UNIVERSIDADE FEDERAL DO PARANÁ

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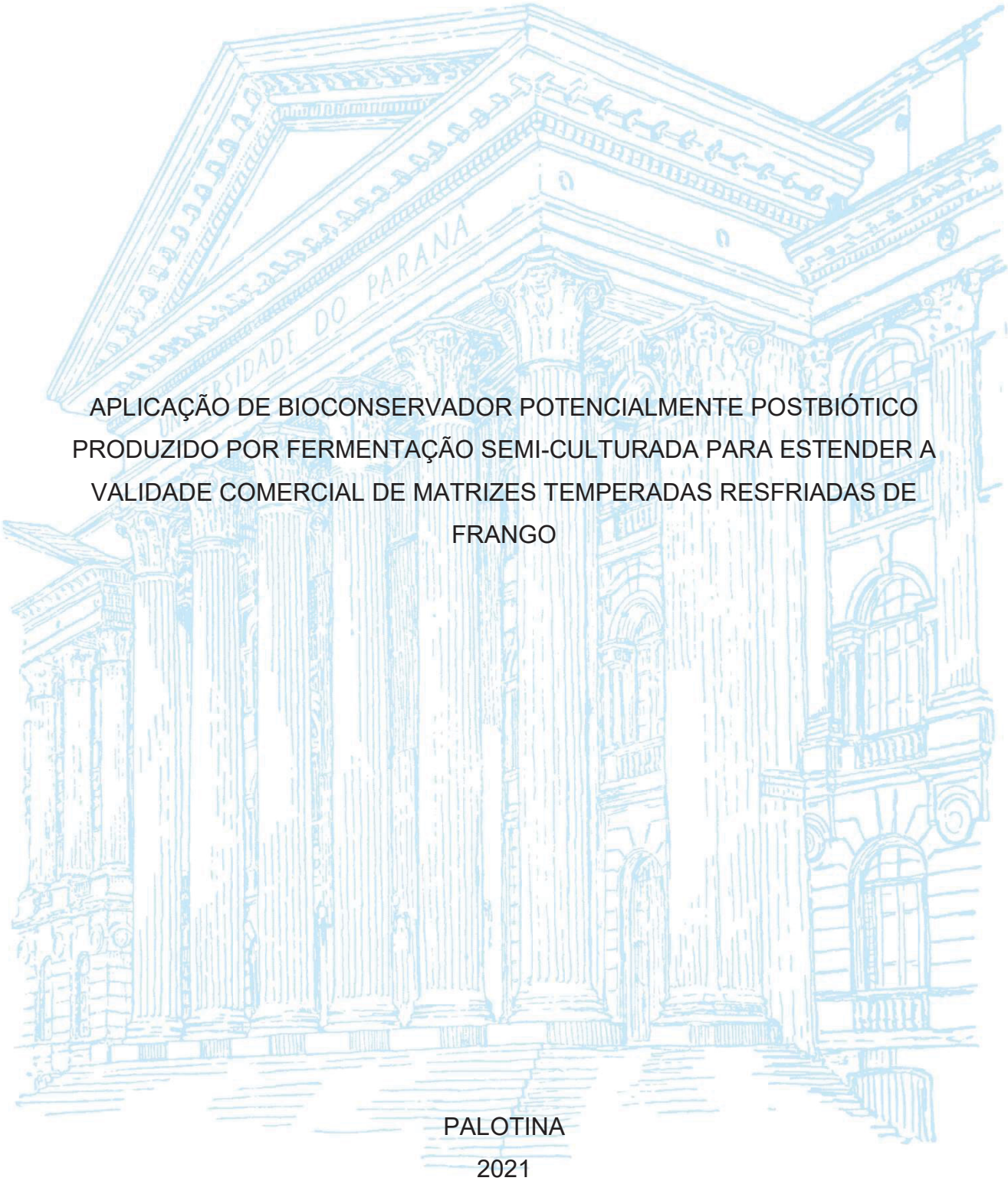

APLICAÇÃO DE BIOCONSERVADOR POTENCIALMENTE POSTBIÓTICO PRODUZIDO POR FERMENTAÇÃO SEMI-CULTURADA PARA ESTENDER A VALIDADE COMERCIAL DE MATRIZES TEMPERADAS RESFRIADAS DE FRANGO

PALOTINA

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Dissertação apresentada como requisito parcial à conclusão do Programa de Pós-Graduação em Biotecnologia, Setor de Palotina, Universidade Federal do Paraná.

