*, *Enterobacteriaceae* and *Helicobacteriacea*, attenuating the effects promoted by HLD. Administration of live *L. casei* prevented the increase in blood pressure (BP) while inactivated *L. casei* attenuated the BP increase. The results show beneficial effects of live and inactivated *L. casei* 01 and indicate ultrasound inactivation as effective for obtaining a paraprobiotic with functional properties comparable or better, depending on the effect measured, than those of live cells.

Keywords: dyslipidemias; hypertension; probiotics; microbiota; ultrasonics.

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1 INTRODUÇÃO

A definição de probióticos alega que os efeitos benéficos à saúde do hospedeiro são proporcionados através do consumo de quantidades adequadas de células viáveis (HILL et al., 2014). Entretanto, estudos recentes indicam que mesmo as células não cultiváveis de alguns microrganismos probióticos, descritos como paraprobióticos, podem exercer tais efeitos (DESHPANDE et al., 2018; SOMBOLESTANI et al., 2018; WARDA et al., 2019).

Os paraprobióticos apresentam vantagens em relação aos probióticos, pois facilitam a incorporação em matrizes alimentares que passam por processamentos que afetariam a viabilidade celular de células vivas (AGUILAR-TOALÁ et al., 2018). Desta forma, o uso de paraprobióticos elimina a necessidade de adicionar os microrganismos em etapas de pós-processamento, e a necessidade de mantê-los vivos nos substratos, durante a vida de prateleira do produto (ALMADA et al., 2021a; PIMENTEL et al., 2021). Ainda, a possibilidade de uso de células probióticas inativadas para a funcionalização de alimentos facilita o processamento, transporte e armazenamento e permite em alguns casos uma vida de prateleira ampliada. Outro fator importante relacionado ao uso de paraprobióticos é a administração segura para indivíduos imunodeprimidos ou com barreira intestinal comprometida (ALMADA, et al., 2021b; BARROS et al., 2020).

Vários tratamentos têm sido empregados para obtenção de células paraprobióticas como tratamentos térmicos (ANANTA e KNORR, 2009, PARK et al., 2018), alta pressão (ANANTA e KNORR, 2009), irradiação (KAMIYA et al., 2006), raios ultravioletas (GAYÁN et al., 2013) e ultrassom (POSADAS et al., 2017). O ultrassom já se mostrou um método viável para obtenção de células paraprobióticas de *L. casei* com metabolismo ativo, entre outros cinco métodos de inativação utilizados (calor, pH alto, pH baixo, dióxido de carbono (CO_2) supercrítico e irradiação), as células de *L. casei* inativadas por ultrassom apresentaram as melhores condições morfológicas e fisiológicas (ALMADA et al., 2021a).

Entre os efeitos benéficos à saúde já avaliados do *Lacticaseibacillus casei* 01 destaca-se os anti-hipertensivos e propriedades antioxidantes (BALTHAZAR et al., 2018), melhoras no perfil lipídico e de pressão arterial de indivíduos hipertensos (SPERRY et al., 2018), melhora de aprendizagem e memória em ratos (XIAO et al., 2014). Empregado em matrizes alimentares como queijo (SPERRY et al., 2018), iogurtes (ARYANA et al., 2007), sorvete (BALTHAZAR et al., 2018) e sucos (CHAIKHAM et al., 2012; KINGWATEE et al., 2015). BARROS et al. (2020); BARROS et al. (2021) avaliaram os efeitos de cepas de *L. casei* inativadas por aquecimento ôhmico. O paraprobiótico obtido se mostrou efetivo no controle da glicemia pós-prandial de adultos saudáveis, sendo apenas esse parâmetro investigado.

O hábito alimentar caracterizado pelo consumo aumentado de açúcares, colesterol, ácidos graxos saturados e trans e de excessiva quantidade de calorias causa desequilíbrios nas concentrações séricas de colesterol total, *high-density lipoprotein* (HDL), *low-density lipoprotein* (LDL) e triglicerídeos (BARROSO et al., 2021; SBC, 2017). O consumo excessivo de sódio e gordura saturada está relacionado à disbiose intestinal, aumento do risco de desenvolver doenças como diabetes *mellitus* tipo 2 e hipertensão (BARROSO et al., 2021; DARROUDI et al., 2018).

Considerando o exposto, o presente trabalho tem como objetivo geral avaliar os efeitos do consumo de *L. casei* 01 e seu paraprobiótico inativado por ultrassom sobre parâmetros do metabolismo lipídico, glicídico, cardiometabólicos e microbiota de ratos alimentados com dieta hiperlipídica.

2 REFERENCIAL TEÓRICO

2.1 HEALTH BENEFITS AND TECHNOLOGICAL EFFECTS OF *Lacticaseibacillus casei*-01: AN OVERVIEW OF THE SCIENTIFIC LITERATURE

A abordagem da dissertação refere-se à utilização do *Lacticaseibacillus casei* 01 e seu paraprobiótico e seus benefícios para a saúde. A revisão de literatura publicada no periódico *Trens in Food Science and Technology*, aborda os aspectos gerais, classificação e nomenclatura do *L. casei* 01. Também são discutidos os benefícios para a saúde do hospedeiro associados ao consumo de células vivas e inativadas desta cepa, e seu importante papel no desenvolvimento de alimentos probióticos lácteos e não lácteos.

Informações complementares sobre paraprobióticos e a tecnologia de ultrassom como alternativa para sua obtenção, que dão suporte ao desenho experimental desta dissertação estão apresentados após a revisão já publicada.

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Health benefits and technological effects of *Lacticaseibacillus casei*-01: An overview of the scientific literature

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ABSTRACT

Background: Probiotics have been widely studied due to their health benefits and technological effects. In recent years, paraprobiotic and postbiotics derived from probiotic strains stood out. The recently reclassified *Lacticaseibacillus casei*-01 (former *Lactobacillus casei*-01) may exert different health effects on the host and has an important role in the development of probiotic dairy and non-dairy foods.
Scope and approach: This review presents the general aspects, the classification and nomenclature of *L. casei*-01 and summarizes the available scientific literature about this strain from 2011 to 2020. The health benefits associated with consumption of live and inactivated *L. casei*-01 cells, the factors influencing its survival in food products, and the impact of its addition on the quality parameters of dairy and non-dairy products are discussed.
Key findings and conclusions: The regular consumption of foods added with *L. casei*-01 promotes antihypertensive and anti-hyperglycemic effects, and improves lipid profile in human. The incorporation of *L. casei*-01 in dairy matrices can improve the quality parameters, while in non-dairy products the desired characteristics can be achieved if suitable *L. casei*-01 concentrations and other factors are optimized. However, the physiological and technological effects of paraprobiotics or postbiotics derived from *L. casei*-01 remain unexplored.

1. Introduction

The food pattern established in recent years is focused on the consumption of foods that have functionality for health and on the development of new food products, with emphasis on the use of probiotic microorganisms, paraprobiotic microorganisms, and postbiotics (Aguilar-Toalá et al., 2018; Almada et al., 2016; Barros et al., 2020; Hill et al., 2014).

Probiotics have been defined as non-pathogenic live microorganisms that, when ingested in adequate amounts, can confer health benefits to individuals (Hill et al., 2014). On the other hand, paraprobiotics are inactivated (non-viable) microorganisms that confer health benefits to the consumers (Almada et al., 2016). Otherwise, postbiotics are defined as soluble factors (products or metabolic by-products) secreted by live bacteria or released after bacterial lysis, which can benefit the host. The soluble factors include peptides, short-chain fatty acids, teichoic acids, enzymes, organic acids, vitamins, among others (Aguilar-Toalá et al., 2018).

The most studied probiotic bacteria species belong to the *Lactobacillus* genus (Alves et al., 2016; Salvetti et al., 2018). Lactobacilli are key players in the industry, food, and human and animal health-related fields; they contribute to fermented food production, food texture, and food preservation (Duar et al., 2017; Sun et al., 2015). Over the decades, lactobacilli have been widely administered in foods and dietary supplements because of their safety, efficacy, and commercial interests (Alves et al., 2016). Several species have shown beneficial physiological effects to the host (Costa, Brandao, et al., 2019; Sperry et al., 2018; Yadav et al., 2019), however, the physiological effects evidenced by probiotic microorganisms cannot be generalized to bacteria from the same species or genus, being, therefore, strain-specific (Bagon et al., 2018).

The enormous genomic diversity among the more than 260 species belonging to the genus *Lactobacillus* and the continuous addition of new species let taxonomists revise the current classification (Salvetti et al.,

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2018). Recently, the genus was reclassified into 25 genera, including the emended genus *Lactobacillus* based on phenotypic, genotypic, and ecological features (Zheng et al., 2020). This reclassification presents itself as a unique occasion to better explain the former *Lactobacillus* genus' universe, better understand the specificities among the new groups, and develop better targeted products with a deeper functional vision, and ultimately, better efficiency of specific strains.

Lacticaseibacillus casei (former *Lactobacillus casei*) is one of the most studied species due to its industrial, commercial, and applied health potential (Hill et al., 2018; Zheng et al., 2020). Particularly, the strain *L. casei*-01 has been traditionally used as a probiotic culture in dairy products (Balthazar et al., 2018; Costa, Paula, et al., 2019; Zheng et al., 2020), however it has also become a strategy to obtain non-dairy probiotic matrices (Miranda, Paula, et al., 2019). Recently, *L. casei*-01 has also been incorporated in whey-protein or polysaccharide-based edible coatings applied in meat and bakery products or fruits (Dianin et al., 2019; Pereira et al., 2018; Pruksarojanakul et al., 2020).

In vivo studies with the *L. casei*-01 strain have proven beneficial effects on the host's health, both human and animal. Its regular consumption proved to result in anti-hypertensive effects, antioxidant properties, and improved lipid profile (Balthazar et al., 2018; Sperry et al., 2018). The *in vivo* studies are related to the inclusion of *L. casei*-01 in dairy matrices (Balthazar et al., 2018; Cordeiro, Souza, et al., 2019; Grom et al., 2020; Sperry et al., 2018), but also in vegetable matrices, highlighting fruit juices (Chaikham et al., 2012; Kingwatee et al., 2015). *L. casei*-01 is generally added in encapsulated, lyophilized, or activated by propagation forms (Miranda, Paula, et al., 2019).

Inactivated L. *casei*-01 cells have also been explored by researchers as paraprobiotics for incorporation in food products. The utilization of paraprobiotics has many advantages compared to the live probiotics, such as less or no interaction with the components of the food, easier processing (allows the addition before the heat treatment), higher safety (reduction of the risk of infection, microbial translocation, and inflammatory responses), and easier transport and storage (Almada et al., 2016; Guimarães et al., 2019). Therefore, *L. casei*-01 as paraprobiotics may be used in products susceptible to technological alterations by the probiotic culture that could compromise their acceptance by consumers. Besides, they could be options when probiotic addition after heating treatment is not possible due to technological or microbiological characteristics, in products targeted for immunocompromised consumers, or needing long-distance transportation, which precludes probiotic survival.

Although many technological and health benefits have been claimed to *L. casei*-01, no previous studies have summarized or discussed these findings. Thus, this review aims to provide an overview of the classification and nomenclature of *L. casei*-01 and summarize the health benefits associated with its consumption as live or inactivated cells. Furthermore, the factors influencing its survival in food products and the impact of its addition on the quality parameters of dairy and non-dairy food products are discussed. The information summarized in this review may assist in developing strategies to design new probiotic, paraprobiotic or postbiotic products with beneficial effects without compromise technological aspects and identifying perspectives for further studies.

2. Methodology

A narrative bibliographic review was carried out using the PubMed, Scopus, and Lilacs platforms as scientific databases. The keywords used as descriptors were: "*Lactobacillus casei* 01", "Lc-01" and "*Lacticaseibacillus casei*", individually or in conjunction with the terms "gut microbiota", "probiotic", "paraprobiotic", "postbiotic", "beneficial effects", "clinical trial", "intervention", "supplementation", "functional food", "physicochemical", "bioactivity", "viability", "gastrointestinal resistance", "probiotic survival", "shelf-life", "*in vitro* digestion", "sensory profile" and "acceptance", as search strategy. It was selected articles published from 2011 to 2020, written exclusively in English language. Papers that did not coincide with the theme, case series, case-control, and repeated papers available on different platforms were excluded. The articles' selection and screening process to form the writing base of this overview was independently performed by two authors and summarized in Fig. 1.

A total of 77 articles were researched and collected, subsequently screened regarding duplicity. Five were excluded from the 55 articles selected for the entire reading because they comply with at least one of the exclusion criteria. A total of 50 studies were evaluated and categorically subdivided considering: I – *L. casei*-01 isolated or as an additive with a focus on the health benefit on consumers after a probiotic food consumption; and II- articles in which *L. casei*-01 strain or its metabolites are used on the processing of food matrices, focusing on the technological and sensory aspects.

3. L. casei-*01: General characteristics and nomenclature issues*

The microorganisms most commonly used in the formulation of products with probiotic characteristics (supplements or foods) belong to the genus *Lactobacillus* (Hill et al., 2014). Restricted by fastidious growth requirements, *Lactobacillus* species are found in nutrient-rich habitats associated with food (fermented or spoiled), feed, plants, and as part of the animal (invertebrate and vertebrate) and human microbiota. Due to its economic importance, the metabolism, genetics, and phylogeny of lactobacilli have been extensively studied. In addition to the beneficial effects already proven, these microorganisms can still be used to give aroma, flavor, and texture to food products, in addition to assisting in their bioconservation (Duar et al., 2017).

Lactobacillus was primarily described in 1901 with *Lactobacillus delbrueckii*, an indispensable species in yogurt production. In 1935, the Japanese researcher Dr. Shirota discovered the strain *Lactobacillus casei* Shirota with proven health benefits (it can populate and form antimicrobial substances in the small intestine and can improve the activity and quantity of macrophages). Due to these effects, *Lactobacillus casei* Shirota was one of the first commercial probiotics, and it was sold for the first time in 1955 in Japan (Yerlikaya, 2014).

The species included in the genus *Lactobacillus* increased since the beginning of the 21st century, mainly due to the new sequencing methods (Hammes & Hertel, 2006; Zheng et al., 2020). The first *Lactobacillus* genome sequence was reported in 2002. The findings suggested that the genes, which provide an important part of *Lactobacillus*' interaction with its environment, form a lifestyle adaptation region in the chromosome (Duar et al., 2017; Kleeberzem et al., 2002). Over the years, scientific and technological advances have enabled the development of numerous researches and discovering new species of microorganisms within the genus and its applications. Nevertheless, bacteria can divide to create a second-generation (vertical transmission of the genes) and/or continuously exchange genetic material through conjugations with other bacteria (horizontal transmission), which can occur in the ecosystem and, especially, in the human intestinal microbiome (Jeong et al., 2019). Therefore, the numerous scientific genetic analyzes carried out in recent years show an enormous heterogeneity among the more than 260 species of the genus *Lactobacillus* (Park et al., 2018; Salvetti et al., 2018).

For this reason, based on various genetic approaches and markers (mean nucleotide, amino acid, and genetic nucleus amino acid identity, central genome phylogeny, signature genes, and metabolic or ecological criteria), Zheng et al. (2020) developed a new classification that disperses the species of the *Lactobacillacea* family under *Lactobacillus*, *Paralactobacillus*, *Pediococcus*, and 23 new genera.

The new classification aims to create new genera with a better homogeneity, which respects the rules of homogeneity between organisms (the international prokaryote nomenclature code includes 65 rules designed to assess the correctness of a microbial name, Parker et al., 2015). Furthermore, the genera should be stable in the long term and offer space for future species' accommodation based on the source of

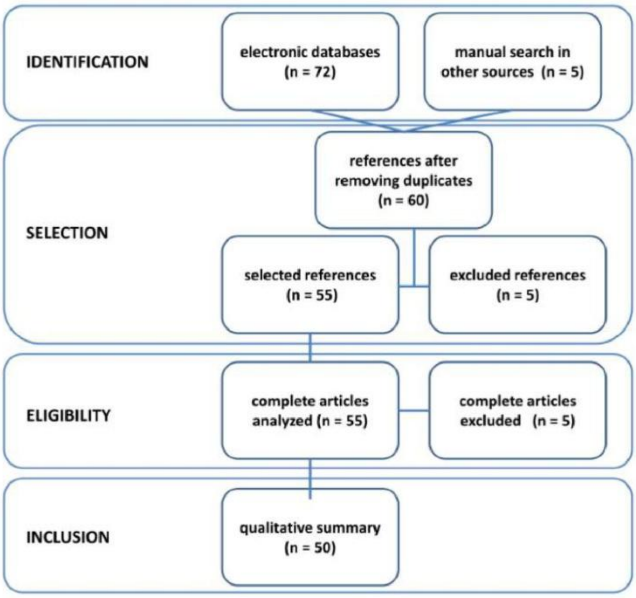

Fig. 1. Flowchart for the selection of articles included in the review with number of studies/articles at each stage of selection.

isolation. The new classification may provide a better ecological and functional vision, improving the way the industry develops products defining boundaries in the bacterial world (Duar et al., 2017; Salvetti et al., 2018).

Bacteria from the *L. casei* group comprises the closely related *L. casei*, *L. rhamnosus*, and *L. paracasei* species. The bacteria in this group have been widely studied for their health-promoting properties. Several beneficial functions to the human organism have been attributed to the regular consumption of foods containing bacteria from this group. Such bacteria are often used in the food industries to improve different products' quality (Gänzle et al., 2019; Hutkins, 2019). The genus has considerable economic importance as it harbors several species used as starter cultures in dairy fermentations and as probiotics (Salvetti et al., 2012; Zheng et al., 2020). Even with the importance of these microorganisms to the industry, the taxonomy of bacteria in this group is complex (Duar et al., 2017; Gänzle et al., 2019; Hutkins, 2019; Sun et al., 2015; Zheng et al., 2015). Particularly, *L. casei* accounts for an unclear taxonomy over the past decades. The high levels of DNA homology among strains belonging to *L. casei* subspecies led to a reclassification of *L. casei* subsp. *casei* (most strains) as *Lactobacillus paracasei* (Collins et al., 1989). Thus, most genomes designated as *L. casei* in the NCBI database corresponds to *L. paracasei* (Salvetti et al., 2012; Wuyts et al., 2017).

The recently proposed genus *Lacticaseibacillus* (*L. casei* group) by reassessment of its species' genetic and phylogenetic relationship remained relatively heterogeneous, mainly concerning the average amino acid identity values. Therefore, indicating that the species *L. casei*, *L. rhamnosus*, and *L. paracasei* are nomadic and can currently adapt to a wide variety of niches (Zheng et al., 2020).

Lacticaseibacillus genus comprises lactic acid bacteria capable of colonizing various natural and human-made environments (Hansen & Lessel, 1971). Strains of *Lacticaseibacillus* are homofermentative, but not all species metabolize pentoses *via* the phosphoketolase pathway. The mol % G + C content of DNA is between 46 and 58. The genome size ranges from 1.93 to 3.14 Mbp. The strains are non-motile, oxidase negative, often producing D (−)- and L (+)-lactic acid from glucose. The temperature range for growth is variable but usually from 10 to 45 °C. Some subspecies from *Lacticaseibacillus* can survive until 70 °C for 40 s. Lys–d-Asp is the most common type of peptidoglycan (Gänzle et al., 2019; Hutkins, 2019; Zheng et al., 2020).

The *L. casei* (originally described by Hansen & Lessel, 1971) includes strains with catalase activity (Hansen & Lessel, 1971; Wuyts et al., 2017), genome size of 2.83 Mbp and mol% G + C content of DNA of 46.5. In this group, stands out the strain *L. casei*-01. This strain can be considered a safe microorganism, as a previous study reported susceptibility to all tested antibiotics (penicillin G, ampicillin, amoxicillin, piperacillin, ampicillin/sulbactam, amoxycillin/clavulanate potassium, cefalotin, cefoperazone, cefazolin, cefuroxime sodium, meropenem, azithromycin, clarithromycin, erythromycin, doxycycline, tetracycline, clindamycin, chloramphenicol, rifampicin) and lack of biogenic amines production (except for tyramine) (Zhang et al., 2013). Furthermore, the oral administration did not have adverse effects on the body weight of mice, and no bacterial translocation associated with the treatment was observed. Over the last decades, a range of health benefits has been associated with *L. casei*-01. Fig. 2 summarizes the main health effects associated with this strain.

4. Health benefits of L. casei-01

4.1. L. casei-01 as live cells

Table 1 presents the main studies assessing the *in vitro* and/or *in vivo* (in human clinical studies and murine model) physiological effects of the probiotic strain *L. casei*-01 added into food matrices. As the health effect is strain-specific, it is important to observe which effects could be

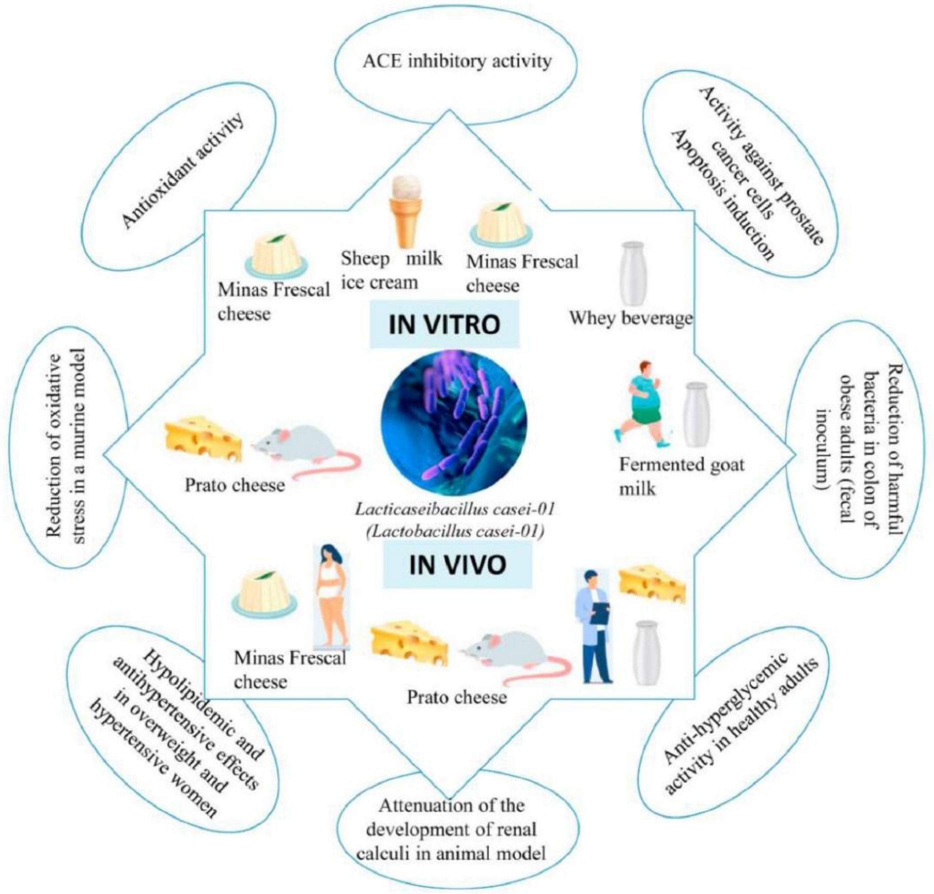

Fig. 2. Main health effects of *Lactobacillus casei*-01 reported in *vitro* and/or *in vivo* studies. Images: Freepik.

associated with this strain.

In vitro tests showed that the addition of *L. casei*-01 to ice creams (9 log cfu/mL) increased the formation of beneficial microbial metabolites in gut model, such as propionate, acetate, lactic acid, and butyrate, and decreased the content of toxic ammonia. Furthermore, it stimulated *Lactobacillus* and *Bifidobacterium* counts and inhibited the harmful microorganisms (clostridia and fecal coliforms). The effect of *L. casei*-01 was more pronounced than that of *L. acidophilus* La-5 (Chaikam & Rattanasena, 2013). Stimulation of *Lactobacillus* and *Bifidobacterium* genera and reduction of *Megamonas, Prevotella*, and *Succinivibrio* genera was also observed by Casarotti et al. (2020) after a two-week treatment with low-fat fermented goat milk with *L. casei*-01 (6 log cfu/mL) and passion fruit by-product (1%). The authors used fecal inoculum from obese individuals, and analyses were performed in a Simulator of Human Intestinal Microbial Ecosystem (SHIME) system. Therefore, the *in vitro* tests indicate the possible mechanism of action of *L. casei* in the modulation of intestinal microbiota, by increasing the number of beneficial microorganisms (*Lactobacillus* and *Bifidobacterium*), decreasing the number of harmful microorganisms (*Megamonas, Prevotella* and *Succinivibrio*), and production of beneficial microbial metabolites (propionate, acetate, lactic acid, and butyrate). The decrease in the number of harmful microorganisms may be attributed to the antimicrobial activity of *L. casei*-01. However, the antimicrobial activity of this strain is not clearly understood. Parsaeimehr et al. (2017) observed an *in vitro* antimicrobial activity of *L. casei*-01 against *Staphylococcus aureus*. Chaikham et al. (2013) observed that *L. casei*-01 produced antibacterial substances (organic acids, fatty acids, and hydrogen peroxide) and reduced the counts of *S. aureus* TISTR118, *E. coli* TISTR780, *M. luteus* TISTR884, *B. cereus* TISTR687, and *En. Faecalis* TISTR1482. Lin et al. (2015) observed that *L. casei*-01 inhibited the growth and biofilm formation of *Streptococcus mutans*, mainly through the acid production. They also suggest that this strain could produce bacteriocins with a molecular weight lower than 3000 Da. However, they could not observe differences in the SDS-PAGE for this strain compared to control. In this way,

Table 1
Main studies assessing the *in vitro* and/or *in vivo* (in human and murine model) physiology effects of the probiotic strain *Lacticaseibacillus casei*-01.

Type of study	Objectives	Treatments	Assays	Main results	References
Animal model	Co-supplementation of *L. casei*-01 with a fermented whey beverage (FWB) against infection by *Salmonella* Typhimurium in a murine model	FWB (starter culture) PFWB (starter and *L. casei*-01) 0.1 mL for 30 days (10 days before infection and 20 days after infection) Probiotic concentration: 8–9 log cfu/mL		FWB presented better effect on animal survival (70%), translocation of the pathogen to the liver (2 out of 10), histopathology (fewer lesions), and inflammation than PFWB	Cordeiro, Souza, et al. (2019)
		Type of animal model: female BALB/c mice	First set: inflammatory and histological analysis 10 days post-infection		
		Number of animals: 30	Second set: clinical signs, weight loss, and mortality 20 days post-infection		
Animal model	Effect of prato cheese added with *L. casei*-01 in the prevention of dextran sodium sulphate (DSS)-induced ulcerative colitis in mice	(Conventional cheese) *Lactococcus lactis* ssp. *lactis* and *Lc. lactis* ssp. *cremoris*			Cordeiro, Lemos, et al. (2019)
		(Probiotic cheese) *Lactococcus lactis* ssp. *lactis*, *Lc. lactis* ssp. *cremoris* and *L. casei*-01	Weight, food intake, liquid intake		
		500 µg for 15 days	Colitis macroscopy analysis		
		Probiotic concentration: 9.47 log cfu/mL	Colitis histomorphological analysis		
		Type of animal model: Female C57/BL6 inbred mice	Secretory IgA	Reduced weight loss	
		Number of animals: 30-36	Colon cytokines expression	No reduction of the inflammation scores	
Animal model	Probiotic L. *casei*-01 Prato cheese against the inflammatory and oxidative damage in mice organs induced by cigarette smoke exposure	(CS) Exposed to cigarette smoke and fed regular chow; (CS + C) Smoke + fed daily conventional cheese *ad libitum* (CS + PC) Cigarette smoke + fed daily probiotic (*L. casei*-01) cheese *ad libitum* (C) Ambient smoke-free air + fed regular chow Exposure to 12 commercial cigarettes per day (*via* an inhalation chamber; 10 mg tar, 0.9 mg nicotine, and 10 mg monoxide) for 5 days 30 g of cheese for 5 days Probiotic concentration: 9.33 log cfu/g Type of animal model: C57BL/6 male mice Number of animals: 40	Broncho alveolar lavage (BAL), blood, gut and liver homogenates - for biochemical assays	Probiotic Prato cheese consumption reduced oxidative stress in the lungs, gut, and liver	Vasconcelos et al. (2019)
Animal model	Probiotic Prato cheese containing *L. casei*-01 on urolithiasis in a murine model	(NC) Naive Control; (CaOx-C) Control of calcium oxalate (CC) Calcium oxalate with conventional cheese (PC) Calcium oxalate with probiotic cheese 2 g/day/animal of cheese during 25 days		Only the PC group presented a significant reduction in the size of the pellets, radiological examination confirmed the role of PC in preventing kidney stone development	Martins et al. (2018)
		Probiotic concentration: 8.13 log cfu/g	Urinalysis - volume, density, pH, urea, creatinine		
		Type of animal: male *Rattus novergicus*, Wistar lineage	Serum urea, creatinine, sodium, potassium and magnesium		
		Number of animals: 24	Latero-lateral radiographs of the abdominal cavity		
Human clinical study	Yogurt supplemented with probiotic bacteria (*L. casei*-01) on the prevention of antibiotic-associated diarrhea (ADD) in hospitalized patients	(Placebo-yogurt) *S. thermophilus* and *L. delbrueckii* subsp. Bulgaricus (Probiotic yogurt) La-5, Bb-12 and *L. casei*-01 (No yogurt) unblinded control 200 mL of yogurt within 48 h of beginning the antibiotic therapy and up to 5 days after stopping the antibiotic	Patients were followed up with for 1 month to determine occurrence of diarrhea		Velasco et al. (2019)

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Table 1 (*continued*)

Type of study	Objectives	Treatments	Assays	Main results	References
		Probiotic concentration: 6 log cfu/mL of La-5, 7 log cfu/mL of *L. casei*-01, and 8 log cfu/mL of Bb-12 Subject type: Adults Number of subjects: 314 Type of study: double-blind placebo controlled trial		The administration of the probiotic yogurt did not alter the duration of diarrhea, maximum number of bowel movements, or admission because of diarrhea The combined probiotic strains LA-5, BB-12 and LC-01 do not have an effect in the prevention of AAD in hospitalized patients	
Human clinical study	*L. casei*-01 on Minas Frescal cheese to investigate the hematological and clinical effects on hypertensive overweighed women	(Treatment group) Probiotic Minas Frescal cheese (*L. casei* 01) (Control group) Probiotic-free Minas Frescal cheese (negative control) 50 g of cheese for 28 days Probiotic concentration: 8.32 log cfu/g Subject type: hypertensive overweighed women Number of subjects: 30 Type of study: randomized double blind	Hematological indices (leukocytes, lymphocytes and platelets) Inflammatory indices (white blood cells) Metabolic indices (lipids: TC, LDL-C, HDL-C, and TG)	*L. casei*-01 improved the total cholesterol, low-density lipoprotein-cholesterol, high-density lipoprotein cholesterol, triglyceride, diastolic and systolic pressure, hemoglobin, and hematocrit count	Sperry et al. (2018)
Human clinical study	Effect of different probiotic-enriched dairy matrices containing *L. casei*-01 on *in vitro* and *in vivo* anti-hyperglycemic activity	(1) 50 g of white bread + 300 mL of water (control) (2) 50 g of white bread + 50 g of probiotic Prato cheese (3) 50 g of white bread + 300 mL of probiotic-enriched dairy beverage (4) 50 g of white bread + 50 g of probiotic Minas Frescal cheese Probiotic concentration: 7–8 log cfu/g Subject type: healthy adults Number of subjects: 15 Type of study: repeated measure design	*In vitro:* Inhibition of α-glucosidase and α-amylase activities *In vivo:* Effects of different probiotic foods on postprandial glycemia	*In vitro:* Prato cheese presented the highest α-amylase and α-glucosidase inhibition *In vivo:* The consumption of Prato cheese resulted in a lesser increase in blood glucose level compared with the consumption of bread alone, Minas Frescal cheese, and whey dairy beverage, with glycemic indices similar to that observed for the control.	Grom et al. (2020)
In vitro	Effect of the *L. casei*-01 and inulin (10 g/100 g) addition on sheep milk ice cream during storage (−18 °C, 150 days)	(Control) 10% w/w sheep milk cream (Probiotic) 10% w/w sheep milk cream + *L. casei*-01 (Synbiotic) 10% w/w inulin + *L. casei*-01 Probiotic concentration: 6 log cfu/mL	Caco-2 cell adhesion Bioactivity	A high adhesion (>50%) of *L. casei*-01 to Caco-2 cells Increased ACE-inhibitory activity Increased antioxidant activity	Balthazar et al. (2018)
In vitro	Effect of the addition of *L. casei*-01 on the quality parameters of Minas Frescal cheese	(Treatment group) probiotic Minas Frescal cheese (*L. casei*-01) (Control group) probiotic-free Minas Frescal cheese (negative control) Probiotic concentration: 8.32 log cfu/g	Starter and probiotic counts Physicochemical parameters Water mobility (TD-NMR) Bioactivity Microstructure	Higher antioxidant and ACE inhibitory activities	Sperry et al. (2018)
In vitro	Effect of probiotic fermented sheep's milk added with *L. casei*-01 on enamel demineralization and microorganism counts in a mixed biofilm model	(1) Sheep milk (SM) (2) Fermented sheep milk with starter culture (FSMS) (3) Fermented sheep milk with probiotic culture (FSMP)		No prevention of hardness loss No differences in total microorganism or *Streptococcus* counts	Nadelman et al. (2019)

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Table 1 (*continued*)

Type of study	Objectives	Treatments	Assays	Main results	References
		(4) Fermented sheep's milk with starter and probiotic culture (FSMSP)	Percentage of surface hardness loss (%SHL) Total microorganism and *Streptococcus counts*	Probiotic product was not able to inhibit the growth of *Streptococcus* spp.	
		Probiotic concentration: 9.2–9.9 log cfu/g	Mean internal mineral density loss	Tendency to control enamel internal demineralization	
In vitro	The antiproliferative and apoptotic effects of probiotic whey dairy beverages in human prostate cancer cell lines (PC-3 and DU-145)	Conventional whey beverage without addition of probiotic strain (CTL)		Decrease in the PC-3 cells in G0/G1 and S Increase in G2/M phase (50.0 and 100.0 μg/mL)	Rosa et al. (2020)
		Whey beverages containing *Lactobacillus acidophilus* La-05, *Lactobacillus acidophilus* La-03, *Lactobacillus casei*-01, and *Bifidobacterium animalis* Bb-12	Cell viability	Extensive apoptosis induction in both cells' lines	
		Probiotic concentration: 7–8 log cfu/mL	Apoptosis	Whey beverage added with *L. casei*-01 might be a better candidate against prostate cancer cells	
In vitro	Impact of a two-week treatment with low-fat fermented goat milk with *L. casei*-01 and passion fruit by-product (1%) on the modulation of gut microbiota from obese individuals using the Simulator of Human Intestinal Microbial Ecosystem (SHIME) system	a) Adaptation (1 week), b) Treatment (2 weeks) and c) Washout (1 week) periods.	Microbial composition	Reduction of genera *Prevotella*, *Megamonas* and *Succinivibrio*	Casarotti et al. (2020)
		Treatment: 200 mL twice a day	Short-chain fatty acids	Increase of *Lactobacillus* and *Bifidobacterium* genera	
		Probiotic concentration: 6 log cfu/mL	Ammonia	No effect on short-chain fatty acids and ammonia	
In vitro	Effects of low-fat ice cream supplemented with *L. casei*-01 and *L. acidophilus* LA-5 on modulating of colon microbiome using an *in vitro* gut model	(TI) Low-fat ice cream	Short-chain fatty acids	Increased formation of beneficial microbial metabolites (propionate, acetate, lactic acid, and butyrate)	Chaikam and Rattanasen (2017)
		(TII) Low-fat ice cream + *L. casei*-01	Lactic acid	Decreased content of ammonia. Stimulation of lactobacilli and bifidobacteria	
		(TIII) Low-fat ice cream + *L. acidophilus*	Ammonia	Inhibition of clostridia and fecal coliforms	
		Probiotic concentration: 9 log cfu/mL	Microbial communities	Effect of L. *casei*-01 was more pronounced than that of *L. acidophilus* La-5	

the mechanisms underlying the antimicrobial activity of *L. casei*-01 should be better evaluated in future studies to close this gap.

The addition of *L. casei*-01 to ice creams (6 log cfu/mL) and cheeses (8.32 log cfu/g) also resulted in a higher inhibition of the angiotensin-converting enzyme (ACE) activity, which was associated with the proteolytic activity of this culture and formation of bioactive peptides (Balthazar et al., 2018; Sperry et al., 2018). In a clinical study (randomized double-blind pilot trial), the consumption of Minas Frescal cheese added with *L. casei*-01 (50 g for 28 days, 8.32 cfu/g) could improve the lipid profile, blood pressure, and hematological parameters of hypertensive overweight women ($n = 30$), as lower total cholesterol ($p = 0.018$), low-density lipoprotein-cholesterol ($p = 0.022$), and triacylglycerides ($p = 0.030$) levels, reduced diastolic ($p = 0.001$) and systolic ($p = 0.001$) pressure, and higher high-density lipoprotein-cholesterol ($p = 0.015$), and hemoglobin ($p = 0.012$) and hematocrit ($p = 0.001$) counts were observed compared to the women that consumed conventional Minas Frescal cheese (Sperry et al., 2018). The authors reported that the anti-hypertensive properties could be associated with the formation of bioactive peptides by probiotic cultures in the cheeses (Sperry et al., 2018). Furthermore, the ACE-inhibitory activity (*in vitro*) and anti-hypertensive properties (*in vivo*) were correlated (Sperry et al., 2018).

Whey beverages added with *L. casei*-01 (7–8 log cfu/mL) could act against prostate cancer cells (PC-3 and DU-145, Rosa et al., 2020), as decreases in the PC-3 cells in G0/G1 and S and increases in G2/M phase at concentrations of 50.0 and 100.0 μg/mL were observed ($p < 0.05$), as well as extensive apoptosis induction in both cells' lines. In this study, the whey beverage added with *L. casei*-01 showed higher inhibition of prostate cancer cells than whey beverages added with *L. acidophilus* La-05, *L. acidophilus* La-03, and *Bifidobacterium animalis* Bb-12. The *in vitro* tests indicate the possible mechanism of action of *L. casei* against cancer cells by decreasing their numbers and promoting apoptosis.

The addition of *L. casei*-01 to food products can result in a reduction of oxidative stress. Vasconcelos et al. (2019) evaluated the effect of probiotic prato cheese (50 g for 5 days, 9.33 log cfu/g) against oxidative and inflammatory damages in C57BL/6 male mice ($n = 40$) by exposure to cigarette smoke. The mice consuming the probiotic prato cheese had fewer bronchoalveolar lavage leukocytes, reactive oxygen species, and lipid peroxidation in the bronchoalveolar lavage and gut compared to mice consuming conventional cheese or regular chow ($p < 0.05$). Furthermore, they had lower lactate dehydrogenase levels in plasma, peroxynitrite expression, and inducible nitric oxide synthase ($p < 0.05$). The results of the mice exposed to cigarette smoke and that consumed probiotic prato cheese was similar to the mice that was not exposed to the smoke, demonstrating that the probiotic food reduced the oxidative stress (gut, liver, and lungs).

The consumption of probiotic (*L. casei*-01) prato cheese (2 g/day/animal of cheese for 25 days, 8.13 log cfu/mL) was also associated with the attenuation of the development of renal calculi in animal model (male *Rattus novergicus*, Wistar lineage, $n = 24$) with surgical implant of calcium oxalate tablets (CaOx) compared to those that consumed the conventional prato cheese (Martins et al., 2018). A reduction in the size of the pellets and excretion of minerals in the urine (potassium, calcium, and magnesium) was observed after probiotic cheese consumption ($p < 0.05$), and the prevention of kidney stone development was observed in the radiological examination. The results indicated attenuation of renal calculi development by the interaction of gut bacteria with the precursors of crystal formation. However, the mechanism of action was not completely evidenced, demonstrating a need for further investigations.

Dairy products with *L. casei*-01 (7–8 log cfu/g) could reduce the postprandial glycemia ($p < 0.01$) of healthy adults ($n = 15$) in a repeated measure design, being the effect dependent on the dairy matrix (Grom et al., 2020). Minas Frescal cheese (50 g), prato cheese (50 g) or dairy beverage (300 mL) were consumed with bread (50 g), and prato cheese had a better performance, resulting in a lower increase in the postprandial blood glucose compared to the consumption of only bread ($p < 0.01$). Prato cheese presented a more suitable chemical composition (higher protein and lipid contents) and, probably, a higher number of bioactive peptides formed during the ripening process by the *L. casei*-01. The consumption of dairy beverages also positively impacted the postprandial blood glucose, probably because of whey proteins.