Orientadora: Profa. Dra. Ivonete Rossi Bautitz.
Coorientador: Prof. Dr. André Fioravante Guerra

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação BIOTECNOLOGIA da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de **CAROLYNE LUCIANE DE ALMEIDA GODOY** intitulada: **APLICAÇÃO DE BIOCONSERVADOR POTENCIALMENTE POSTBIÓTICO PRODUZIDO POR FERMENTAÇÃO SEMI-CULTURADA PARA ESTENDER A VALIDADE COMERCIAL DE MATRIZES TEMPERADAS RESFRIADAS DE FRANGO**, sob orientação da Profa. Dra. IVONETE ROSSI BAUTITZ, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de mestra está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

PALOTINA, 21 de Dezembro de 2021.

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Dedico este trabalho aos meus pais, marido e filhos, que me deram suporte, apoio, motivação e incentivo para percorrer cada etapa deste desafio.

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Enfim, agradeço a todos que direta ou indiretamente me auxiliaram na execução deste trabalho.

RESUMO

Objetivou-se neste estudo avaliar o uso de conservador potencialmente pós-biótico (PPCP), produzido em um sistema de fermentação semiculturado com *Lacticaseibacillus paracasei* DTA 83 e *Saccharomyces cerevisiae* var. *boulardii* 17, para estender a validade comercial de linguiça frescal e cortes temperados de frango resfriados. Microrganismos associados com a deterioração dos derivados de frango foram estimulados ao crescimento por incubação em pares dos produtos em duas temperaturas diferentes, com coletas em diferentes tempos. O método turbidimétrico foi realizado para avaliar a susceptibilidade microbiana para o PPCP. PPCP foi adicionado nos derivados de frango para obter efeito inibitório parcial *in situ* sobre os microrganismos deteriorantes e estender a validade comercial. A triagem *in vitro* indicou inibição total do crescimento microbiano em concentrações acima de 3.0 % de PPCP. Embora esta concentração tenha apresentado notável potencial inibitório, a sua adição pode impactar o custo da formulação. Assim, a aplicação de doses com inibição microbiana parcial podem ser uma estratégia adequada para o uso de PPCP em derivados de frango. Os resultados demonstraram que o gerenciamento da cadeia de frio e co-utilização de PPCP estenderam a validade comercial dos derivados de frango, sugerindo uma alternativa tecnológica para bio-controlar o crescimento microbiano em matrizes cárneas.

Palavras-chave: Bio-conservador. Biocontrole. Metabiótico. Benéfico. Substâncias.

ABSTRACT

This study aimed to evaluate the use of potentially postbiotic-containing preservative (PPCP), produced in a semi-culture fermentation system with *Lacticaseibacillus paracasei* DTA 83 and *Saccharomyces cerevisiae* var. *boulardii* 17, to extend the use-by date of raw chicken sausages and semi-finished chicken products. Microorganisms associated with the spoilage of chicken products were stimulated to grow by pair incubation of the products at two different temperatures and with collection at different times. The turbidity method was performed to evaluate the microbial susceptibility to PPCP. PPCP was added in chicken products to obtain an *in situ* partial inhibitory effects on spoilage microorganisms to extend the use-by date. The *in vitro* trial showed total inhibition of the microbial growth by adding above 3.0% of PPCP. Although this concentration showed a remarkable inhibitory potential, its addition can severely impact the formulation cost. Thus, the application of doses with partial microbial inhibition may be a suitable strategy for the use of PPCP in chicken products. The results revealed that cold chain management and co-use of PPCP in chicken products extended the proposed use-by date, suggesting an alternative food preservation technology for the use of naturally derived compounds.

Keywords: Biopreservative. Biocontrol. Metabiotic. Beneficial. Compounds.

SUMMARY

1 INTRODUCTION

The presence of spoilage microorganisms in food represents a critical issue with repercussions on massive food waste worldwide. Food shelf-life is the period over which a food maintains its safety and quality under reasonably foreseeable conditions of distribution, storage, and use. Consumer's eating habits have changed over the decades in pursuit of a healthier diet. Since consumers have taken up the increasing concept of using food to manage health, several industries are focusing on replacing added artificial preservatives. Biopreservation is one of the alternative food preservation technologies for the use of naturally derived compounds. Nowadays, they are of increasing interest for the food industry and consumers.