However, previous studies observed that fermented dairy beverage consumption with *L. casei*-01 (0.1 mL for 30 days [10 days before infection and 20 days after infection], 8–9 log cfu/mL) did not have effect against *Salmonella enterica* ssp. *enterica* serovar Typhimurium infection in a murine model (female BALB/c mice, n = 30), as the product without the probiotic culture presented a higher survival of animals (70 *vs* 50%), lower translocation of the pathogen to the liver, and fewer lesions (liver and ileum) and inflammations (Cordeiro, Souza, et al., 2019). Furthermore, the consumption of yogurts (200 mL of yogurt within 48 h of beginning the antibiotic therapy and up to 5 days after stopping the antibiotic) added with *L. casei*-01 (7 log cfu/mL together with *Lactobacillus acidophilus* La-5 [6 log cfu/mL), *B. animalis* subsp. *lactis* Bb-12 [8 log cfu/mL]) did not protect hospitalized patients (n = 314, mean age 76 years) against antibiotic-associated diarrhea in a double-blind placebo controlled trial (Velasco et al., 2019), as the administration of the probiotic yogurt did not alter the duration of diarrhea, the maximum number of bowel movements, or admission because of diarrhea compared to the conventional yogurt ($p > 0.05$). Furthermore, the consumption of prato cheese (250 mg for 15 days, 9.47 log cfu/mL) with *L. casei*-01 did not ameliorate the symptoms of dextran sodium sulphate (DSS)-induced ulcerative colitis in mice (female C57/BL6 inbred mice, n = 30–36) (Cordeiro, Lemos, et al., 2019), as no reductions in the inflammation scores were observed compared to conventional prato cheese. Finally, Nadelman et al. (2019) observed that fermented sheep milk added with *L. casei*-01 (9.2 log cfu/mL) could not prevent *Streptococcus* proliferation in a dental biofilm model compared to conventional sheep milk ($p > 0.05$), suggesting that this strain was unable to compete with the pathogenic culture under the circumstances of the tooth surface. On the other hand, a tendency to control internal enamel demineralization was observed.

The human clinical trials with products added with *L. casei* demonstrated that it improved the lipid profile, blood pressure, and hematological parameters of hypertensive overweighed women, and the control of postprandial glycemia in healthy individuals, demonstrating its probiotic properties. Furthermore, *in vitro* and animal model tests indicate possible effects on intestinal modulation, oxidative stress, and attenuation of renal calculi development. However, clinical trials are needed to prove them. The mechanisms of action of *L. casei* may be related to the increase in the number of beneficial microorganisms (*Lactobacillus* and *Bifidobacterium*) in the gut, decrease of the number of harmful microorganisms (*Megamonas*, *Prevotella* and *Succinivibrio*), production of beneficial metabolites (propionate, acetate, lactic acid, and butyrate) and formation of bioactive peptides by probiotic cultures. Human clinical trials were restricted to only 3 studies (Grom et al., 2020; Sperry et al., 2018; Velasco et al., 2019), and only one was a double-blind placebo-controlled trial (Velasco et al., 2019). This demonstrated the need for further studies.

4.2. *L. casei-01* as paraprobiotic or postbiotics

The effect of paraprobiotic and postbiotics from *L. casei*-01 was evaluated by Liu et al. (2011). The authors studied the effect of cell fractions (heat-treated cells [paraprobiotic], crude cell walls, intracellular extracts), and exopolysaccharides (EPS) on the proliferation of human intestinal epithelial cells, human colon cancer cell (HT-29), and intestine cells (407). The paraprobiotic cells, crude cell walls, intracellular extracts, and EPS of *L. casei*-01 possessed antiproliferative activities on human colon carcinoma cell line HT29 and the ability to reduce the cytotoxicity of 4-nitroquinoline 1-oxide (4-NQO) against intestine 407 cells. EPS provided the highest antiproliferation in the cancer cells and anticytotoxic activity. Therefore, EPS could exert bioanticytotoxic and blocking effects by both repairing the 4-NQO-damaged cells and adjusting the function of intestine 407, thus reducing cytotoxicity of 4-NQO.

Other studies have focused on the positive effects of *L. casei* as paraprobiotics, but they did not include the strain *L. casei*-01. Furthermore, no study evaluated it as a postbiotics source (Cukrowska et al., 2010; Ostad et al., 2009). In this sense, it is worth pointing out that considering the importance of this strain and its wide industrial application, researches on this field are important to increase the possibilities of consumers' access to the reported effects. Future studies are also needed to assess the *L. casei*-01 postbiotics profile and their safety dose. They may represent a valid and safer alternative to avoid risks related to live probiotic bacteria, conferring to postbiotics specific practical applicability and functionality to become a prominent strategy.

5. *Impact of the addition of* L. casei-*01 on the quality parameters of food products*

Fig. 3 summarizes the main effects of *L. casei*-01 on the quality parameters of food products. The survival of *L. casei*-01 and the impact on the quality parameters of food products is dependent on the processing steps, food matrix, ingredients used, the form of addition, the concentration of the probiotic culture, presence of starter cultures, type of package, storage temperature, and addition of prebiotic components, among others. Table 2 presents the main studies assessing the impact of probiotic strain *L. casei*-01 on quality parameters and its survival in food matrices.

5.1. *Probiotic survival*

The minimum probiotic population suggested in the food at the time of consumption is 10^6-10^7 cfu/mL or g (Hill et al., 2014). Recently, there was a tendency to consider a minimum concentration of 10^9 cfu per food portion to compensate 1–2 log decrease during digestion (Ranadheera et al., 2019). However, it is important to mention that the probiotic viability should be defined based on the expected health effect and considering the food carrier. Suitable *L. casei*-01 counts during storage have been observed in yogurts (Costa, Paula, et al., 2019; Januário et al., 2017; Pimentel et al., 2012), cheeses (Oliveira et al., 2014; Sperry et al., 2018; Vasconcelos et al., 2019), dairy flans (Mantovani et al., 2020), milk beverages (Alves et al., 2020; Mantovani et al., 2020), ice creams (Balthazar et al., 2018), juices (Costa et al., 2017; Miranda, Paula, et al., 2019; Okina et al., 2018; Santos et al., 2019), soyogurt (Kemsawasd & Chaikham, 2018), riceberry and sesame-riceberry milk ice creams (Kemsawasd & Chaikham, 2020), among other products. Therefore, in a general view, *L. casei*-01 is resistant in a diversity of food matrices.

Food processing can have steps that contribute positively or negatively to the *L. casei*-01 survival. The presence of a fermentative step can increase the probiotic counts in the products. Mantovani et al. (2020) added *L. casei*-01 at the same concentration (0.2 g/kg) to chocolate flan, passion fruit flan, and chocolate fermented milk beverage. They observed higher counts in the latter, mainly because the probiotic culture participated in the fermentative process, a step presented only in this food matrix. The overrun, a step present in the ice cream processing, can decrease the *L. casei*-01 viability, mainly because of the incorporation of oxygen in the matrix (Balthazar et al., 2018).

The type of starter culture and its form of use could impact the survival of the *L. casei*-01. El-Dieb et al. (2012) evaluated the effect of the type of the starter culture (YC-Fast 1, YC-380 and YC-180) and its form (live or heat-shocked) on the *L. casei*-01 survival in bio-yogurts. The type of starter culture did not impact probiotic viability, but the utilization of heat shock starter culture enhanced it. The competition for

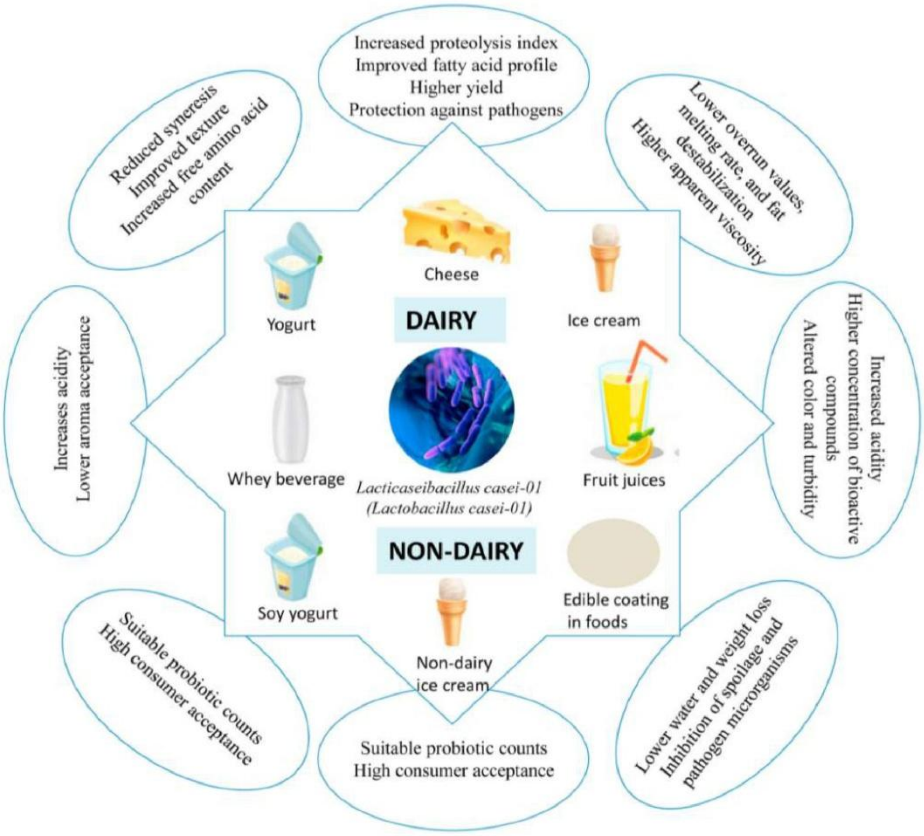

Fig. 3. Main technological effects of *Lactobacillus casei*-01 in dairy and non-dairy products. Images: Freepik.

nutrients between *L. bulgaricus* and *S. thermophilus* from the starter culture and *L. casei*-01 probably inhibited the probiotic culture. Furthermore, the microorganisms of the starter culture may have produced antimicrobial substances that affected probiotic survival. Therefore, in using live starter cultures, it is advisable to evaluate its compatibility with *L. casei*-01 in the product.

The chemical composition of the food matrix has an important influence on the *L. casei*-01 survival. In a general view, products with a solid structure, high pH (close to neutral), and without starter cultures (competitive microorganisms) present a higher probiotic survival (Mantovani et al., 2020). Furthermore, the presence of citric acid (can be used as energy source), vitamin C (can reduce the dissolved oxygen), and fibers (can provide physical protection) can improve the *L. casei*-01 survival (Miranda, Paula, et al., 2019). Finally, concentrations of proteins higher than 0.3% are recommended, as *L. casei*-01 requires proteins and amino acids for its maintenance (Okina et al., 2018). However, it is important to mention that the probiotic viability should be evaluated in each food matrix.

The ingredients used in the food formulation can impact positively or negatively on the *L. casei*-01 survival. The addition of fruits and derivatives should be carefully studied, as they can inhibit the probiotic culture due to the acidity and presence of carotenoids, fatty acid esters, and aroma compounds (Mantovani et al., 2020). The addition of green tea to the milk or mulberry leaf extract to soymilk could stimulate the growth, acidification, and metabolism of *L. casei*-01, resulting in higher counts (Kemsawasd & Chaikham, 2018; Ma et al., 2015). Prebiotic components (inulin, oligofructose, polydextrose, among others) can be added to food products to increase the probiotic survival during storage, as they are substrates available for the probiotic cultures, and can provide a physical protection (Balthazar et al., 2018; Pimentel, Madrona, Garcia, & Prudencio, 2015). However, it is important to mention that other studies reported no impact of the prebiotic components on the *L. casei*-01 viability (Costa et al., 2017; Miranda, Silva, et al., 2019; Santos et al., 2019). The positive effect seems to be related to the food matrix's conditions, as the prebiotic components helped the *L. casei*-01 in products with unsuitable chemical composition (low protein content) or harsh conditions (low pH values, high acidity, and high dissolved oxygen).

The food packaging material has an important role in the *L. casei*-01 viability, as this strain is microaerophilic, and, thus, the exposure to

Table 2
Main studies assessing the impact of probiotic strain *Lacticaseibacillus casei*-01 on quality parameters and its survival in food matrices.

Objectives	Matrices Dairy and non-dairy products	Assays	Main results	References
Effect of the addition of *L. casei*-01 and/or long-chain inulin (20 g/L) on the quality parameters of yogurts during refrigerated storage	Yogurt (Whole) Whole milk (Skimmed) Skimmed milk (Prebiotic) Skimmed milk + inulin (Probiotic) Skimmed milk + *L. casei*-01 (Synbiotic) Skimmed milk + *L. casei*-01 + inulin Probiotic concentration: 8 log cfu/mL	Physical and chemical characteristics Texture parameters Probiotic culture survival Sensory acceptance	No effect on the pH, titratable values, sensory profile, and sensory acceptance Less syneresis Increased firmness and gumminess Suitable probiotic counts (>10^7 cfu/g) during storage	Pimentel et al. (2012, 2013)
Effect of the addition of *L. casei*-01, *L. acidophilus* La-5 and/or *B. lactis* BB-12 on the quality of a Brazilian goat semi-hard cheese (Coalho) during 21 days of storage at 10 °C	Goat semi-hard cheese (C1) Conventional, starter culture) (C2) Starter + *L. acidophilus* La-5 (C3) Starter + *L. casei*-01 (C4) Starter + *B. lactis* BB-12 (C5) Starter + *L. acidophilus* La-5 + *L. casei*-01 + *B. lactis* BB-12) Probiotic concentration: 7 log cfu/g	Yield Gross composition Physicochemical analysis Proteolysis and meltability Texture profile Color parameters Sensory evaluation	Higher yield Higher syneresis (day 21) Higher titratable acidity (day 21) Higher acceptance (appearance, flavor, taste, texture, and general impression)	Oliveira et al. (2012)
Effect of the addition of *L. casei*-01 (10^8 cfu/g) on the quality parameters of Minas Frescal cheese	Minas Frescal cheese (Treatment group) Probiotic Minas Frescal cheese (*L. casei*-01) (Control group) Probiotic-free Minas Frescal cheese (negative control) Probiotic concentration: 8.32 log cfu/g	Starter and probiotic countsn Physicochemical parameters Water mobility (TD-NMR) Bioactivity Microstructure	No change in the proximate composition and microstructure More suitable fatty acid profile Lower pH Higher proteolysis, and organic acid levels Suitable probiotic counts (>10^8 cfu/g)	Sperry et al. (2018)
Effect of the *L. casei*-01 (10^6 cfu/mL) and inulin (10 g/100 g) addition on sheep milk ice cream during storage (−18 °C, 150 days)	Sheep milk ice cream (Control) 10% w/w sheep milk cream (Probiotic) 10% w/w sheep milk cream + *L. casei*-01 (Synbiotic) 10% w/w inulin + *L. casei*-01 Probiotic concentration: 6 log cfu/mL	Probiotic count Survival after SGIC Caco-2 cell adhesion Bioactivity and microstructure Physical characteristics (color parameters, thermal analysis and organic acids/volatile compounds)	Suitable probiotic counts (>10^6 cfu/mL) during storage Lower overrun, melting rate, fat desestabilization Higher apparent viscosity No effect on the color parameters Production of several volatile compounds, such as carboxylic acids, alcohols, aldehydes and ketones Interaction between probiotic bacteria and inulin	Balthazar et al. (2018)
The efficacy of probiotic Prato cheese against the inflammatory and oxidative damage in mice organs induced by cigarette smoke exposure	Prato cheese (C) Conventional (with starter bacteria only) (PC) Probiotic (with starter and probiotic bacteria) Probiotic concentration: 9.33 log cfu/g	Probiotic viability Gross composition and minerals	No effect on the gross composition and minerals Suitable probiotic counts (>10^8 cfu/g)	Vasconcellos et al. (2019)
The impact of the addition of *L. casei*-01 and/or carbonation (CO_2) on the chemical composition, physicochemical characteristics, probiotic survival, and sensory acceptance of passion-fruit flavored whey dairy beverages (70% milk/30% whey) during storage (30 d/4 °C)	Whey dairy beverage (CONTROL) Without L. *casei*-01 or carbonation (PRO) With L. *casei*-01 (CARB) With carbonation (PRO-CARB) With L. *casei*-01 and carbonation Probiotic concentration: 7.2–7.7 log cfu/mL	Physical and chemical characteristics Probiotic culture survival Sensory acceptance	No impact on the chemical composition, pH values, and acceptance (flavor and overall impression) of the products, but increased the acidity, and decreased the aroma acceptance	Alves et al. (2020)
Effect of the addition of oligofructose (20 g/L) as sugar substitute and *L. casei*-01 (10^8 cfu/mL) as a probiotic on the physicochemical characteristics, probiotic viability and on the acceptability during refrigerated	Apple juice (PUR) Pure (SAC) Sucrose (PRE) Oligofructose (PRO) Probiotic	Physical and chemical characteristics Texture parameters	No effect on the chemical composition, density, acceptability and purchase intent Glass package more appropriate than the plastic package in maintaining the viability of the probiotic culture, with no effect on the physicochemical characteristics and acceptability of juices	Pimentel, Madrona, Garcia, and Prudencio (2015)

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Table 2 (*continued*)

Objectives	Matrices Dairy and non-dairy products	Assays	Main results	References
storage (4 °C for 28 days) in plastic or glass packages	(SYNB) Oligofructose + probiotic		Suitable probiotic counts (>10^6 cfu/mL) during storage for 14 (PRO-Plastic), 21 (SYNB-Plastic and PRO-Glass) and 28 (SYNB-Glass) days	
	Glass or plastic packages	Probiotic culture survival		
	Probiotic concentration: 8 log cfu/mL	Sensory acceptance		
Effect of the addition of oligofructose (20 g/L) or sucralose (0.03 g/L) as sugar substitutes and *L. casei*-01 as a probiotic on the sensory profile and acceptance of clarified apple juice	Apple juice (PUR) Pure (SAC) Sucrose (PRE) Oligofructose (PRO) Probiotic (SYNB) Oligofructose + probiotic (SUC) Sucralose			Pimentel, Madrona, and Prudencio (2015)
	(SUC–P) Sucralose + probiotic	Sensory profile	Probiotic increased the turbidity of the juice	
	Probiotic concentration: 8 log cfu/mL	Sensory acceptance	No impact on the acceptance (appearance, aroma, flavor, texture and overall impression)	
Effect of the addition of *L. casei*-01 and/or oligofructose (20 g/L) and/or ascorbic acid (0.24 g/L) on the quality parameters of orange juice during refrigerated storage	Orange juice (PURE) Pure juice (PRO) Juice added probiotic culture (ACID) Juice added acid ascorbic			Costa et al. (2017)
	(PRE) Juice added oligofructose	Physical and chemical characteristics		
	(PRO-ACID) Juice added probiotic culture and ascorbic acid	Texture parameters	Juices with probiotic culture showed physicochemical characteristics, acceptance and storage stability similar to the pure juices; but lower turbidity and yellow color	
	(SYNB) Juice added probiotic culture and oligofructose	Probiotic culture survival	Suitable probiotic counts (>10^6 cfu/mL) during storage	
	Probiotic concentration: 7 log cfu/mL	Sensory acceptance	No effect on the sensory acceptance	
The influence of mulberry leaf extract (MLE) on viability of *L. casei*-01 and *L. acidophilus* LA-5 in soyoghurt during refrigerated storage (4 °C) for 30 days	Soyoghurt (Control) plain-soyoghur) (LC-S) soyoghurt + *L. casei*-01			Kemsawasd and Chaikham (2018)
	(LA-S) soyoghurt + *L. acidophilus* LA5	Physiochemical (DPPH-radical scavenging activity assay, FRAP assay, total flavonoids, pH, syneresis, water holding capacity)		
	(LC-SM and LA-SM) soyoghurts + probiotics + MLE	Survival probiotics	MLE: increased antioxidative effects of probiotic-soyoghurts and minimized probiotic cell loss over prolonged storage	
	Probiotic concentration: 8.7–8.9 log cfu/mL	Sensory evaluation	All samples showed similar scores for all sensory attributes regardless MLE or probiotic	
The effect of the probiotic addition (*L. casei*-01, 2% of the biomass) on the physicochemical characteristics and antioxidant capacity of white grape juice during refrigerated storage (4 °C/28 days)	White grape juice	Physicochemical characteristics, antioxidant capacity and probiotic survival (product and in SGIC)	Probiotic addition altered the color parameters (darker-reddish coloration) and decreased the total phenolic compounds	Okina et al. (2018)
	(GJ) control		Improved the color stability and maintained the antioxidant activity	
	(GJP) probiotic culture		Suitable probiotic viability (>10^9 cfu/200 mL) during 21 days of storage in the products	
	Probiotic concentration: 12 log cfu/200 mL		Survival for 28 days of refrigerated storage after SGIC	
Effect of coatings with L. casei-01 and Bifido*bacterium* strains on the quality and safety of packed sliced ham foreseeing extension of shelf-life and a potential carrier for viable probiotic cells	Ham (Control) Without bacteria		Decreased water and weight loss	Pereira et al. (2018)
	(Cba) With *B. animalis* Bb-12®	The viability of incorporated probiotic bacteria	No color change	
	(Clc) With L. *casei*-01	Sliced ham characterization (water activity, color, weight loss, pH)	Consumer: preference for the sliced coated ham	
	Probiotic concentration: 8 log cfu/mL	Consumer study	Suitable probiotic counts (>10^8 cfu/mL) during storage	
Effect of the direct addition of the freeze-dried *L. casei*-01 and prebiotics on the	Sugarcane juice (PUR) Pure juice			Santos et al. (2019)