Substances from lactic acid bacteria and *S. cerevisiae* are affirmed by U.S. Food and Drug Administration (FDA) as Generally Recognized as Safe (G.R.A.S.) in section 21 CFR184, being useful to control the frequent development of pathogens and spoiling microorganisms in food and foodstuff. FDA also prescribe those conditions for their use are prescribed in the referent regulations and are predicated on the use of nonpathogenic and nontoxicogenic strains of the respective organisms and on the use of current good manufacturing practice (184.1(b))'.

Lacticaseibacillus paracasei DTA 83 and *Saccharomyces cerevisiae* var. *boulardii* 17 have been reported as candidate strains to deliver probiotics in food matrices. Otherwise, concerns on the administration of viable cells to have been raised in the current literature. While they can play a benefic role in the host, the invasive potential of these microorganisms may limit their use to healthy people. Therefore, delivery probiotics to health-impaired individuals or when medical institutions are considered is still a matter of discussion. Indeed, postbiotic effect derived from G.R.A.S microorganisms can be safe in all situations.

Under this background, this study aimed to produce potentially postbiotic-containing preservative (PPCP) in a semi-culture fermentation system with *L. paracasei* DTA 83 and *S. boulardii* 17 to extend use-by date of raw chicken sausage and semi-finished chicken products. In addition, three logistic distribution routes (R) were drafted to evaluate the impact of the cold chain management on use-by date of the products.