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Table 2 (*continued*)

Objectives	Matrices Dairy and non-dairy products	Assays	Main results	References
physicochemical characteristics, probiotic survival and on the acceptance of pasteurized sugarcane juice	(PRO) With L. *casei*-01 (OLIGO) With oligofructose (POLY) With polydextrose		Increased the acidity, turbidity, luminosity and green color	
	(SYNB–O) With oligofructose + *L. casei*-01	Physicochemical evaluation (moisture, protein, lipid, ash, carbohydrate, pH and TSS)	Decreased the acceptance	
	(SYNB–P) With polydextrose + *L. casei*-01	Texture parameters (firmness, consistency, cohesiveness and viscosity)	Suitable probiotic counts (>10^9 cfu/mL) during storage	
	Probiotic concentration: 9 log cfu/mL	Probiotic survival	Directly adding LC-01 (easier and faster manufacturing process, high probiotic counts)	
Evaluate the effect of the probiotic addition methodology (10^8 cfu/mL, direct addition of the lyophilized commercial culture, probiotic activated or probiotic encapsulated) on the physical and chemical characteristics and sensory acceptance of orange juice during refrigerated storage	Orange juice	Physical and chemical characteristics		Miranda, Paula, et al. (2019)
	(PURE) Pure juice	Rheological parameters	Direct: Characteristics and sensory acceptance similar to pure	
	(DIRECT) Lyophilized commercial culture	Volatile compounds profile	Activated: Higher content of organic acids, absence of important volatile compounds and lower sensorial acceptance	
	(ACTIVATED) Probiotic activated by propagation	Probiotic culture survival	Encapsulated: More consistent products, decreased organic acid	
	(ENCAPSULATED) Probiotic encapsulated in alginate	Sensory acceptance of	content and lower sensorial acceptance	
	Probiotic concentration: 8 log cfu/mL	orange juice	Suitable probiotic counts (>10^7 cfu/mL) during storage	
Effect of the addition of *L. casei*-01 (2 g/L) and/or oligofructose (20 g/L) on the quality parameters of orange juice and hibiscus tea mixed beverage during refrigerated storage	Orange juice and hibiscus tea mixed beverage (PUR) No addition			Miranda, Silva, et al. (2019)
	(PRO) With 2 g/L of the *L. casei*-01	Physical and chemical characteristics		
	(PRE) With 20 g/L of oligofructose	Rheological parameters	Probiotic addition increased the turbidity and altered the color	
	(SYN) With 2 g/L of the *L. casei*-01 probiotic culture and 20 g/L of oligofructose	Probiotic culture survival	Probiotic addition improved the nutritional value and the stability of the rheological parameters and had no impact on the sensory acceptance	
	Probiotic concentration: >7 log cfu/mL	Sensory acceptance	Suitable probiotic counts (>10^7 cfu/mL) during storage	
Evaluation of the influence of the dairy matrix on the survival of *L. casei*-01 during refrigerated storage and survival under SGIC	Chocolate fermented milk beverage			Mantovani et al. (2020)
	Chocolate flan	Physicochemical analysis (pH, moisture, lipids, protein, ash and carbohydrates)		
	Passion fruit flan	Microorganisms viability during refrigerated storage	All matrices provided suitable counts of *L. casei*-01 during storage	
	Probiotic concentration: 6.65–7.72 log cfu/g	Probiotic survival under SGIC	The type of matrix had impact on the initial probiotic counts in the products and on the probiotic resistance to the SGIC	
Examine the changes of probiotic populations in riceberry and sesame-riceberry milk ice creams	Riceberry milk ice cream (LC-R) Riceberry milk ice cream (LC-RI) Riceberry milk ice cream containing inulin			Kemsawasd and Chaikham (2020)
	(LC-SR) Sesame-riceberry milk ice cream		*L. casei*-01 was more resistant to frozen storage compared to *L. acidophilus* LA-5	
	(LC-SRI) Sesame-riceberry milk ice cream containing inulin	Probiotic survival, pH and titratable acidity	Suitable probiotic counts (>10^6 cfu/mL) during storage (4 °C/30 days)	
	Probiotics used: *L. casei*-01 and *L. acidophilus* LA-5	Antioxidant activity	Suitable probiotic counts (>10^5 cfu/mL) during SGIC	
	Probiotic concentration: 10 log cfu/mL	Sensory evaluation	Suitable sensory acceptance (scores higher than 7 in a 9-point hedonic scale)	

oxygen can decrease its survival. The utilization of glass packages favored the *L. casei*-01 survival due to the low oxygen permeability, and they were used in most of the studies (Costa et al., 2017; Miranda, Paula, et al., 2019; Okina et al., 2018; Santos et al., 2019). Pimentel, Madrona, Garcia, and Prudencio (2015) evaluated the effect of the type of package (glass or polyethylene terephthalate [PET]) on the quality parameters and *L. casei*-01 survival in apple juices and observed that the glass package was more appropriate to maintain the viability of the probiotic culture, with no effect of the type of package on the physicochemical characteristics and sensory acceptance. The result was associated with the higher permeability of the plastic to oxygen, resulting in decreased *L.* casei-01 survival. The products' shelf life could be increased in 7 days using the glass packages (based on probiotic counts > 10^6 cfu/mL). As the industries prefer plastic packages, the authors suggest utilizing material with selective permeability, packages with increased thickness, or the addition of oxygen absorbers to the package.

The encapsulation of the *L. casei*-01 can result in increased survival, but the effect is dependent on the food matrix and the material used as encapsulant agent. The utilization of alginate in the microencapsulation had no protective effect on *L. casei*-01 in orange juices, mainly because of its porous structure, which allows acid diffusion inside the capsules (Miranda, Paula, et al., 2019). On the other hand, the utilization of alginate beads coated with chitosan at 1.5% increased the probiotic counts by approximately 0.4 logs in orange juice (Krasaekoopt & Watcharapoka, 2014).

The probiotic culture should survive the simulated gastrointestinal conditions (SGIC) in sufficient numbers (10^6-10^7 cfu/mL or g), but survivals of 10^2-10^5 cfu/mL or g could be considered satisfactory (Milette et al., 2013). Products with lower pH values generally result in a lower probiotic survival, mainly because the cells are more injured in these products (Mantovani et al., 2020). *L. casei*-01 survival to SGIC was observed in previous studies with white grape juice (Okina et al., 2018), ice cream (Balthazar et al., 2018), yogurts (Costa, Paula, et al., 2019), dairy flans and chocolate whey beverage (Mantovani et al., 2020), cheese (Oliveira et al., 2014), riceberry and sesame-riceberry milk ice creams (Kemsawasd & Chaikham, 2020), and soyogurt (Kemsawasd & Chaikham, 2018). Therefore, in a general view, *L. casei*-01 is resistant to the SGIC.

5.2. *Technological and sensory properties*

5.2.1. *Dairy products*

The probiotic culture is commonly added to dairy products in the freeze-dried form and after the pasteurization step (Costa, Paula, et al., 2019; Januário et al., 2017; Pimentel et al., 2012). However, in some cases, it can be added as supplement (Ranadheera et al., 2019). *L. casei*-01 generally has no impact on the chemical composition (moisture, protein, fat, carbohydrates) and mineral content (sodium and calcium) of dairy products (Sperry et al., 2018; Vasconcelos et al., 2019).

The impact of the *L. casei*-01 on the acidity (pH and titratable acidity values) is dependent on the food matrix, as no effect was observed in yogurts (Pimentel et al., 2012), while decreased pH values and/or higher acidity (higher lactic and acetic acids levels) were observed in Minas Frescal cheeses (Sperry et al., 2018) and whey beverages (Alves et al., 2020). Therefore, during the fermentation step or storage, the *L. casei*-01 probably used lactose to form the organic acids (Sperry et al., 2018). The impact of the increased acetic acid content on the sensory acceptance needs evaluation, as this compound can promote a pungent and vinegar flavor to the products (Miranda, Paula, et al., 2019). It is also important to evaluate if the higher acidity of the products added with *L. casei*-01 could increase their shelf life, which would be of technological importance, mainly for fresh cheeses that present short shelf life.

In yogurts, *L. casei*-01 could reduce syneresis, which is the most important defect in this type of product. This effect was attributed to the production of EPS by the probiotic culture, which can act as a food stabilizer, contributing to the product's structure and preventing wheying off (Pimentel et al., 2012). Furthermore, an increase in the firmness and gumminess was observed, as the EPS can increase the viscosity, interaction of the food components, and water retention (Pimentel et al., 2012). These results are interesting for the industries, as the consumers prefer yogurts without visible whey and with increased consistency, mainly because of the several Greek-type yogurts available in the market. *L. casei*-01 could also increase the free amino acid content in fermented milks supplemented with green tea, which are the most important precursors of volatile aroma compounds (Ma et al., 2015).

In cheeses, the proteolysis index can be increased by *L. casei*-01 addition, which is positive, contributing to increasing the digestibility of the products and forming bioactive compounds. However, it is important to control this parameter, as the free amino acids can alter the cheese flavor (bitter, malty, sweet) and/or originate volatile compounds, resulting in off-flavors (Sperry et al., 2018). *L. casei*-01 is also able to alter the fatty acid profile of the products, with increases in the levels of medium and long-chain fatty acids, monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). Therefore, the probiotic addition increases the unsaturation degree of the fatty acids, which is considered a universal conserved adaptation of this type of microorganism and is interesting from the health point of view (Sperry et al., 2018). The yield of cheeses can be increased by *L. casei*-01 addition (Oliveira et al., 2012). Finally, *L. casei*-01 could be used as protective culture for delaying the growth of *S. aureus* and *L. monocytogenes* in goat coalho cheese, as this culture presented the highest inhibition rates compared to cheeses inoculated with *B. lactis* and *L. acidophilus* (Oliveira et al., 2014).

In ice creams, the product added with *L. casei*-01 had lower overrun, melting rate, fat destabilization values, and higher apparent viscosity (Balthazar et al., 2018). The lower melting rate is important because a product that melts fast can cause discomfort and an undesirable situation to the consumers during consumption. However, lower overrun values are associated with dense and heavy ice creams (Silva et al., 2020). It is important to mention, however, that the differences observed in the study could not be only attributed to the addition of *L. casei*-01, as the milk used in the conventional ice cream was not fermented, while the milk used in probiotic ice cream was fermented. The utilization of fermented milk provided lower incorporation of air, resulting in a more compact structure that melted in a longer time (Balthazar et al., 2018). Therefore, more studies are necessary to evaluate the impact of the addition of *L. casei*-01to ice creams.

The effect of the addition of *L. casei*-01 on the sensory characteristics is dependent on the food matrix. Probiotic and conventional yogurts were similar on the color, brightness, syneresis, firmness (appearance and texture), homogeneity (appearance and texture), acid aroma, fermented milk aroma, acid taste, sweet taste, creaminess, and sensory acceptance to the conventional product (Pimentel et al., 2013). Oliveira et al. (2012) reported that the goat coalho cheese added with *L. casei*-01 had higher acceptance on appearance, flavor, taste, texture, and general impression than the conventional cheese. The improved sensory characteristics were associated with the higher proteolysis in the probiotic products, with the formation of flavor compounds and alterations on the texture parameters. However, Alves et al. (2020) reported no effect of *L. casei*-01 addition on the acceptance in flavor and overall impression of passion-fruit whey dairy beverages; however, it decreased the aroma acceptance.

5.2.2. *Non-dairy products*

In non-dairy products, such as fruit juices, *L. casei*-01 is usually added as an activated culture. For that, the freeze-dried probiotic culture is incubated at suitable conditions (usually 37 °C/15 h, three times) in Man Rogosa and Sharp (MRS) broth (Pimentel, Madrona, Garcia, & Prudencio, 2015) or orange juice (Costa et al., 2017), separated in a refrigerated centrifuge, and added to the juices as a biomass (Okina et al., 2018) or in a saline solution (Costa et al., 2017; Pimentel, Madrona, Garcia, & Prudencio, 2015) after the heat treatment. This methodology promotes minor changes in the products, but the activation process is long (24–54 h), and specific equipment (refrigerated centrifuge) is needed to separate the biomass, which precludes the manufacture of probiotic fruit juices by small and medium-sized industries (Costa et al., 2017). Recently, there was a tendency of including *L. casei*-01 in the freeze-dried form after the heat treatment, which can reduce the processing time and the post-processing contamination (Miranda, Paula, et al., 2019).

The addition of *L. casei*-01 has no impact on the chemical composition (moisture, protein, carbohydrates, fat) of the non-dairy products (Costa et al., 2017; Miranda, Silva, et al., 2019; Santos et al., 2019). Furthermore, several studies reported no effect on the sensory acceptance of the products such as orange-juice-hibiscus tea mixed beverage

(Miranda, Silva, et al., 2019), apple juice (Pimentel, Madrona, & Prudencio, 2015), orange juice (Costa et al., 2017), and soyogurt (Kemsawasd & Chaikham, 2018). Pimentel, Madrona, and Prudencio (2015) evaluated the sensory profile of apple juices added with *L. casei*-01 using Quantitative Descriptive Analysis and observed no effect on the evaluated attributes (color, presence of particles, body, apple aroma, sweet aroma, apple flavor, sweet taste, sour taste, and aftertaste), but an increase in the turbidity occurred. However, other studies observed a negative impact of probiotic addition on the products' sensory acceptance (Miranda, Paula, et al., 2019; Santos et al., 2019). The impact of *L. casei*-01 on sensory acceptance seems to be related to the food matrix, the form of probiotic addition, and the probiotic concentration.

The effect of the form of addition of *L. casei*-01 (10^8 cfu/mL, freeze-dried, activated, or microencapsulated in alginate) on the quality parameters of orange juices was evaluated (Miranda, Paula, et al., 2019). The addition of the freeze-dried probiotic culture was considered the most suitable technique, as the probiotic product was similar to the conventional product (physical and chemical characteristics and sensory acceptance). The addition of the probiotic in the activated form (3 incubations, 37 C/15 h, included in the saline solution) increased the organic acid content (lactic, acetic, and citric), and decreased the sensory acceptance. Finally, the microencapsulated probiotic culture's addition increased the consistency, decreased the content of organic acids, and decreased sensory acceptance.

The effect of the concentration of the *L. casei*-01 on the quality parameters of non-dairy products is associated to the food matrix. Probiotic additions of 10^6-10^{10} cfu/g or mL were observed in previous studies, with no significant impact on the sensory acceptance for riceberry and sesame-riceberry milk ice creams (10^{10} cfu/g, Kemsawasd & Chaikham, 2020), orange juice and hibiscus tea mixed beverage (10^7 cfu/mL, Miranda, Silva, et al., 2019), soyoghurt (10^8 cfu/g, Kemsawasd & Chaikham, 2018), orange juice (10^7 cfu/mL, Costa et al., 2017), and apple juice (10^8 cfu/mL, Pimentel, Madrona, Garcia, & Prudencio, 2015). However, in sugar cane juice, *L. casei*-01 addition (10^9 cfu/mL) increased the acidity, turbidity, luminosity, and green color, and decreased the acceptance during the storage period (Santos et al., 2019).

The impact of the addition of *L. casei*-01 on the acidity (pH values and titratable acidity) of non-dairy products is controversial, as no impact was observed in orange juice and riceberry and sesame-riceberry milk ice creams (Costa et al., 2017; Kemsawasd & Chaikham, 2018; Miranda et al., 2019a, 2019b), while higher acidity was observed for apple and sugar cane juices (Pimentel, Madrona, Garcia, & Prudencio, 2015; Santos et al., 2019). These results may be related to the products' buffering capacity (Miranda, Paula, et al., 2019). The higher acidity is associated with the metabolic activity of the *L. casei*-01 during fermentation (in the case of fermented products) or storage of the products and mainly associated with the consumption of sugars and production of organic acids (Mantovani et al., 2020), such as lactic acid, acetic acid, and citric acid (Miranda, Paula, et al., 2019). The increased acidity had no impact on the sensory acceptance of apple juices (Pimentel et al., 2015a, 2015b), but decreased the acceptance of sugarcane juices (Santos et al., 2019).

The addition of *L. casei*-01 can result in an increase in the bioactive compounds (total phenolic compounds [TPC] content and vitamin C) and antioxidant activity of the products. In some cases, the higher concentration of the bioactive compounds was associated with the probiotic products' increased acidity, as the phenolic compounds are more stable at this condition (Santos et al., 2019). Furthermore, *L. casei*-01 has enzymes and can produce acids that promote the degradation of the fruit cell materials, helping release the phenolics from the complexed forms, increasing their concentration (Miranda, Silva, et al., 2019). This is important from the health and technological points of view. The phenolic compounds have antioxidant activity, and they are associated with the color, aroma, astringency, and oxidative stability of some products (Santos et al., 2019). Furthermore, it can be considered a cost-effective type of biofortification, with potential benefits for the manufacturers and consumers (Ranadheera et al., 2019). However, other studies reported no effect on the TPC or vitamin C contents and/or antioxidant activity (Costa et al., 2017; Kemsawasd & Chaikham, 2018; Miranda, Paula, et al., 2019).

The impact of the *L. casei*-01 addition on the color and turbidity of the products depends on the food matrix, the form of addition, and probiotic survival. Alterations on these parameters were reported for fruit juices (Costa et al., 2017; Pimentel, Madrona, Garcia, & Prudencio, 2015; Santos et al., 2019). In clarified fruit juices (apple and white grape juices), the probiotic addition increased the turbidity values (Okina et al., 2018; Pimentel, Madrona, Garcia, & Prudencio, 2015), while in cloudy products (orange juice), a decrease in this parameter occurred (Miranda, Paula, et al., 2019). Products with a low *L. casei*-01 survival have, generally, higher alterations on the color parameters, as the accumulation of lysed or killed probiotic cells alters these characteristics (Costa et al., 2017).

The addition of *L. casei*-01 to food products generally has no impact on the texture parameters (Costa et al., 2017; Santos et al., 2019) or rheological properties (Miranda et al., 2019a, 2019b). However, the addition of the probiotic culture in the microencapsulated form can result in a more consistent product because of larger particles (Miranda, Paula, et al., 2019). This increased consistency is associated with decreases in the sensory acceptance of the products' texture (Miranda, Paula, et al., 2019). Therefore, when using microencapsulated probiotic cultures, it is advisable to use capsules of small size.

The L. *casei*-01 probiotic culture can contribute positively or negatively to the aroma profile of fruit juices. The formation of α-terpineol (moldy and pungent aroma and oxidized taste) was observed, and 1-nonanol (fat aroma) in orange juices. However, decanal (citrus aroma and coriander notes), 1-hexanol (fruity aroma), α-phellandrene (fruit and mentholated notes), octanal and nonanal (typical aroma), *o*-cimene (fruity and lemon aroma), alpha-cubebene (herbaceous and waxy), and gamma-terpinene (sweet and citrus aroma) were also observed (Miranda, Paula, et al., 2019). The form of addition, probiotic concentration, and food matrix are variables that impact the observed results.