2 LITERATURE REVIEW

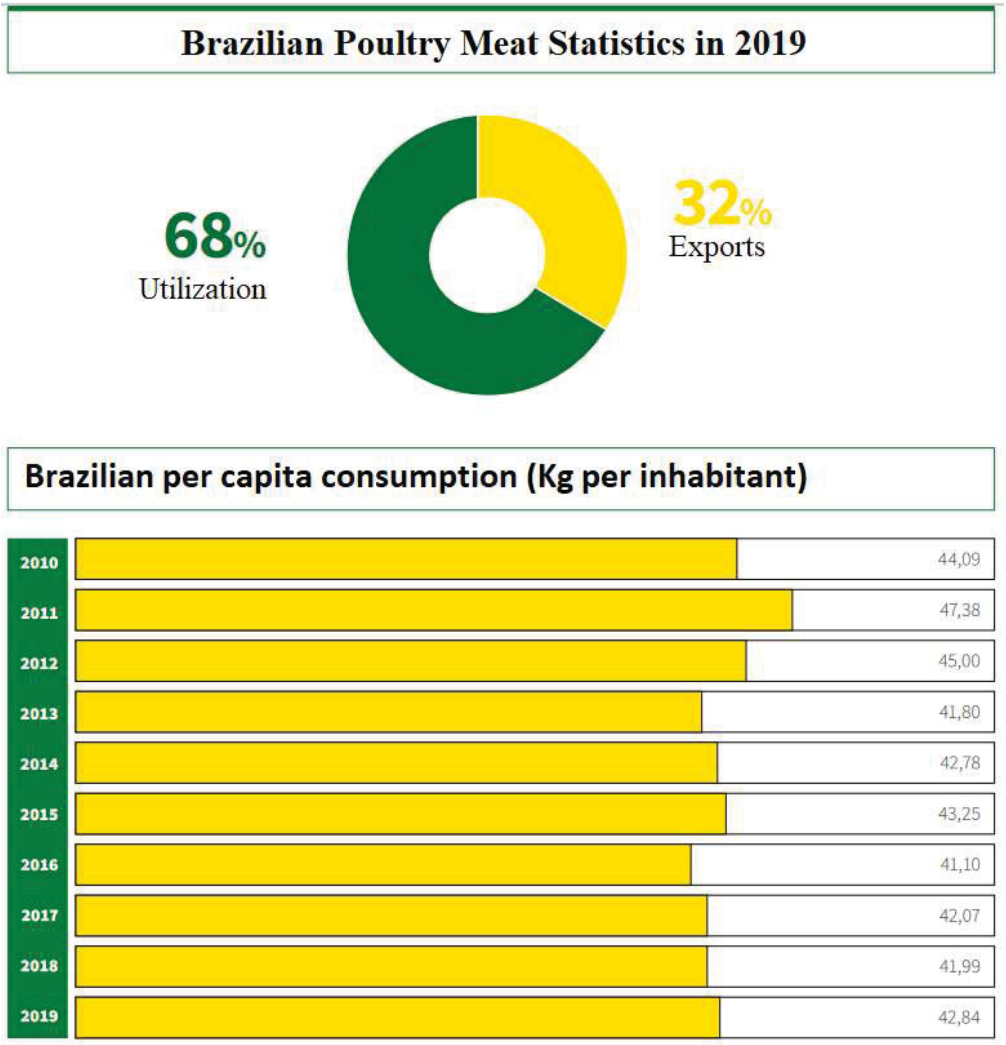
2.1 OVERVIEW OF POULTRY PRODUCTION AND TENDENCY OF THE SECTOR

World poultry meat production reached 133 million tonnes in 2020, up 1,3 percent year-on-year. World total meat production was of about 337 million tonnes (in carcass weight equivalent) to the same year, similar to the previous year. Increased poultry and ovine meat outputs compensated for pig and bovine meat production contractions. Poultry meat's relative affordability and shorter production cycle were the two critical enabling factors contributing to the sector's better performance than the bovine, ovine and pig meat sectors. Much of the output expansion occurred in China, the United States of America, Brazil, South Africa and the European Union. Poultry meat imports in China surged by nearly 55 percent from 2019, rising to a record level of 2,2 million tonnes, secured from many countries, including Brazil, the United States of America, the Russian Federation, Thailand, Argentina, the European Union and Chile [1].

Brazilian total meat production in 2020 was at 28,620 thousand tonnes (carcass weight equivalent). Regarding production of poultry meat, Brazil was the fourth largest producer in 2020, about 14,363 thousand tonnes in carcass weight equivalent was produced in 2020. China was the largest producer in the world (77,918 thousand tonnes), followed by the European Union (48,459 thousand tonnes), and the United States of America (48,112 thousand tonnes). Brazilian poultry meat production has been destinated to consumption (10,288 thousand tonnes) and to exports (4,080 thousand tonnes) (FIGURE 1 and 2). Only 5 thousand tonnes was imported by Brazil in 2020 [1][2].

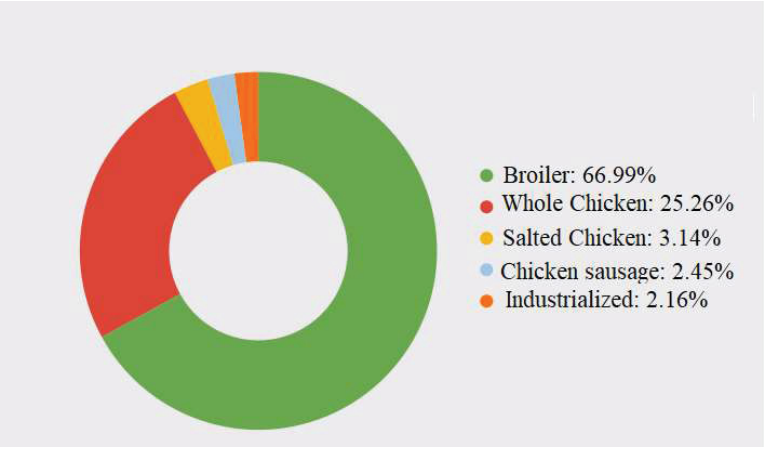
According to Brazilian Association of Animal Protein (ABPA), the southern of Brazil is the largest poultry slaughterer. The state of Paraná slaughtered 34.69%, followed by the state of Santa Catarina (15.40%) and Rio Grande do Sul (14.32%) (FIGURE 3).