5.2.3. Edible coatings

L. casei-01 could be included in the coating and used in food products. Pereira et al. (2018) used a coating of whey protein isolates added with *L. casei*-01 to preserve sliced ham during storage (45 days at 4 °C). Probiotic counts were maintained higher than 10^8 cfu/g throughout storage time, enabling the slice of ham to act as a suitable carrier for the *L. casei*-01. The utilization of the coating resulted in products with lower water and weight loss. There was inhibition of *Staphylococcus* spp., Enterobacteriaceae, *Pseudomonas* spp., and yeasts/molds during storage. The consumers preferred the ham with the coating. Pruksarojanakul et al. (2020) used a coating of konjac glucomannan added with *L. casei*-01 and inulin to preserve bread buns during storage (8 days at room temperature). Probiotic counts were maintained higher than 10^7 cfu/portion throughout storage time. There was inhibition of molds, and no effect on the color parameters. Dianin et al. (2019) used a coating of whey protein isolate with *L. casei*-01 to preserve tomatoes and grapes during storage (21 days at 25 °C). Suitable probiotic counts ($>10^6$ cfu/g) were observed for 14 days, as well as a positive effect on the ripening process of the tomatoes and grapes (lower total soluble solids values).

6. Perspectives and future studies

L. casei-01 demonstrated to have well diffused applications in food industry as an ingredient with technological properties and proven benefits. Solid information is available about the positive effects of *L. casei*-01 on human and/or animal health through modulation of intestinal microbiota, activity against pathogenic bacteria, or metabolic effects to attenuate impacts of chronic diseases as hypertension and dyslipidemia, among others. The impact of the addition of *L. casei*-01 on the quality parameters of food products discloses enormous suitability from this strain to survive into both dairy and non-dairy food matrices. In dairy products, its addition can promote improvements on the quality parameters, while in non-dairy products, the desired characteristics can be achieved if the suitable concentration of the *L. casei*-01, form of addition, and other factors are studied and established.

However, although *L. casei*-01 is marketed in a lyophilized powder and demonstrated health effects and suitable technological applications in a diversity of food products; up to date, we did not find any commercialized food product with a clear description that this strain was used. We observed that several products include *L. casei* as probiotic culture, but the strain is not fully described. Furthermore, we observed that a clear description of the probiotic strain is performed mainly in food products that use trademark strains, such as *Bifidobacterium* BB-12® and *Lactobacillus acidophilus* La-5®. We hope that our revision incentive the precise description of this strain in the label of food products due to its high potential to promote different health benefits.

Clinical trials are needed to better explore the metabolic effects and the mechanisms underlying the physiological effects associated to *L. casei*-01. Furthermore, there is a gap about the potential effect of paraprobiotics and postbiotics derived from *L. casei*-01 and the feasibility of their use in processed food products. Finally, this strain should be included in more products, which would clarify if the benefits promoted by *L. casei*-01 are impacted by the matrices, allowing enlargement of the rational use of this strain in industrial processes and by consumers for specific purposes.

In conclusion, this was the first study to provide an overview of the health benefits associated with the consumption of live or inactivated *L. casei*-01, which demonstrated, mainly, modulation of intestinal microbiota and activity against pathogenic bacteria in a gut model, and attenuation of hypertension and dyslipidemia in human. Furthermore, a discussion about the factors affecting *L. casei* survival in food products and the impacts of its addition on the quality parameters of dairy and non-dairy products was performed. Improvements in the quality parameters of dairy products have been reported, while non-dairy products need a careful evaluation of the process parameters to obtain products with suitable technological and sensory properties. The information summarized in this review may assist in developing strategies to design new probiotic, paraprobiotic, or postbiotic products with beneficial effects without compromise technological aspects and identifying perspectives for further studies.

Declaration of interest statement

Authors declare no conflict of interest.

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2.2 PARAPROBIÓTICOS

Fatores que limitam o uso de probióticos em matrizes alimentares incluem, baixa resistência térmica, as propriedades físico-químicas da matriz alimentar e as condições de armazenamento do produto. Condições desfavoráveis podem afetar a sobrevivência dos probióticos e estes necessitam de contagens mínimas de células viáveis para que se estabeleça o efeito esperado ao consumir o produto (AGUILAR-TOALÁ et al., 2018; MARCIAL-COBA et al., 2019). Neste sentido é mais vantajosa incorporação de paraprobióticos em alimentos, pois esses têm uma menor interação com os componentes dos produtos alimentícios quando comparados aos probióticos, podem ser adicionados antes de processamentos térmicos pois não é necessário mantê-los vivos durante a vida de prateleira do produto, facilitando também a manipulação, armazenamento e transporte de produtos com células probióticas inativadas (ALMADA et al., 2021a; PIMENTEL et al., 2021).

O termo paraprobiótico foi definido devido ao significado de seu prefixo "para" que traduzido do grego significa atípico, diferenciando da definição tradicional de probiótico. Assim, como definição, paraprobióticos são "células microbianas não viáveis (intactas ou quebradas) ou frações celulares que, quando administradas (oral ou topicamente) em quantidades e frequência adequadas, conferem benefícios ao consumidor humano ou animal" (TAVERNITI et al., 2011). Portanto, células não viáveis (não cultiváveis) e células microbianas com atividades imunológicas ativas podem fornecer benefícios à saúde do hospedeiro (ALMADA et al., 2016).

Os paraprobióticos podem fornecer tais benefícios por diferentes interações com o organismo, mesmo que os efeitos e mecanismos de ação destes na interação com o intestino e outros órgãos sistêmicos ainda não sejam completamente compreendidos (AGUILAR-TOALÁ et al., 2018; ALMADA et al., 2016). Paraprobióticos de *L. casei* já geraram melhorias de motilidade gastrointestinal, no estudo de HARA et al., (2018) células de Lc327 inativadas pelo calor (liofilizada, fórmula comercial) aumentaram a síntese de 5-hidroxitriptamina, um regulador da função gastrointestinal em camundongos. Tratamento com *L. plantarum* inativados por calor (Lp nF1, formulação em pó, comercial) reduziu significativamente a replicação viral nos pulmões de camundongos infectados com *influenza* A (PARK et al., 2018). E *L. paracasei* MCC1849 inativado por calor (100 °C por 30 min) demonstrou potencial de aumentar as respostas imunes adquiridas por meio do aumento na produção de IgA antígeno-específico em tecidos intestinais de ratos (ARAI et al., 2018).

Paraprobióticos são obtidos de microorganismos probióticos inativados após serem expostos a condições extremas que alteraram sua estrutura celular, danificaram seu DNA ou membrana celular. A inativação acontece quando as células são incapazes de se multiplicarem in vitro, ou seja, quando não crescem no plaqueamento em meio de cultura adequado (RAZ e RACHMILEWITZ, 2005). Entretanto existem condições em que as estruturas celulares de microorganismos não cultiváveis podem ter atividade biológica no hospedeiro. (ALMADA et al., 2016; PATEL e DENNING, 2013). A inativação de culturas probióticas vem sendo realizada usando tecnologias clássicas (aquecimento ou mudanças de pH) e tecnologias emergentes, como irradiação (ALMADA et al., 2021a), aquecimento ôhmico (ALMADA et al., 2016), alta pressão (CUEVAS-GONZÁLEZ et al., 2020) e ultrassom (SHIN et al., 2010).

No estudo de ALMADA et al. (2021a), foram avaliadas condições de inativação com ultrassom e outros cinco métodos (calor, alto e baixo pH, irradiação gama e dióxido de carbono supercrítico) para obtenção de paraprobióticos de *L. acidophilus, L. casei* e *B. animalis.* Para avaliação do impacto de cada método em diferentes condições nas células dos microorganismos foram usados plaqueamento, citometria de fluxo e análises de microscopia eletrônica de varredura. Os resultados demonstraram o ultrassom como o método mais viável, dentre os testados, para obtenção de paraprobióticos de *L. casei* com metabolismo ativo. Embora a bactéria probiótica tenha perdido a capacidade de se multiplicar em meio de cultura, esta ainda manteve algum metabolismo celular.

2.2.1 Ultrassom e paraprobióticos derivados

Ultrassons são ondas sonoras com frequências acima de 16-20 kHz (ZHANG et al., 2019). É possível gerar ondas de ultrassom em campos estáveis através de equipamentos (transdutores), que geram energia na forma de vibrações ultra-sônicas. E a sua aplicação em produtos alimentares pode ser feita utilizando sonotrodos ou banhos ultra-sônicos (ARVANITOYANNIS et al., 2017) O ultrassom pode ser programado com combinações variadas dos parâmetros que incluem a potência (W), frequência (kHz) do aparelho, tempo de processamento e duração (GUIMARÃES et al., 2019).

O processo de ultrassom induz alterações físicas e químicas nos alimentos e pode ser usado na tecnologia alimentar para melhorar propriedades físico-químicas de produtos lácteos (CHANDRAPALA e ZISU, 2018; AKDENIZ e AKALIN, 2019), como tecnologia de preservação de produtos alimentícios (OJHA et al., 2017) e para produção de paraprobióticos (ALMADA et al., 2021a, 2021b) devido à sua capacidade de causar danos (reduzir a espessura e até mesmo quebrar) às membranas da parede celular e ao DNA de bactérias (LYE, 2012; PANIWNYK, 2017). Os probióticos são inativados por processos de rupturas físicas, vazamento do conteúdo celular, alteração da bicamada da membrana celular e danos ao DNA através de micro eventos (aumento na temperatura, pressão e de ondas de energia de cisalhamento) que ocorrem em um sistema de geração de ultrassom (GUIMARÃES et al., 2019).

Em seu estudo, STAREK et al. (2021) utilizaram ultrassom (40 W, 20 kHz por 5 min e 28 W, 20 kHz por 10 min) para inativação de mesófilas, microrganismos aeróbios, bactérias ácido lácticas, coliformes e leveduras em suco de tomate. O tratamento resultou em um produto livre de microorganismos deteriorantes, mesmo após 10 dias de armazenamento e ainda manteve as propriedades químicas e estruturais do suco de tomate.

LUO et al. (2021) avaliaram a aplicação de paraprobiótico do *Clostridium butyricum* CBG01 inativado por ultrassom (300 W, 20 kHz por 20 min) na melhora do crescimento, respostas imune e resistência a doenças em camarões, e seus resultados demonstraram que tanto as células vivas quanto as inativadas por ultrassom de *C. butyricum* CBG01 melhoraram o desempenho de crescimento, taxa de sobrevivência e respostas imunológicas dos animais.

Estudos também avaliaram cepas probióticas inativadas por ultrassom como alternativa ao uso de antibióticos na prevenção e tratamento de infecções causadas por bactérias patogênicas (POSADAS et al., 2017), e em efeitos à saúde de hospedeiros, que demonstraram efeitos hipocolesterolêmicos em ratos tratados com paraprobiótico de *Bifidobacterium longum* (SHIN et al., 2010).

Entretanto a eficiência do processo (sobrevivência microbiana e manutenção de atividade enzimática) está relacionada a parâmetros como a frequência das ondas, potência, o tempo de exposição, e os microrganismos alvo pois, diferentes cepas apresentam diferentes respostas ao processo (ALMADA et al., 2021a; GUIMARÃES et al., 2019). O ultrassom de baixa frequência (20-50 kHz) é relacionado à maior eficiência do processo de fermentação de produtos alimentares. As taxas de inativação de bactérias deteriorantes em alimentos são observadas utilizando tempos de exposição mais longos (>100 min) durante o processo (GUIMARÃES et al., 2019).

Apesar de ALMADA et al. (2021a) apresentarem resultados sugerindo o ultrassom como um método viável para obter paraprobióticos de *L. casei* com metabolismo ativo. No estudo de ALMADA et al. (2021b) o consumo diário de *L. casei* inativados por ultrassom (792 W, 20 kHz por 40 min), não afetou os parâmetros bioquímicos (níveis de glicose e triglicerídeos) de ratos *Wistar* comparado ao grupo controle. Demonstrando que os benefícios à saúde gerados por paraprobióticos é cepa dependente, mas também dependente do método de inativação. Sendo necessário mais estudos para avaliação de outros parâmetros bioquímicos com utilização de cepas de *L. casei* inativados por ultrassom.

Tratamentos com ultrassom, combinados ou não a outros tratamentos como processos térmicos e alterações de pressão, vêm demonstrando serem mais vantajosos para aplicação em produtos alimentares, uma vez que alcançam a inativação de microorganismos deteriorantes enquanto mantêm ou até aprimoram as propriedades organolépticas e nutricionais de produtos lácteos e não lácteos (GUIMARÃES et al., 2019, LUO et al., 2021; STAREK et al., 2021; WANG et al., 2020).

3 MATERIAIS E MÉTODOS

3.1 PREPARAÇÃO DA CEPA PROBIÓTICA

A cultura probiótica comercial *Lacticaseibacillus casei* 01 (*L. casei* 01) foi obtida de Chr. Hansen (Valinhos, SP, Brasil). A suspensão celular foi preparada incubando as culturas probióticas em caldo MRS (de Man, Rogosa e Sharp) (HiMedia, Mumbai, Índia) sob anaerobiose (Anaerobic System Anaerogen, Oxoid Ltda., Wade Road, UK) a 37 °C em *overnight*. As células foram centrifugadas a (4500 g, 15 min, 4 °C) e lavadas duas vezes com tampão fosfato-salino (PBS; pH 7,2), ressuspensas no mesmo diluente, e homogeneizado usando um vortex (30 s) para obtenção suspensões celulares padrões que correspondiam a contagens viáveis de 9 log UFC/mL (densidade óptica de 1,0 a 660 nm). As suspensões celulares foram preparadas diariamente para a administração aos animais.

3.2 INATIVAÇÃO DO PROBIÓTICO POR ULTRASSOM

Para obter o *L. casei* inativado (paraprobiótico), a suspensão celular (9 log UFC/mL) foi tratada por ultrassom usando um disruptor celular (DES500, Unique, Campinas, Brasil, 20 kHz, 40 min, 792 W). As condições empregadas para a inativação das células de *L. casei* foram baseadas em um estudo prévio para obtenção de células com paraprobióticos (ALMADA et al., 2021a), e foram preparadas e armazenadas semanalmente sob refrigeração (4 °C) até a administração aos animais.

3.3 DESENHO EXPERIMENTAL

Todas as experiências foram previamente aprovadas pela Comissão de Ética no Uso de Animais de Laboratório (CEUA, UFPB, Paraíba, Brasil, protocolo No. 1254111219). Foram incluídos no estudo 24 ratos *Wistar* de 90 dias (peso de 303,27 ± 26,0 g) obtidos do Biotério da Universidade Federal de Pernambuco (UFPE, Recife, Brasil). Os procedimentos experimentais foram sempre realizados de acordo com as diretrizes de cuidados para o uso de animais de laboratório. Os ratos foram mantidos em gaiolas coletivas de polipropileno em ciclos de 12 h de luz-escuro, 22 ± 1 °C, e 60-70% de umidade relativa do ar.

Os ratos foram distribuídos aleatoriamente em 4 grupos de 6 animais cada (3 ratos por gaiola). Grupo controle (CTL) recebeu uma dieta padrão de acordo com o *American Institute of Nutritio*n (Tabela 1, AIN- 93 M) (REEVES et al., 1993) e os grupos de dieta hiperlipídica (DHL) receberam uma dieta rica em gordura adquirida na empresa Rhoster (Tabela 1,Rhoster®, SP, Brasil).

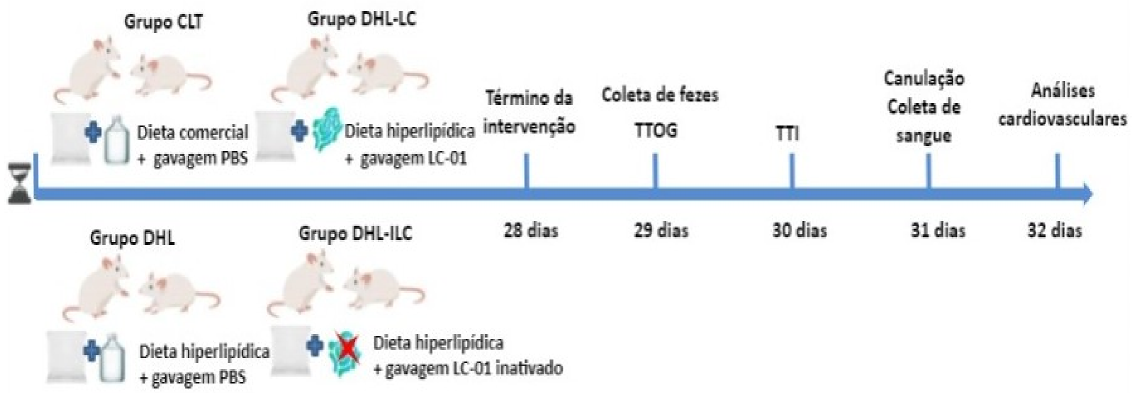

Figura 1. Esquema do estudo experimental. Grupo controle (CTL): alimentados com dieta comercial + gavagem de tampão fosfato-salino (PBS; pH 7,2); grupo dieta hiperlipídica (DHL): Dieta hiperlipídica + PBS; grupo dieta hiperlipídica e *L. casei* vivo (DHL-LC): Dieta hiperlipídica + células vivas de *L. casei;* grupo dieta hiperlipídica e *L. casei* inativado (DHL-ILC): Dieta hiperlipídica + *L. casei* inativado por ultrassom. Coleta de fezes, testes de tolerância à glicose (TTOG) e insulina (TTI), canulação, coleta de sangue e análises cardiovasculares realizados ao final da intervenção (28 dias).

A suspensão (1 mL) contendo probióticos (9 log UFC/mL) ou paraprobiótico foi administrada diariamente por gavagem orogástrica durante 28 dias, sempre no mesmo horário (às 14 h). Dietas, água potável e ração foram fornecidas *ad libitum* durante todo o período experimental. O peso corporal era monitorado semanalmente, e a ingestão de alimentos avaliada a cada dois dias.

Nutrientes	Dieta Padrão	Dieta hiperlipídica
Caseína (85)*	20	19,86
Amido dextrinizado	13	15,5
Celulose	5	5
Sacarose	10	6
Amido de milho	39,7	33,6
Óleo de soja	7	3
Banha de porco	0	6
Gordura vegetal hidrogenada	0	5
Ácido cólico (sigma)	0	0,5
Colesterol (sigma)	0	0,5
Colina	0,25	0,25
Metionina	0,3	0,3
Formulação de vitaminas	1	1
Formulação de minerais	3,5	3,5
T-BHQ	0,014	0,014

Tabela 1. Composição nutricional dietética (g/100 g) da dieta comercial padrão (AIN- 93 M) e dieta hiperlipídica (Rhoster®, SP, Brasil).*A caseína com 85% de pureza.

3.4 TOLERÂNCIA À GLICOSE E À INSULINA E ANÁLISES BIOQUÍMICAS

Os testes de tolerância oral à glicose (TTOG) e de tolerância à insulina (TTI) foram realizados em ratos em jejum de 8 h. Para o TTOG, uma carga de glicose (2 g por kg de peso corporal) foi administrada por gavagem oral. Antes da administração de glicose e após 15, 30, 60, 90 e 120 min, foram coletadas amostras de sangue das veias caudais. Após 24 h de TTOG, uma injeção intraperitoneal de insulina (0,75 UI por kg de peso corporal) foi realizada para avaliar o TTI. A concentração de glicose no sangue foi medida antes (0 min) e após 30, 60, 90 e 120 min após a injeção de insulina. A medição da concentração de glicose no sangue foi realizada com um glicosímetro Accu-Check (Bayer®). A resistência à glicose e à insulina foi avaliada utilizando a área sob a curva (AUC) dos gráficos (glicose × tempo).

As medições do soro foram realizadas 24 h após experiências com homeostase de glicose. Os animais foram submetidos a jejum noturno, anestesiados com cetamina (80 mg/kg) e xilazina (10 mg/kg) para inserção de cateteres de polietileno na artéria e veia femoral. Amostras de sangue (aproximadamente 2 mL) foram coletadas por cateter venoso para medidas bioquímicas. O sangue foi centrifugado a 3000 g durante 15 min à temperatura ambiente e as medidas bioquímicas foram realizadas utilizando kits colorimétricos enzimáticos apropriados, de acordo com as instruções do fabricante (Bioclin, Belo Horizonte, Minas Gerais, Brasil) para análise de colesterol total (CT), colesterol HDL, colesterol LDL, e concentrações de triglicerídeos.