FIGURE 1 – SUMMARY OF BRAZILIAN POULTRY MEAT STATISTICS AND PER CAPITA CONSUNPTION



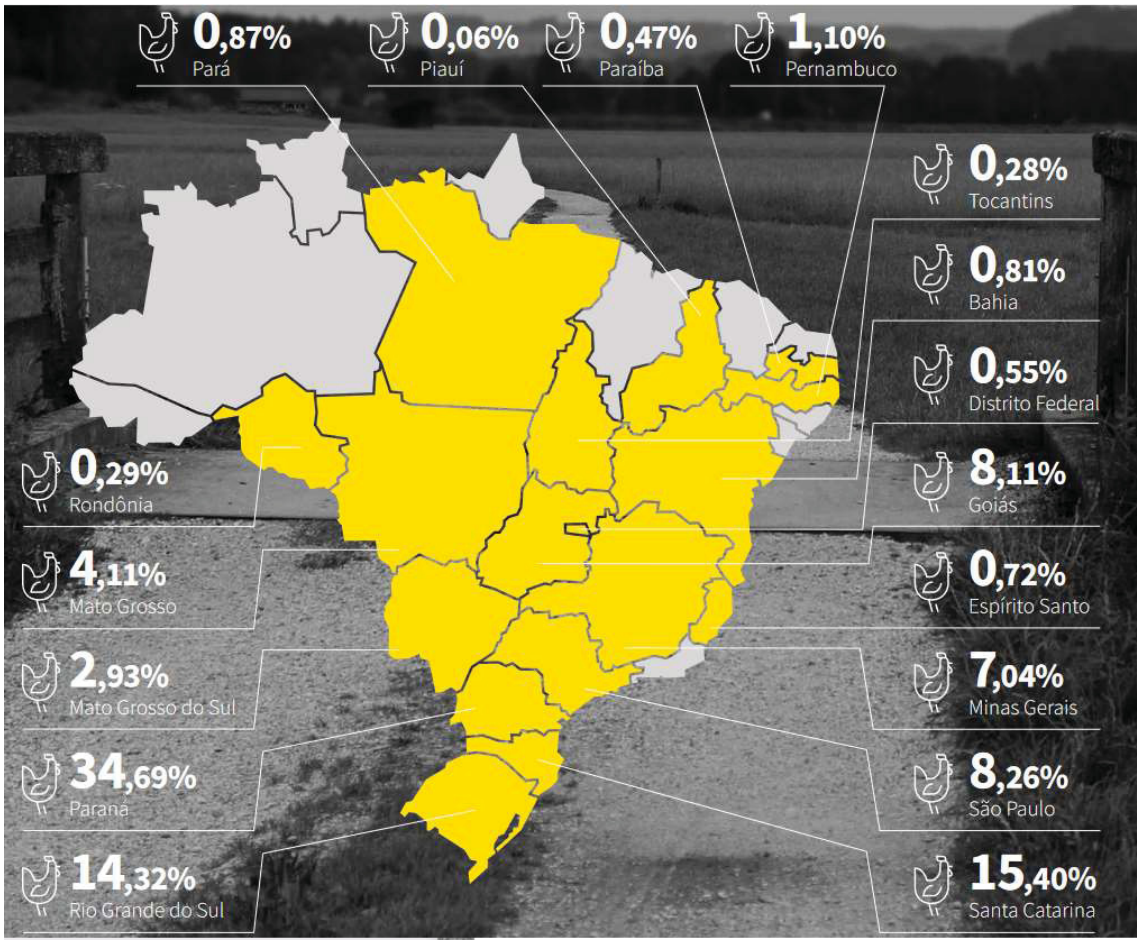
SOURCE: [2] (adapted).

FIGURE 2 – BRAZILIAN POULTRY MEAT EXPORT IN 2019



SOURCE: [2] (adapted).

FIGURE 3 – RANKING OF BRAZILIAN POULTRY MEAT PRODUCTION IN 2019 BY FEDERATIVE STATES



SOURCE: [2].

Estimated growth for global meat production up to 16% is expected in 2025 [3] and up to 19% in 2030 [1], among them the poultry meat will represent the biggest growth. The factors that contribute to the high consumption of this food in the country are its availability, cost-effectiveness, ease and versatility of cooking, as well as its nutritional quality, such as vitamins, minerals, protein and has a low level of saturated fat, because it is concentrated in the skin that can be easily removed [4].

Poultry meat industry in Brazil has become very competitive with the vertical integration model. The evolution and expansion can be attributed to new commercial and productive dynamics, especially in the southern region of the country [5]. The Western of the state of Paraná (Brazil) contributes for the increase of the sector. Agricultural cooperatives model that was developed in the

state, coupled with an integrated management model, provides income stability to producers [6].

Chicken products has been for decades approached as commodity [6]. Therefore, manufacturers need to provide innovative products to consumers, specially to the supply of natural and functional products. It is worth noting that poultry meat is equally consumed in all social classes and of about 80% of the Brazilians intake poultry twice a week [2].

Kumari and colleagues evaluated the quality and shelf life of chicken meat cutlets by incorporating functional ingredients like mushrooms, sesame seeds and wheat gluten at optimized levels. The authors observed that mushroom, sesame seeds and wheat gluten in chicken meat cutlets had significantly higher moisture, ash, fat, protein, crude fiber, cooking yield, sensory attributes, lower free fatty acids, peroxide value, lower shrinkage and shear force in comparisons to control samples. Additionally, the products containing functional ingredients had significantly higher acceptability than control chicken meat cutlets [7].

Chappalwar et al. (2020) developed low-fat chicken patties by incorporating mango peel powder as fat replacer to replace vegetable oil in the formulation. The emulsion pH, emulsion stability, water activity, fat and cholesterol content of mango peel treated chicken patties were significantly lower, however, cooking yield, moisture content, fat retention and moisture retention values were significantly higher than control. All mineral content decreased significantly in treatments except potassium and phosphorous content. Incorporation of mango peel powder had a significanteffect on textural and colour parameters. Sensory scores decreased significantly in treatments, however, the product was well acceptable up to 2% of mango peel powder incorporation [8].

Probiotic strains have been extensively used in the meat poultry chain. Soni et al. (2021) evaluated *Bacillus velezensis* ZBG17 performance as antibiotic growth promoter substitute in broiler chickens. The authors reported that ZBG17 completely inhibited *Salmonella enterica* and *Escherichia coli* within 6 h and 8 h in liquid co-culture assay, respectively. In addition, dietary supplementation of ZBG17 significantly improved feed utilization efficiency and humoral immune response in broiler chickens, suggesting its prospective application as a direct-fed microbial in broiler chickens.

Arif et al. (2021) carried out feeding trial was conducted to evaluate the effects of *Bacillus*-based probiotics on growth performance, intestinal histo-morphology, gut microbial population and immune response in broilers. Dietary inclusion of *B. licheniformis* significantly improved body weight gain in broilers. In line with noted by Soni et al. (2021), probiotics increased the population of *Bacillus* spp. and decreased the population of *Clostrium perfringens, Salmonella* spp. and *Escherichia coli* in the jejunum and ileum in broiler birds on day 21 and 35 [10].

Ahiwe et al. (2021) reported in a systematic review of the literature that there is convincing evidence that probiotic and prebiotic S. cerevisiae products can replace in-feed antibiotics in broiler chicken production; however, there is a need for more testing in order to achieve consistency. A combination of appropriate yeast products alongside proper husbandry practices and bio-security measures could significantly reduce the observed inconsistencies, maximize broiler productivity and pave the way to a global antibiotic-free era in meat production. The study reported by is in line with increasing consumer demand for antibiotics-free poultry products [11].

2.2 BENEFICIAL MICROBES AND METABOLITIES

The first observation of the benefic role played by some selected bacteria is attributed to Eli Metchnikoff, the Russian born Nobel Prize winner by its work at the Pasteur Institute at the beginning of the last century. Metchnikoff suggested the dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes [12].

The first scientific mention of the word ‘probiotic’ was done by Lilly and Stillwell (1965). The authors used the term meaning ‘unknown growth-promoting factors produced by microorganisms. Later, this effect was attributed to certain peptides produced by *Colpidium campylum* with impact to prolong the logarithmic growth phase of Tetrahymena pyriformis. To date, the term probiotic is reserved to live microbes with beneficial effect on the host; however, the first meaning attributed by Lilly and Stillwell is closely related with the prebiotic concept.

The first use of the term probiotic referring to the beneficial effect of microbes is attributed to Élie Metchnikoff. The author provided a definition as ‘Organisms and substances which contribute to intestinal microbial balance’. Although the mention of organisms was included in the author’s definition, they have not restricted the term and ‘substances’ were also included in the definition. Moreover, the authors did not indicate the host which the probiotic acts neither about the viability of the microorganisms [14].