3.5 ANÁLISES CARDIOVASCULARES

Os cateteres arteriais foram tunelizados da parte de trás do pescoço para facilitar a conexão ao transdutor de pressão. Após a cirurgia, os animais receberam uma dose de cetoprofeno (5 mg/kg, anti-inflamatório) por via subcutânea. Posteriormente, os ratos passaram por um período de recuperação cirúrgica de 24 h. A pressão arterial (PA) e a frequência cardíaca (FC) foram registradas em animais conscientes através da conexão da cânula arterial a um transdutor de pressão (ML866/P, Instrumentos AD, Power Lab, Bella Vista, NSW, Austrália) (GUIMARÃES et al., 2017).

A pressão arterial pulsátil (PAP) e a FC foram registradas por 40-60 min sob condições basais, e os valores de PA sistólica (PAS), PA diastólica (PAD), PA média (PAM) e FC foram calculados *offline* selecionando-se 10 min para cada animal (LabChartTM Pro, ADInstruments, Bella Vista, NSW, Austrália). Usando o mesmo período de 10 min de linha de base dos registros PA e FC, as análises espectrais no domínio da frequência PAS e intervalo de pulso (IP) foram avaliadas usando software de computador (CardioSeries-v.2.4; http://www.danielpen-teado.com). Os espectros PAS foram integrados nas bandas de baixa frequência (LF; 0,2-0,75 Hz) e alta frequência (HF; 0,75-3 Hz). Além disso, a relação LF/HF foi utilizada para avaliar o índice simpático-vagal. Finalmente, a sensibilidade espontânea do barorreflexo (SBRS) foi calculada através de um método de sequenciamento (GUIMARÃES et al., 2017).

3.6 ANÁLISES DA MICROBIOTA FECAL

As amostras fecais foram coletadas no 29° dia de experimento de acordo com as recomendações da empresa (Neoprospecta, Santa Catarina - Brasil), armazenadas à temperatura de congelamento (-18 °C) e enviadas para análises da microbiota.

O DNA foi extraído por um procedimento exclusivo usando a técnica de *beads* magnética (Neoprospecta Microbiome Technologies, Brasil). A diversidade bacteriana foi avaliada através de sequenciamento de alto rendimento das regiões V3/V4 do gene 16S rRNA empregando primers 341F (CCTACGGGRSGCAGCAG) (WANG et al., 2009) e 806R (GGACTACHVGGGGTWTCTAAT) (CAPORASO et al., 2012). As bibliotecas 16S rRNA foram sequenciadas usando o Sistema MiSeq Sequencing (Illumina Inc., EUA) utilizando os primers Illumina padrão fornecidos no kit, 300 Cycles (sequenciamento Paired-end 200 bp). Depois disso, as sequências foram demultiplexadas e submetidas a um filtro de qualidade, baseado na soma dos erros de probabilidades das bases de DNA, permitindo um máximo de 1% dos erros acumulados. Posteriormente, adaptadores e primers Illumina foram removidos das sequências de DNA.

Todos os procedimentos para análise de bioinformática foram apresentados em detalhes em KAMIMURA et al. (2020); VIEIRA et al. (2019). Em resumo, a qualidade dos dados foi verificada usando FastQC (v0.11.9). As sequências *paired-end* foram unidas utilizando o software FLASH (versão 1.2.11). Posteriormente, os dados foram filtrados utilizando o software Trimmomatic 0,39 SE (single end e minlength de 300 bp) (BOLGER et al., 2014). As sequências quiméricas foram verificadas usando o algoritmo UCHIME2 (EDGAR et al., 2011). Após o controle de qualidade, a atribuição taxonômica das sequências foi obtida usando o *Quantitative Insights into Microbial Ecology* (QIIME), software versão 1.9.1 (CAPORASO et al., 2010). A taxonomia foi atribuída para cada Unidade Taxonômica Operacional (OTU) em relação à base de dados SILVA 132 (QUAST et al., 2013). A partir dos resultados obtidos, foram realizadas análises utilizando o software QIIME para cálculo dos índices de riqueza e alfa diversidade: Shannon, Simpson (KIM et al., 2017; LEMOS et al., 2011) Good's coverage e Chao1 (CHAO, 2002). O índice de distância Bray-Curtis foi usado para construir uma matriz de similaridade taxonômica (métrica de distância UniFrac ponderada e não-ponderada) para atribuir a diversidade beta (LOZUPONE e KNIGHT, 2005).

3.7 ANÁLISES ESTATÍSTICAS

Os resultados foram expressos em média ± desvio padrão. O teste de Kolmogorov-Smirnov foi utilizado para avaliar a normalidade dos dados. A análise de variância unidirecional (ANOVA) seguida do pós-teste de Tukey foi utilizada para os parâmetros bioquímicos e dados de diversidade alfa. Para análise dos resultados dos testes TTOG e TTI foram utilizados o teste ANOVA bidirecional e o pós-teste de Bonferroni. Para os resultados de HF e abundância relativa a nível familiar, os dados foram analisados através do teste Kruskal-Wallis. O teste Dunn pós-teste também foi utilizado para os dados de HF. Para todas as análises, o nível de significância foi fixado em 5% ($p < 0,05$). Os dados foram analisados e todos os gráficos foram feitos usando o software GraphPadPrism 7® (GraphPad Software Inc. San Diego, CA, EUA).

Os testes não paramétricos PERMANOVA usando dados de diversidade beta foram realizados no software QIIME versão 1.9.0. A análise de componentes principais (PCA) foi realizada para enfatizar a variação e mostrar padrões nos conjuntos de dados usando o software XLSTAT. Apenas as OTUs com valores de abundância superiores a 0,1% ($p < 0,05$) em pelo menos seis amostras foram incluídas na análise PCA. A matriz de PCA foi composta de 4 colunas (grupos) e 22 linhas (ordem, família e gênero). O recurso e o agrupamento de amostras foram analisados simultaneamente usando a ferramenta de análise de dados exploratória do *heatmap* no software XLSTAT versão 2020.1.3 (Adinsoft, Paris, França) usando OTUs com valores de abundância superiores a 0,1%. Os arquivos das sequências foram depositadas no Arquivo de Leitura Sequencial do *National Centre for Biotechnology Information* (NCBI) sob o *Bio-Project* ID PRJNA718826.

4 RESULTADOS

Os resultados desta dissertação estão apresentados em forma de um artigo original no apêndice A.

O Artigo I (APÊNDICE A) intitulado "LIVE AND ULTRASOUND-INACTIVATED *Lacticaseibacillus casei* MODULATE THE INTESTINAL MICROBIOTA AND IMPROVE BIOCHEMICAL AND CARDIOVASCULAR PARAMETERS IN MALE RATS FED A HIGH-FAT DIET" avaliou-se os efeitos da ingestão *Lacticaseibacillus casei* 01 e seu paraprobiótico derivado de tratamento com ultrassom (20 kHz, 40 min) durante 28 dias sobre parâmetros de saúde (bioquímicos e cardiovasculares) e de microbiota intestinal (sequenciamento de *amplicon* de RNA ribossomal 16S) em ratos alimentados com dieta hiperlipídica. Vinte e quatro ratos Wistar foram divididos em quatro grupos com seis animais em cada: CTL (dieta padrão), HFD (dieta hiperlipídica), HFD-LC (dieta hiperlipídica e *L. casei* vivo) e HFD-ILC (dieta hiperlipídica e *L. casei* inativado). O consumo de *L. casei* vivo e inativado causou redução dos níveis de colesterol (total e LDL) e controlou resistência à insulina nos animais alimentados com dieta hiperlipídica (DHL), promoveu modulação da microbiota intestinal pelo aumento e/ou diminuição significativa em grupos bacterianos específicos, atenuando efeitos causados pela DHL. Os resultados mostram efeitos benéficos das células probióticas vivas e inativadas e indicam que a inativação por ultrassom produz um paraprobiótico com propriedades de saúde semelhantes ou melhoradas em comparação com células vivas. O *L. casei* inativado pode ser usado para melhorar hipertensão (PAS, PAM e PAD) e colesterol (colesterol total e LDL).

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APÊNDICES

APÊNDICE A – ARTIGO I

LIVE AND ULTRASOUND-INACTIVATED *Lacticaseibacillus casei* MODULATE THE INTESTINAL MICROBIOTA AND IMPROVE BIOCHEMICAL AND CARDIOVASCULAR PARAMETERS IN MALE RATS FED A HIGH-FAT DIET.

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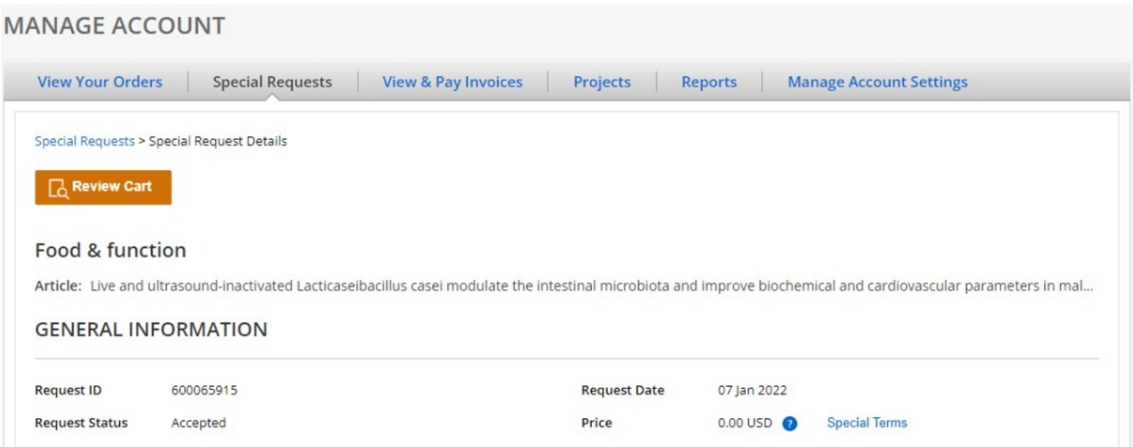

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Live and ultrasound-inactivated *Lacticaseibacillus casei* modulate the intestinal microbiota and improve biochemical and cardiovascular parameters in male rats fed a high-fat diet†

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This study aimed to evaluate the effects of ingestion of live (9 log CFU mL^{-1}) and ultrasound-inactivated (paraprobiotic, 20 kHz, 40 min) *Lacticaseibacillus casei* 01 cells for 28 days on healthy parameters (biochemical and cardiovascular) and intestinal microbiota (amplicon sequencing of 16S ribosomal RNA) of rats fed a high-fat diet. Twenty-four male Wistar rats were divided into four groups of six animals: CTL (standard diet), HFD (high-fat diet), HFD-LC (high-fat diet and live *L. casei*), and HFD-ILC (high-fat diet and inactivated *L. casei*). The administration of live and ultrasound-inactivated *L. casei* prevented the increase ($p < 0.05$) in cholesterol levels (total and LDL) and controlled the insulin resistance in rats fed a high-fat diet. Furthermore, it promoted a modulation of the intestinal microbial composition by increasing ($p < 0.05$) beneficial bacteria (*Lachnospiraceae* and *Ruminoccocaceae*) and decreasing ($p < 0.05$) harmful bacteria (*Clostridiaceae*, *Enterobacteriaceae*, and *Helicobacteriacea*), attenuating the effects promoted by the HFD ingestion. Only live cells could increase ($p < 0.05$) the HDL-cholesterol, while only inactivated cells caused attenuation ($p < 0.05$) of the blood pressure. Results show beneficial effects of live and inactivated *L. casei* 01 and indicate that ultrasound inactivation produces a paraprobiotic with similar or improved health properties compared to live cells.

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1. Introduction

Probiotics are live microorganisms, which confer health effects to the host when consumed in adequate amounts.[1] *Lacticaseibacillus casei* consumption incorporated or not into food products has been associated with several health effects, including microbiota modulation, anti-carcinogenic properties,[2] reduction of oxidative stress,[3] hypolipidemic and hypotensive properties,[4] and reduction in postprandial glycemia.[5]

Paraprobiotics are defined as inactivated microbial cells (non-viable) that, when administered confer health benefits to the host.[6] Paraprobiotics have advantages over probiotics, such as the easiness of processing, transport, and storage, safety administration to individuals with compromised immunity or gut barrier, longer shelf life, and fewer changes in the quality parameters when incorporated into food products.[5,7] The inactivation of probiotic cultures can be carried out using classical technologies (heating or pH changes) and emerging technologies, such as irradiation,[7] ohmic heating,[6] high pressure,[8]

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and ultrasound.[9] Ultrasound may be used to inactivate probiotics by promoting a physical disruption, leakage of the cellular content, alteration of the cell membrane bilayer, and DNA damage.[10]

Previous studies have reported health benefits associated with the consumption of paraprobiotics included or not into food products, such as relief of constipation with heat-inactivated *L. gasseri*,[11] improvements in the gut microbiota composition with heat-inactivated *L. paracasei*,[12] and reductions in glycemia and cholesterol concentrations with irradiation-inactivated *Bifidobacterium animalis*.[7] Only two studies have focused on evaluating the effect of inactivated *L. casei* strains,[5,13] but they investigated only postprandial glycemia and the probiotics were inactivated by ohmic heating.

High-fat diets (HFD) can increase adipose tissue and induce cardiovascular and metabolic disorders, such as type-2 diabetes, atherosclerosis, arterial hypertension, and stroke, mainly in genetically susceptible individuals.[14] Furthermore, HFD may induce gut dysbiosis.[15] The inclusion of beneficial components in the host diet may correct the health damage caused by HFD consumption,[14] and probiotic administration may prevent cardiovascular disorders.[15] However, little is known about the effects of paraprobiotics on disorders caused by HFD consumption. Therefore, the present study aimed to evaluate the effects of live and ultrasound-inactivated *L. casei* on biochemical and cardiovascular parameters and intestinal microbiota using amplicon sequencing of 16S ribosomal RNA in male rats fed a high-fat diet.

2. Materials and methods

2.1 Probiotic strain preparation

The commercial probiotic culture *Lacticaseibacillus casei* 01 (LC-01) was obtained from Chr. Hansen (Valinhos, SP, Brazil). The cell suspension was prepared by incubating the probiotic cultures on De Man Rogosa and Sharp broth (HiMedia, Mumbai, India) at 37 °C overnight under anaerobiosis (Anaerobic System Anaerogen, Oxoid Ltda., Wade Road, UK). The cells were harvested by centrifugation (4500*g*, 15 min, 4 °C) and washed twice with phosphate-buffered saline (PBS; pH 7.2), resuspended and homogenized using a vortex (30 s) in the same diluent to obtain standard cell suspensions that corresponded to viable counts of 9 log CFU mL^{-1} (optical density at 660 nm of 1.0). Cell suspensions were daily prepared for administration to animals.

2.2 Probiotic inactivation by ultrasound

To obtain the inactivated *L. casei* (paraprobiotic), the cell suspension (9 log CFU mL^{-1}) was treated by ultrasound using a cell disruptor (DES500, Unique, Campinas, Brazil, 20 kHz, 40 min, 792 W). The conditions employed for inactivation of *L. casei* cells were based on a previous study to obtain cells with paraprobiotics,[16] and were weekly prepared and stored under refrigeration (4 °C) until administration to the animals.

2.3 Experimental design

All experiments were previously approved by the Ethical Commission on Animal Use (CEUA, UFPB, Paraiba, Brazil, protocol No. 1254111219). Twenty-four male Wistar rats of 90 days (weight of 303.27 ± 26.0 g) obtained from the Bioterium of the Federal University of Pernambuco (UFPE, Recife, Brazil) were included in the study. Experimental procedures were always performed in accordance with care guidelines for the use of laboratory animals. Rats were kept in collective polypropylene cages (3 rats per cage) in a 12 h light–dark cycles, 22 ± 1 °C, and 60–70% relative air humidity.

The rats were randomly assigned to 4 groups of 6 animals each as follows CTL group which received a standard diet according to American Institute of Nutrition (AIN-93 M) and PBS (Table S1†);[17] HFD group which received a high-fat diet acquired in Rhoster company (Table S1,† Rhoster®, SP, Brazil) and PBS, HFD-LC group which received the HFD and *L. casei* live cells in PBS and, HFD-ILC group which received the high-fat diet and ultrasound-inactivated *L. casei* in PBS. The suspension (1 mL) containing probiotics (9 log CFU mL^{-1}) or paraprobiotics was daily administrated by orogastric gavage for 28 days. Diets, drinking water, and feed were provided *ad libitum* throughout the experimental period.

Body weight was monitored weekly, and food intake was assessed every two days. Insulin and glucose tolerance, biochemical tests, cardiovascular parameters, and fecal microbial ecology analysis were performed at the end of the experiment (28 days).

2.4 Glucose and insulin tolerance and biochemical analysis

The oral glucose tolerance (OGTT) and insulin tolerance (ITT) tests were performed in rats 8-hour fasting. For OGTT, a glucose load (2 g per kg of body weight) was administered by oral gavage. Before glucose administration and after 15, 30, 60, 90, and 120 min, blood samples were collected from the caudal veins. After 24 h of OGTT, an intraperitoneal injection of insulin (0.75 IU per kg of body weight) was performed to evaluate the ITT. The blood glucose concentration was measured before (0 min) and after 30, 60, 90, and 120 min after the insulin injection. The measurement of blood glucose concentration was performed with an Accu-Check glucometer (Bayer®). The glucose and insulin resistance were evaluated using the area under the curve (AUC) of the graphs (glucose × time).

Serum measurements were performed 24 h after glucose homeostasis experiments. The animals were subjected to overnight fasting, anesthetized with ketamine (80 mg kg^{-1}) and xylazine (10 mg kg^{-1}) for insertion of polyethylene catheters in the femoral artery and vein. Blood samples (approximately 2 mL) were collected by venous catheter for biochemical measurements.[18] The blood was centrifuged at 3000*g* for 15 min at room temperature and biochemical measurements were performed using appropriate enzymatic colorimetric kits, according to the manufacturer's instructions (Bioclin, Belo Horizonte, Minas Gerais, Brazil) for analysis of total cholesterol (TC), HDL-cholesterol, LDL-cholesterol, and triglycerides concentrations.

2.5 Cardiovascular analyses

Arterial catheters were tunneled from the back of the neck to facilitate connection to the pressure transducer. After surgery, the animals received a dose of ketoprofen (5 mg kg^{-1} ip, anti-inflammatory) subcutaneously. Subsequently, the rats underwent a 24 h surgical recovery period. Blood pressure (BP) and heart rate (HR) were recorded in conscious animals by connecting the arterial cannula to a pressure transducer (ML866/P, AD Instruments, Power Lab, Bella Vista, NSW, Australia).[18]

The pulsatile arterial pressure (PAP) and HR were recorded for 40–60 min under baseline conditions, and the values of systolic AP (SAP), diastolic AP (DAP), mean AP (MAP), and HR were calculated offline by selecting 10 min for each animal (LabChartTM Pro, ADInstruments, Bella Vista, NSW, Australia). Using the same 10 min period of baseline of AP and HR records, the spectral analyzes in the SAP frequency and pulse interval domain (PI) were evaluated using appropriate computer software (CardioSeries-v.2.4; http://www.danielpenteado.com). The SAP spectra were integrated in the low-frequency waves (LF; 0.2–0.75 Hz) and high-frequency waves (HF; 0.75–3 Hz) bands. Besides, the LF/HF ratio was used to assess the sympathetic-vagal index. Finally, spontaneous baroreflex sensitivity (SBRS) was calculated using a sequencing method.[18]

2.6 Intestinal microbiota analysis

DNA was extracted by an exclusive procedure using a magnetic bead (Neoprospecta Microbiome Technologies, Brazil). The bacterial diversity was assessed *via* high-throughput sequencing of 16S rRNA V3/V4 region employing 341F (CCTACGGGRSGCAGCAG)[19] and 806R (GGACTACHVGGGTWTCTAAT)[20] primers. The 16S rRNA libraries were sequenced using the MiSeq Sequencing System (Illumina Inc., USA) using the standard Illumina primers provided in the kit, 300 Cycles (paired-end sequencing with 200 bp). After that, the sequences were demultiplexing and submitted to a quality filter, based on the sum of the DNA bases probabilities errors, allowing a maximum of 1% of accumulated errors. Subsequently, Illumina adapters and primers were removed from the DNA sequences.

All the procedures for bioinformatic analysis were presented in detail.[21,22] Briefly, the data quality was checked using FastQC (v0.11.9). The paired-end sequences were joined using flash version (FLASH-1.2.11). Subsequently, the data was filtered with Trimmomatic 0.39 option SE (single end and min-length of 300 bp).[23] Chimeric sequences were checked using the UCHIME2 algorithm.[24] After that, were assessed the taxonomy assignment to the representative sequences using Quantitative Insights into Microbial Ecology (QIIME), version 1.9.1, software.[25] Taxonomy was assigned to each OUT against the SILVA 132 database.[26] In all samples, OTU abundance normalization for the same OUT number was performed. The data were used to assess the observed OTUs and calculated richness using QIIME: α-diversity indices, including Shannon and Simpson diversity indices[27,28] Good's coverage, Chao1 richness.[29] Bray–Curtis distance index was used to construct a taxonomic similarity matrix (weighted and un-weighted UniFrac distance metric) to assignment the beta diversity.[30]

2.7 Statistical analysis

The results were expressed as mean ± standard deviation. Kolmogorov Smirnov test was used to assess the normality of data. The One-Way analysis of variance (ANOVA) followed by Tukey post-test was used for the biochemical parameters and alpha diversity data. Two-way ANOVA and Bonferroni's *post hoc* test was used to analyze the results of the OGTT and ITT tests. For results of HF and relative abundance at the family level the data were analyzed using the Kruskal–Wallis test. Dunn post-test was also used for HF data. For all analyses, the significance level was set at 5% ($p < 0.05$). The data were analyzed, and all the graphics were made using the GraphPadPrism 7® software (GraphPad Software Inc. San Diego, CA, USA).

Non-parametric PERMANOVA tests using beta diversity data were carried out in QIIME version 1.9.0 software. Principal component analysis (PCA) was performed to emphasize variation and show patterns in the datasets using XLSTAT software. Only OTUs with abundance values higher than 0.1% ($p < 0.05$) in at least six samples were included in PCA analysis. The matrix of PCA was composed of 4 columns (groups) and 22 lines (order, family, and genus). Feature and sample clustering were simultaneously analyzed using the heat map exploratory data analysis tool in the XLSTAT software version 2020.1.3 (Adinsoft, Paris, France) using OTUS with abundance values higher than 0.1%. Raw sequence reads were deposited in the Sequence Read Archive of the National Centre for Biotechnology Information (NCBI) under the Bio-Project ID PRJNA718826.

3. Results

3.1 Diet intake, body weight, and biochemical parameters

Similar diet intake and weight gain were observed in rats fed HFD (HFD group) and standard diet (CLT group). The daily administration of live or ultrasound inactivated *L. casei* to rats fed HFD did not modify the food intake (from 795.2 to 803.0 g) or the weight gain (from 31 to 38 g) compared to CTL group ($p \geq 0.05$, Table 1).

Rats fed a HFD diet showed increases in the serum levels of TC and LDL-cholesterol and decreases in the HDL-cholesterol compared to rats of CTL group ($p < 0.05$, Fig. 1a–c). Daily administration of live or ultrasound inactivated *L. casei* prevented the increase in the serum TC and LDL-cholesterol levels in rats fed a HFD ($p < 0.05$, Fig. 1a and c). An increase in the serum HDL-cholesterol was observed only in rats fed a HFD receiving live *L. casei* (HFD-LC group) ($p < 0.05$, Fig. 1b). No changes were observed in serum triglycerides in rats fed a HFD receiving or not *L. casei* (live or ultrasound inactivated) in comparison to the CTL group ($p \geq 0.05$, Fig. 1d).

Table 1 Diet intake and body weight of rats fed standard diet and rats fed high-fat diet receiving or not live and ultrasound-inactivated Lacticaseibacillus casei

Group	Diet intake for 28 days (g)	Initial body weight (g)	Final body weight (g)	Weight gain (g)
CTL	792.5 ± 22.04^{ns}	288.6 ± 11.8^{c}	339.1 ± 18.1^{ab}	50.5 ± 18.8^{ns}
HFD	795.2 ± 24.5^{ns}	307.6 ± 6.0^{b}	345.8 ± 10.6^{ab}	38.0 ± 7.92^{ns}
HFD-LC	799.5 ± 25.1^{ns}	294.3 ± 5.8^{bc}	325.0 ± 8.9^{b}	31.0 ± 11.95^{ns}
HFD-ILC	803.0 ± 29.1^{ns}	324.6 ± 13.1^{a}	359.1 ± 9.7^{a}	34.5 ± 9.7^{ns}

Data are expressed as means ± SEM. Different letters in the same column means significant difference by Tukey's test ($p < 0.05$). ns = not significant. Groups: control group (CTL), high-fat diet group (HFD), high-fat diet + L. *casei* (HFD-LC) and high-fat diet + ultrasound-inactivated *L. casei* (HFD-ILC).

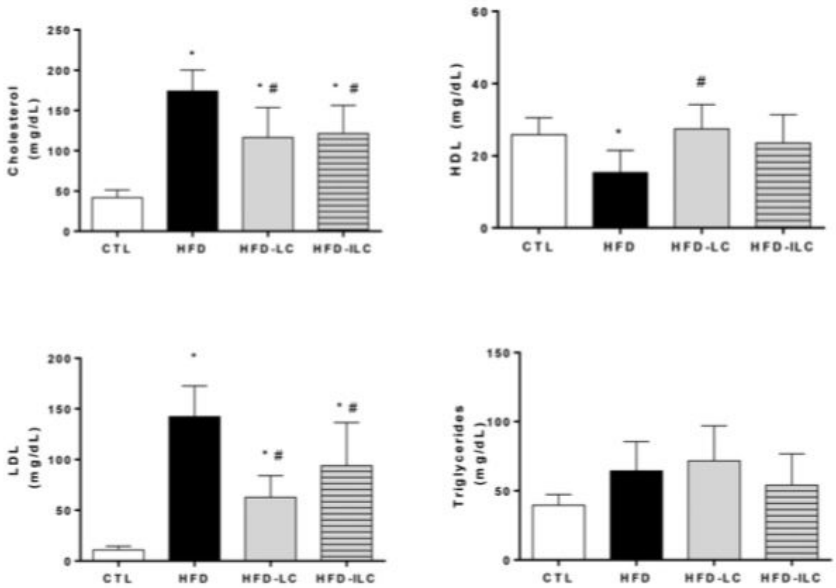

Fig. 1 Effect of *L. casei* and ultrasound-inactivated *L. casei* on total cholesterol (TC), HDL cholesterol, LDL cholesterol and triglycerides (TG) in male Wistar rats fed with high-fat diet for 28 days. Groups: Control group (CTL), high-fat diet group (HFD), high-fat diet + *L. casei* (HFD-LC) and high-fat diet + ultrasound-inactivated *L. casei* (HFD-ILC). Values are means with their standard derivation. * and # $p < 0.05$ *vs.* CTL group using one-way ANOVA and Tukey *post hoc* test.

Rats fed a HFD developed insulin tolerance, as observed by the increased AUC compared to rats fed standard diet (CTL group; $p < 0.05$, Fig. 2a). Administration of live or ultrasound inactivated *L. casei* to rats fed a HFD (HFD-LC and HFD-ILC group) maintained the AUC similar to the CTL group ($p \geq 0.05$, Fig. 2a). Similar glucose tolerance values were observed in rats fed a HFD receiving or not *L. casei* (live or ultrasound-inactivated) and CLT group ($p \geq 0.05$, Fig. 2b).

3.2 Cardiovascular parameters

The animals fed a HFD diet had baseline SAP and MAP higher than those fed a standard diet ($p < 0.05$) (Fig. 3a and c). The administration of the live *L. casei* prevented the increase in SAP and MAP, while the ultrasound-inactivated *L. casei* attenuated these parameters in rats fed on a HFD ($p < 0.05$, Fig. 3a and c). Besides, the administration of ultrasound-inactivated *L. casei* attenuated the DAP in rats fed HFD (Fig. 3b). No differences were observed in HR values of rats fed HFD receiving or not *L. casei* (live or ultrasound inactivated) and CTL group ($p \geq 0.05$, Fig. 3d).

There was a significant increase in LF oscillations of SAP in rats fed HFD when compared with the CLT group ($p < 0.05$, Fig. S1a†). The administration of *L. casei* (live or ultrasound-inactivated) prevented the increase in the LF oscillations of

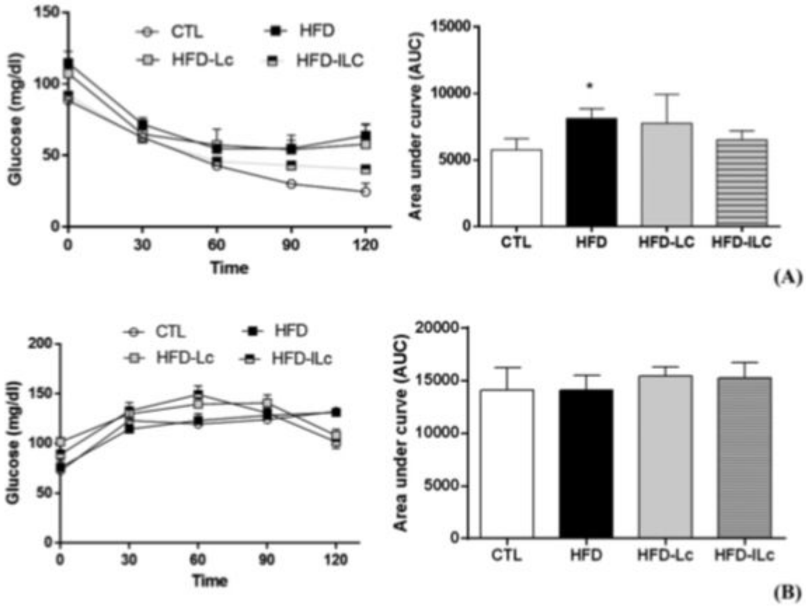

Fig. 2 Effect of *L. casei* and ultrasound-inactivated *L. casei* on insulin tolerance test (A) and oral glucose tolerance (B) in male Wistar rats fed with high-fat diet for 28 days. Groups: Control group (CTL), high-fat diet group (HFD), high-fat diet + *L. casei* (HFD-LC) and high-fat diet + ultrasound-inactivated *L. casei* (HFD-ILC). Values are means with their standard derivation. * $p < 0.05$ *vs.* CTL group using two-way ANOVA and Bonferroni's *post hoc* test or Student's *t*-test.

SAP in rats fed HFD ($p < 0.05$, Fig. S1a†). There were no differences in the HF oscillations of the SAP and the LH/HF ratio of the cardiac interval (Fig. S1b and c†) or in the SBRS values recorded for rats fed HFD receiving or not *L. casei* (live or ultrasound-inactivated) and CLT group ($p \geq 0.05$, Fig. S1d†).

3.3 Intestinal microbiota and taxonomic assignment

A total of 1882.841 readings passed the sequence quality filters applied through the Trimmomatic (0.39) software. An average of 171 167.36 bacterial sequences per sample was obtained after quality filtering and chimera removal (Table S2†). The number OTUs, Good's estimated sample coverage (ESC), Ace, Chao1, Shannon, and Simpson indices were obtained for all the samples (Table 2). Differences were observed among treatments in the OTU numbers and ACE indices ($p < 0.05$). The higher number of OTUs was observed in CTL treatments (1362), and the lower was observed HFD-LC (385) and HFD-ILC (553) (Table 2). Similar patterns were observed for Ace indices among treatments. The estimated sample coverage (ESC) of >91% suggested that bacteria microbial diversity was satisfactorily represented (Table 2). The maximum Chao1 richness values in the samples were observed in CTL and HFD (3398 and 2319, respectively), whereas the lowest value was found in HFD-LC (1050). Differences in Shannon and Simpson indexes were observed among all samples ($p < 0.05$) (Table 2). The higher values of Shannon and Simpson diversity indexes were observed in CTL (7.16 and 0.97, respectively); on the other hand, the lower values were found in HFD-LC (2.29 and 0.60, respectively). Therefore, the administration of live or ultrasound-inactivated *L. casei* resulted in reductions in the intestinal microbiota's diversity and composition in rats fed HFD (HFD-LC and HDF-ILC groups) because of the lower values observed in the parameters (Table 2).

The results of the beta-diversity analysis, built on weighted (Fig. S2a†) and unweighted (Fig. S2b†) uni-Frac distance assessment, suggested that group CTL (on the right side) clustered separately from the other groups (HFD, HFD-LC, and HFD-ILC groups) (on the left side). In fact, when Permanova statistical analysis was performed using the beta-diversity data, statistical differences were observed among groups (CTL, HFD, HFD-LC, HFD-ILC) ($p < 0.001$) (Table S3†).

The taxonomic assignment obtained by 16S rRNA gene sequencing analysis showed that the OTUs belonged to different bacterial phyla in the groups *Firmicutes* (91.3–99.4%), *Bacteroidetes* (0–3.6%), *Euryarchaeota* (0–1.8%), *Actinobacteria* (0.2–1.1%), and *Proteobacteria* (0.1–1.4%) (Fig. 4a). The results

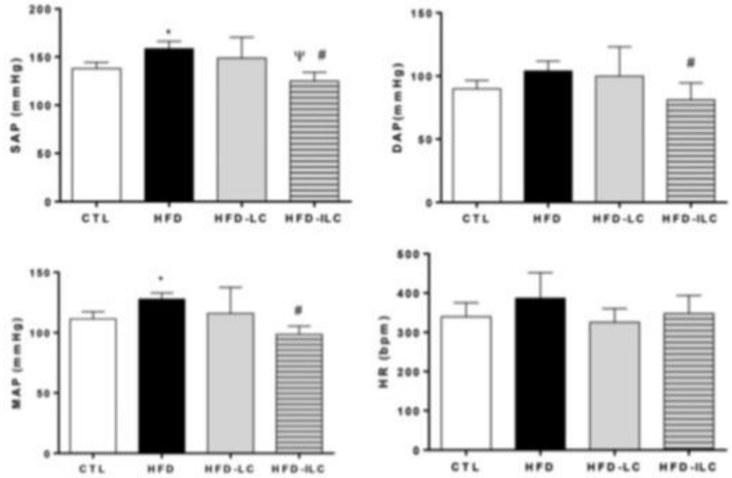

Fig. 3 Effect of *L. casei* and ultrasound-inactivated *L. casei* on systolic arterial pressure (SAP), diastolic arterial pressure (PAD), mean arterial pressure (MAP), and heart rate (HR). Groups: Control group (CTL), high-fat diet group (HFD), high-fat diet + *L. casei* (HFD-LC) and high-fat diet + ultrasound-inactivated *L. casei* (HFD-ILC). *, $p < 0.05$ *versus* CTL group; #, $p < 0.05$ *versus* HFD group, using one-way ANOVA and *post hoc* test from Turkey.

Table 2 The number of OTUs, ESC (estimated sample coverage) ACE, Chao1, Shannon and Simpson indices obtained for all groups

Group	OTUS	ESC	Ace	Chao1	Shannon	Simpson
CTL	1362 ± 383^{a}	0.91 ± 0.02^{b}	3554 ± 1379^{a}	3398 ± 1050^{a}	7.16 ± 0.64^{a}	0.97 ± 0.010^{a}
HFD	962 ± 533^{ab}	0.94 ± 0.03^{ab}	2516 ± 1161^{ab}	2319 ± 959^{ab}	5.34 ± 1.33^{ab}	0.90 ± 0.03^{a}
HFD-LC	385 ± 314^{b}	0.97 ± 0.02^{a}	1138 ± 720^{b}	1050 ± 745^{b}	2.29 ± 0.95^{c}	0.60 ± 0.08^{b}
HFD-ILC	553 ± 213^{b}	0.96 ± 0.01^{ab}	1453 ± 500^{ab}	1390 ± 338^{b}	3.00 ± 0.57^{bc}	0.66 ± 0.04^{b}

Average ± SD. Different letters in the same column means significant difference by Tukey's test ($p < 0.05$). Treatments: Control group (CTL), high fat diet group (HFD), high fat diet + *L. casei* (HFD-LC) and high fat diet + ultrasound-inactivated *L. casei* (HFD-ILC).

showed that the feces of rats fed standard diet (CTL group) presented more bacterial phyla diversity. The intake of a HFD resulted in a decrease in the abundance and diversity of the microbiota, with increases in *Firmicutes* and reductions in the other bacterial groups (Fig. 4a). The administration of live *L. casei* to rats fed HFD (HFD-LC group) decreased *Firmicutes*, and increased *Actinobacteria*, *Proteobacteria* and *Epsilonbacteraeota*, bringing the composition more similar to the CTL group. The administration of ultrasound inactivated *L. casei* increased *Firmicutes* and decreased the other groups.

At the order level, in feces samples from rats that received different diets and live or ultrasound-inactivated *L. casei*, the majority of OTUs were attributed to *Clostridiales* order (ranged from 84.30 to 98.83%) with statistical differences ($p < 0.05$) (Table 3). The feces samples from rats fed standard received (CTL group) showed a different abundance ($p < 0.05$) of *Bacteroidales* (3.65%); *Lactobacillales* (1.15%), *Methanobacteriales* (1.75%), *Desulfovibrionales* (0.30%), *Gastranaerophilales* (0.30%), *Selenomonadales* (0.20%), *Campylobacterales* (0.20%) when comparing to the other groups (Table 3). The ingestion of HFD increased the abundance of *Clostridiales* and *Enterobacteriales* and decreased the abundance of *Bacteroidales*, *Desulfovibrionales*, *Methanobacteriales*, *Gastranaerophilales*, and *Selenomonadales* (Table 3). The administration of live or inactivated *L. casei* increased *Clostridiales* and decreased *Enterobacteriales* and *Campylobacterales* (Table 3). Similar relative abundance of *Erysipelotrichales* and *Coriobacteriales* orders were observed in feces samples from rats fed HFD receiving live *L. casei* (HFD-LC group; 0.47% and 0.23%, respectively) or ultrasound-inactivated *L. casei* (HFD-ILC group; 1.23% and 0.53%) (Table 3).

At the family level, some representative groups were observed in feces samples from rats of all groups (CTL, HFD, HFD-LC, and HFD-ILC), however in different relative abundance (Fig. 4b). *Ruminococcaceae*, *Erysipelotrichaceae*, *Muribaculaceae*, *Methanobacteriaceae* and *Prevotellaceae* were

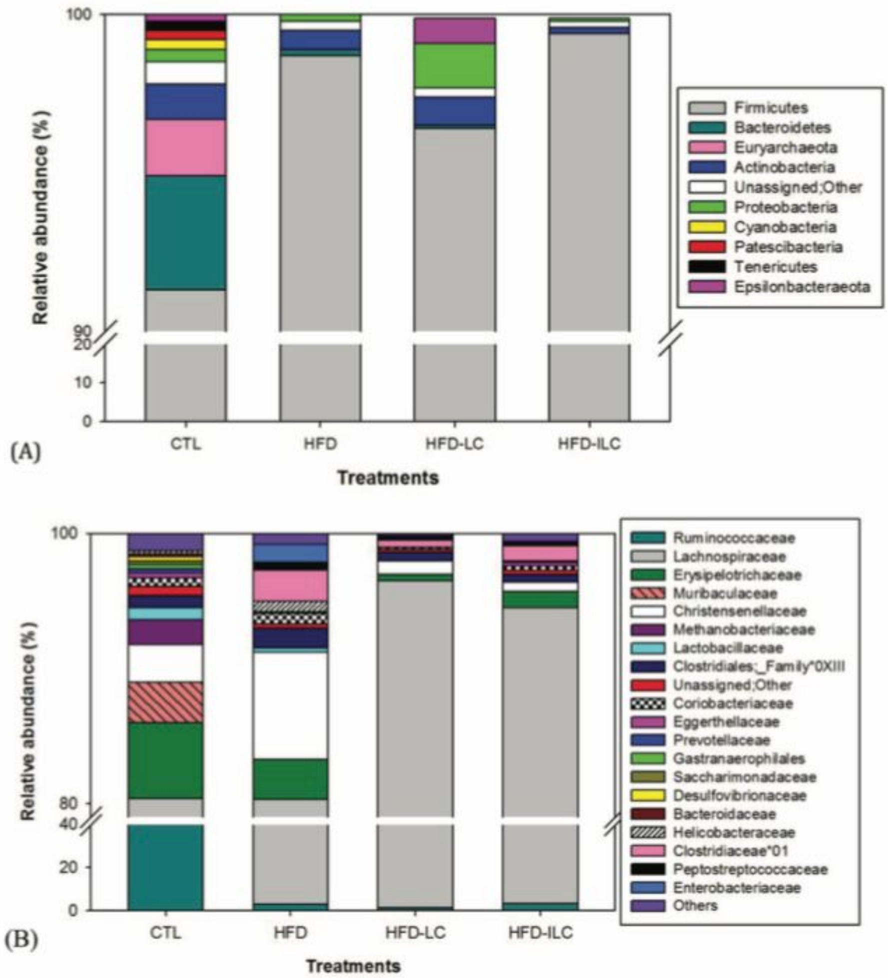

Fig. 4 Relative abundance of intestinal microbiota of rats fed a high-fat diet at phylum (A) and family (B) levels. Groups: CTL group, HFD group, HFD-LC group, and HFD-ILC group.

observed in a higher ($p < 0.05$) relative abundance in CTL group. The ingestion of a HFD reduced the diversity but promoted an increase in *Lachnospiraceae*, *Clostridiaceae 1*, *Peptostreptococcaceae*, and *Enterobacteriaceae*. The administration of live or inactivated *L. casei* increased *Lachnospiraceae* but reduced *Clostridiaceae 1*, *Peptostreptococcaceae*, and *Enterobacteriaceae* to a relative abundance closer to the observed for CTL group ($p < 0.05$). It could be observed that the diversity of microbiota was higher in the HFD-ILC compared to HFD-LC (Table S4†).

A principal component analysis (PCA) was performed to explore the taxonomic assignment obtained by 16S rRNA gene sequencing using OTUs with abundance values above 0.1%. PCA explained approximately 97.73% of the data variation with 77.52 and 20.21% at the first and second dimensions, respectively (Fig. S3†). According to the PCA, *Ruminococcaceae UCG-*

Table 3 Relative abundance of bacterial order inferred from 16 S rRNA gene amplicon sequencing analysis for all groups

Order	CTL	HFD	HFD-LC	HFD-ILC
Clostridiales	84.30 ± 4.2d	92.87 ± 3.06c	98.83± 0.46a	97.23 ± 0.15b
Bacteroidales	3.65 ± 0.55a	0.07 ± 0.05b	0.00 ± 0.00b	0.17± 0.15b
Erysipelotrichales	5.65 ± 2.75a	3.00 ± 2.59ab	0.47± 0.55b	1.23 ± 0.55ab
Coriobacteriales	1.05 ± 0.35a	0.87 ± 0.76ab	0.23 ± 0.15b	0.53 ± 0.23ab
Lactobacillales	1.15 ± 0.45a	0.47 ± 0.28ab	0.07 ± 0.05b	0.13 ± 0.23b
Desulfovibrionales	0.30 ± 0.2a	0.03 ± 0.05b	0.00 ± 0.00b	0.03 ± 0.05b
Methanobacteriales	1.75 ± 1.15a	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b
Gastranaerophilales	0.30 ± 0.1a	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b
Selenomonadales	0.20 ± 0.1a	0.00 ± 0.00b	0.00 ± 0.00b	0.07 ± 0.1b
Campylobacterales	0.20 ± 0.00a	0.80 ± 1.21a	0.00 ± 0.00b	0.00 ± 0.00b
Enterobacteriales	0.00 ± 0.00b	1.33 ± 2.13a	0.10 ± 0.00b	0.13 ± 0.1b

Data are expressed as means ± SEM. Values in lines followed by different letters indicate significant differences between treatments according to Kruskal-wallis nonparametric test ($p < 0.05$). Treatments: Control group (CTL), high fat diet group (HFD), high fat diet + *L. casei* (HFD-LC) and high fat diet + ultrasound-inactivated *L. casei* (HFD-ILC).

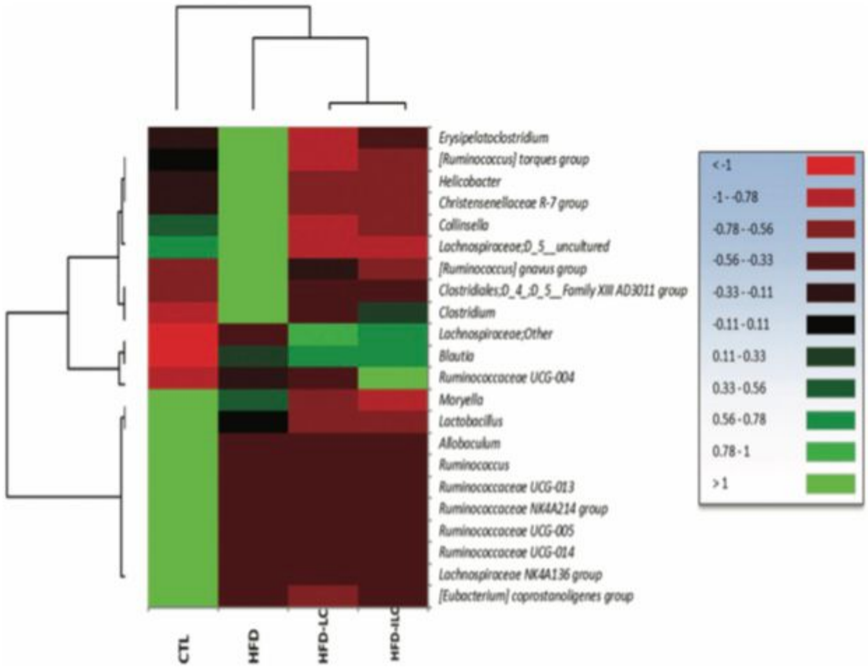

Fig. 5 Heatmap showing microbial taxa at family and genus level with relative abundance obtained by 16S rRNA gene pyrosequencing analysis. Only OTUs with abundance values above 0.1% in at least three samples are shown. Groups: Control group (CTL), high-fat diet group (HFD), high-fat diet + *L. casei* (HFD-LC) and high-fat diet + ultrasound-inactivated *L. casei* (HFD-ILC).

005 were closely related to CTL group, while *Blautia* and *Lachnospiraceae* were related to the HFD, HFD-LC, and HFD-ILC groups (Fig. S3†).

To detect specific patterns along with each taxonomy affiliation among groups, a heat map graphic was performed (Fig. 5). Three distinct clusters were observed. One cluster formed by feces samples of HDF-LC and HDF-ILC groups, despite differences in the relative abundance in specific bacterial microbial groups. The two other distinct clusters were formed by the feces samples of CTL and the HFD groups.

Besides, it was observed that the samples of CLT group clustered completely separated from both HFD and HFD-LC and HFD-ILC groups.

At the genus and family level, the graphic map shows distinct patterns of microbial groups among all the treatments. The feces samples from rats fed standard diet (CTL group) showed a high abundance of specific microbial groups: *Moryella*, *Lactobacillus*, *Allobaculum*, *Ruminococcus* and *[Eubacterium]* caprostanoligenes genera, and *Ruminococcaceae* (UCG-013, NK4A214 group, UCG_005; UCG-014) and *Lachnospiraceae* NK4A136 group. The feces samples from rats fed HFD clustered separately to feces samples from rats fed HFD receiving live (HFD-LC group) and ultrasound-inactivated *L. casei* (HFD-ILC group) and showed higher abundance of *Erysipelatoclostridium*, *[Ruminococcus]*_torques group, *Helicobacter*, *Chsristensenellaceae* R7-group, *Collinsella*, *Lachnospiraceae*;_0_5__uncultured, *[Ruminococcus]*_gnavus group, *Clostridiales*;_0_4;_0_5_Family XIII AD3011_group and *Clostridium*. On the other hand, they presented a lower abundance of microbial groups that characterized the CTL samples (Fig. 5). Samples from HFD-LC and HFD-ILC groups showed similar patterns and clustered separately. The feces samples from rats fed HFD and receiving live and ultrasound inactivated *L. casei* (HFD-LC and HFD-ILC) showed high abundance of specific microbial groups such as: *Lachnospiraceae;* other and Blautia. On the other hand, *Ruminococcaceae UCG-004* was found in higher abundance only in HFD-ILC samples. Furthermore, samples from HFDL-LC and HFD-ILC groups also showed lower abundance of *Allobaculum*, *Ruminococcus* and *[Eubacterium]* caprostanoligenes genera, and *Ruminococcaceae* (UCG-013, NK4A214 group, UCG_005; UCG-014) and *Lachnospiraceae* NK4A136 group.

4. Discussion

The consumption of live cells of *L. casei* incorporated or not into food products has been associated with several health effects, such as microbiota modulation, and anticarcinogenic, antihyperglycemic, antihypertensive, and antihypercholesterolemic properties.[2–5] However, few studies have focused on evaluating inactivated *L. casei* strains.[5,13] Therefore, the present study assessed the effects of live and ultrasound-inactivated *L. casei* on biochemical and cardiovascular properties and gut microbiota of rats fed a high-fat diet (HFD).

4.1 Diet intake and body weight

This study demonstrated that the daily ingestion of a HFD and live or inactivated *L. casei* cells did not alter the diet intake or weight gain. The influence of a HFD consumption or probiotic and paraprobiotic administration in these parameters has been controversial. Eating disorders and promotion of a higher weight gain may occur after a HFD,[9] but reduction in the food consumption to compensate the higher energy density may also be observed.[31] Besides, no effect of HFD on diet intake and/or body weight has also been reported.[32] The administration of probiotic or paraprobiotic may not alter diet intake and body weight,[7,16] but its influence has been reported to be strain-dependent[33] and inactivation method-dependent.[9] In some cases, lower adiposity may be observed after probiotic or paraprobiotic consumption but with no impact on body weight gain, which was attributed to an increase in lean mass.[34] The results were associated with the higher synthesis of protein and lower rates of protein degradation.[34] The lack of effects of live or ultrasound-inactivated *L. casei* on diet intake and weight gain is of interest because there is a growing demand for probiotic and paraprobiotic products that promote health benefits without altering these parameters.[35]

4.2 Biochemical parameters

This study demonstrated that the daily ingestion of a HFD resulted in increases in the TC and LDL-cholesterol and decreases in HDL-cholesterol, which corroborate with results found by Li and co-workers.[36] Hypercholesterolemia, mainly related to the high serum levels of LDL-cholesterol, is the major risk factor for cardiovascular diseases.[37] In addition, decreased serum HDL-cholesterol levels have been reported in individuals with unhealthy lifestyles.[38] The daily ingestion of both live or inactivated *L. casei* prevented the increase in TC and LDL-cholesterol caused by the HFD, while an increase in the HDL-cholesterol was only observed in rats receiving live *L. casei*. Probiotic cultures have been related to hypolipidemic properties, which were associated with the inhibition of cholesterol absorption by binding to the probiotic cells and increasing its excretion, and deconjugation of bile salts by the action of bile salt hydrolase, resulting in an increase in the levels of deconjugated bile salts, and higher excretion in faeces.[39] The cholesterol can attach the probiotic cells in the cell wall peptidoglycans, which contain amino acids with binding ability.[39] The increase in HDL-cholesterol is due to upregulation of the expression of ATPbinding cassette protein A1, which mediates the transportation of cholesterol from cells to apolipoprotein A-1, resulting in the formation of HDL.[32] HDL cholesterol may remove surplus cholesterol from blood to liver, helping to control the serum cholesterol concentrations.[39]

The effect of inactivated probiotics on the lipid levels was reported to be dependent on the inactivation method[9] and probiotic strain.[35] Cellular compounds presented in the dead cells could inhibit the synthesis of cholesterol, bind cholesterol, and prevent its return to the body, or interfere with bile salts recycling and elimination.[9] Furthermore, the utilization of ultrasound as inactivation method could degrade the beta-glucan of the probiotic cell wall, affecting the serum cholesterol levels.[6] Finally, dead cell compounds may modulate the immune system and the gut microbiota, improving the lipid profile.[7] Therefore, the ingestion of *L. casei* as live or ultrasound-inactivated cells exerted positive effects on cholesterol levels. To our knowledge, no previous studies were performed with ultrasound-inactivated *L. casei* that reported hypolipidemic properties.

The daily ingestion of a HFD also resulted in insulin resistance, while the daily ingestion of both live and inactivated

L. casei prevented it. Karamali *et al.*[40] observed that the administration of a probiotic yogurt (*L. acidophilus* LA5 and *B. animalis* BB12, 7 log CFU) for 9 weeks to healthy pregnant women resulted in a positive impact on the markers of insulin resistance. Russo *et al.*[33] reported that the administration of *Lactobacillus fermentum* CRL1446 (8 log CFU day^{-1}) for 14 weeks to Swiss albino mice fed a HFD resulted in reduced fasting glucose and insulin levels and amelioration of the insulin resistance. Probiotic cultures may ameliorate insulin resistance by increasing specific metabolites by modulating the gut microbiota.[35] Furthermore, probiotics may improve the numbers of hepatic natural-killer T (NKT)-cells and secretion of glucagon-like peptide (GLP)-1, upregulate adiponectin, decrease inflammation and glucotoxicity, and increase the sensitivity to insulin.[40] Finally, probiotic consumption may protect the pancreatic β cells, which are important for the production of insulin.[40]

Watanabe *et al.*[41] observed that the administration of heat-inactivated *Lactobacillus brevis* KB290 for 8 weeks to mice fed a HFD resulted in amelioration of the insulin resistance provoked by the HFD. An *et al.*[42] observed that the administration of heat-inactivated *Propionibacterium freudenreichii* for 6 weeks to HFD-induced obese mice resulted in alleviation of insulin resistance and reduced fasting glucose and insulin levels. Paraprobiotic may stimulate the gut microbiota by increasing the abundance of butyrate-producer strains. The main butyrate producing-bacteria belong to the phylum Firmicutes, which was increased in the present study in feces of rats fed ultrasound-inactivated *L. casei* in comparison to all other groups. Particularly *Eubacterium rectale* and *Roseburia* spp. of the family *Lachnospiraceae*, increased in rats fed *L. casei*, have been associated to high butyrate production in the gut.[43] Butyrate may activate GPR41 and GPR43, which induces the secretion of GLP-1 and suppress insulin signaling in adipocytes, contributing to ameliorate insulin resistance.[44] Furthermore, paraprobiotics may increase the expression of adiponectin in the epididymal adipose tissue, contributing to the improvement in the insulin sensitivity.[41,42] Finally, paraprobiotic consumption may reduce the infiltration of macrophages in adipose tissue and downregulate the expression of pro-inflammatory cytokine (TNF-α and IL-6) and the hepatic and serum lipid concentrations, resulting in improvements in the insulin resistance.[45] Therefore, the administration of *L. casei* as live or ultrasound-inactivated cells exerted positive effects on insulin resistance.

4.3 Cardiovascular parameters

Arterial hypertension has been reported in rats fed HFD, and the etiology is complex, involving peripheral and central blood pressure control.[15] We demonstrated that HFD consumption increased the arterial blood pressure, while the administration of live *L. casei* cells prevented this increase, and the administration of inactivated *L. casei* cells attenuated it. The uptake of probiotic cultures has been associated with the prevention of arterial hypertension in rats fed HFD, which was related to the recovery of the community of gut microbiota, production of metabolites with ability to modulate vasodilatation and hypotension and decreases in vascular inflammation and oxidative stress.[15] Paraprobiotic may stimulate the gut microbiota and increase the production of SCFA, which modulate vasodilatation and induce hypotension.[15] Here, the administration of *L. casei* as live cells exerted positive effects on control of blood pressure, while the ingestion of inactivated *L. casei* showed hypotensive properties. To our best knowledge, no previous studies have reported improved blood pressure control for inactivated probiotic cells.

4.4 Intestinal microbiota

The gut microbiota profile is dynamic and can be modified by many factors. At the phylum level, there was the predominance of *Firmicutes* and *Bacteroidetes* in the CTL group's intestinal microbiota. The intestinal microbiota of animals includes *Bacteroidetes*, *Proteobacteria*, *Firmicutes*, *Verrucomicrobia*, and *Archaebacteria*. *Bacteroidetes* and *Firmicutes* represent more than 90%.[46,47] It has been demonstrated that HFD consumption triggers gut microbiota dysbiosis, impairs gut microbiota diversity and composition, related by decreases in the *Bacteroidetes* and increases in *Firmicutes*, and leads to chronic low-grade inflammation.[14,36,46] In the present study, HFD consumption for 28 days decreased *Ruminococcaceae* and *Erysipelotrichaceae* families and increased others *Lachnospiraceae*, *Clostridiaceae 1*, *Peptostreptococcaceae*, *Helicobacteriacea*, and *Enterobacteriaceae*. The decrease in the *Ruminococcaceae* has been attributed to the lower carbohydrate content in the HFD, as the microorganisms of this family are recognized by utilizing plant polysaccharides.[48] Furthermore, *Clostridiaceae*, *Enterobacteriaceae*, and *Helicobacteriacea* can comprise important opportunistic pathogens.[49]

Our results demonstrate that administration of live and inactivated *L. casei* improved the abundance of *Lachnospiraceae* and *Ruminoccocaceae* UCG-004 families, and *Blautia* genera, while it decreased *Clostridiaceae*, *Enterobacteriaceae*, *Peptostreptococcaceae* and *Helicobacteriacea*.

Bacterial groups of the *Lachnospiraceae* family have repeatedly shown ability to produce beneficial metabolites for the host, despite their increased abundance reported in gut of subjects with different diseases.[50] On the other hand, *Ruminococcaceae* is associated with improvements in malnutrition and health recovery.[14] *Ruminococcaceae* (including *Blautia*) also produce SCFA, which can provide energy for intestinal cells and protect gut barriers.[14] A gut microbiota with lower abundance of *Proteobacteria*, and higher amounts of *Ruminococcaceae* is associated with a healthy status.[7] Proteobacteria has been related to the production of pro-inflammatory molecules in the gut and increase host fat storage.[51] Our findings show that the administration of live or inactivated *L. casei* did not entirely restore the effects provoked by the HFD consumption on the gut microbiota, but it is clear that it attenuates them increasing or decreasing specific groups with an important role on the health status.

Overall, more pronounced effects on the gut microbiota were generally observed for the inactivated *L. casei*, suggesting that the functionality of probiotics on intestinal microbiota was preserved or improved by ultrasound inactivation. Nevertheless, the differences at family levels between HFD-LC and HFD-ILC samples may be associated with cell compounds released from the dead cells. Cell surface compounds, such as teichoic acids, proteins, and polysaccharides, may contribute to the interaction of epithelial cells and paraprobiotics.[52] Therefore, inactivated probiotic cells may have improved adhesion capacity and antimicrobial properties.[52]

Furthermore, it is believed that inactivated probiotic cells affect gut functions and change the environmental conditions of the intestinal microbiota, resulting in changes in the microbial composition due to adaptation to the environmental conditions.[53] For inactivated probiotic cells, the peptidoglycan may interact with the intestinal environment, resulting in the activation of Paneth cells of the villi crypts and production of defensins.[54] These features are associated with protection and regulation against pathogenic bacteria.[55] Furthermore, both live and inactivated probiotic cells may adhere to intestinal cells and co-aggregate with possible pathogens.[6]

This study demonstrated that live or inactivated *L. casei* cells' consumption decreased slightly the diversity of microorganisms compared to HFD. This could be related to the suppression of pathogenic microorganisms' growth (*e.g. Proteobacterium* and *Helicobacter pylori*) and favoring the growth of few bacteria with beneficial properties, resulting in decreases in the gut microbiota diversity.[56] A lower diversity is considered a sign of disorder in the intestinal microbiota; however, the inhibition of the growth of pathogenic microorganisms promoted by probiotics and paraprobiotics is associated with a reduction on the overall microbiota diversity. In this way, a healthy intestinal microbiota should consider the diversity of the microbiota but also the microbiota composition and abundance.[57]

5. Conclusion

Our results demonstrate that the consumption of live and ultrasound-inactivated *L. casei* can reduce the cholesterol levels (total and LDL) and control insulin resistance in rats fed a HFD. Besides, it promoted a modulation of the gut microbial composition by increasing and decreasing specific bacterial groups attenuating the effects promoted by the HFD. Live cultures could be a strategy to improve the cholesterol levels (mainly HDL-cholesterol), while the inactivated probiotic culture could be used to improve hypertension (SAP, MAP and DAP) and cholesterol levels (total and LDL-cholesterol). The results indicate that ultrasound is an efficient technology to inactivate probiotic cultures and produce paraprobiotic *L. casei* with health properties similar to live cells.

Author contributions

LRB, JLBA, AGC, TCP, HV and MM participated in the conceptualization of the study; LRB, WKAC, GAH, MPO and JLBA performed the experiments, LRB, WKAC, GAH, JLBA, TCP, MM, MFN and LC worked in the formal analysis of the data; LRB, WKAC, JLBA, LC, TCP, HV and MM wrote the original draft of the manuscript. All authors were involved in review, editing and visualization. MM, JLA and VB provided the resources for development of the study and worked in the supervision; MM and JLA was responsible by the project administration.

Conflicts of interest

The authors declare no conflict of interest.

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