In 1989, Fuller contributed to the field providing another definition to the term as ‘A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance’. The definition by Fuller, expressively contributed to restrict the term probiotic to the use as ‘a live microbial’; otherwise, the term was used to represent the beneficial effect from probiotics to the ‘host animal’. The beneficial effect on the human host was suppressed in the Fuller’s definition [15].

Havenaar et al. (1992) redefined the term as ‘a viable mono or mixed culture of bacteria which, when applied to animal or man, beneficially affects the host by improving the properties of the indigenous flora’. In this definition, the authors have included host ‘man’, beyond ‘animal’. The microbial viability was included in the definition being one of the major contributions of this definition, but it was still lacking information as that related to the dose to play a beneficial effect role in the host [16].

In the Lactic Acid Bacteria Industrial Platform (LABIP) workshop, took place in 1998, a close definition as that proposed by World Health Organization (WHO) was designed as ‘oral probiotics are living micro-organisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition’. Broadening the term to the consumption either as a food component or as a non-food preparation. Although at that occasion ‘opinions differ widely with respect to the requirements needed to substantiate a claim on a beneficial effect of a given bacterial, and there is no consensus on how to define and accredit a viable strain as a probiotic’, the group concluded that well-designed human studies are required, as doubled blinded, placebo-controlled design. Beyond a well-supported in vitro assays [17]. In the occasion, probiotics intended to play a beneficial role out of intestinal tract was not included in this definition, as probiotics to skin or buccal benefits.

In 2001, FAO and WHO proposed a definition of probiotics used today as ‘‘live microorganisms which when administered in adequate amounts confer a health benefit on the host’. A guideline for researchers to evaluate the functional efficiency of probiotic containing foods was recommended into three-stage: safety assessment in *in vitro* and *in vivo* experiments (phase I); evaluation in the Double-Blind, Randomized, Placebo-Controlled trial (phase 2); and post-approval monitoring (phase III). It is noted that along with the ability to obtain statistically significant results of the evaluation, there are practical difficulties of conducting the experiments related to duration, costs, difficulties in selection of target biomarkers and populations. The promising approach for assessing the functional efficacy of a functional food is the concept of nutrigenomics. It examines the link between the human diet and the characteristics of his genome to determine the influence of food on the expression of genes and, ultimately, to human health. Nutrigenomic approaches are promising to assess the impact of probiotics in healthy people [18].

In October 2013, the International Scientific Association for Probiotics and Prebiotics (ISAPP) met to discuss on probiotics. The FAO/WHO definition of a probiotic was reinforced as relevant and sufficiently accommodating for current and anticipated applications. The panel found that the definition of a ‘probiotic advanced by the FAO/WHO in 2001 is sufficiently broad to enable a wide range of products to be developed, and sufficiently narrow to impose some core requirements, the development of metabolic by-products, dead microorganisms, or other microbial-based, nonviable products have potential; however, these do not fall under the probiotic construct’ [19].

As the concept of probiotic are used exclusively to microorganisms, that compounds with stimulus to grow microorganisms are not covered anymore. Gibson and Roberfroid (1995) introduced the concept of prebiotic as ‘nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health’ [20]. In 2016, the International Scientific Association for Probiotics and Prebiotics (ISAPP) convened expert in microbiology, nutrition and clinical research for reviewed the definition and scope of prebiotics and the new definition draft was ‘a substrate that is selectively utilized by host microorganisms conferring a health

benefit' [21]. Thus, prebiotics are substrates used to selectively boost the colonic bacteria in the gut, which can provide beneficial role in the host, especially those belonging to bifidobacterial and lactobacilli group. In line with prebiotic definition, the consumption of prebiotic without hosting beneficial microbe may be vague. The consumption of symbiotic products may be a suitable alternative. Later, this definition was endorsed by FAO in the Technical Meeting on Prebiotics [22].

Gibson and Roberfroid (1995) also introduced the concept of symbiotic as probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare [20].

Over the past decade, based on some evidence supported by researchers on beneficial effects from administration of inactivated or produced compounds by microorganisms, novel terms have been used. To date, the terms have not been endorsed by regulatory agencies. They have been used exclusively in scientific literature as in peer-review papers.

Shenderov (2011) introduced the concept of metabiotics in order to represent structural components of probiotic microorganisms and/or formulation of and/or signaling molecules with a known chemical structure that can optimize host-specific physiological functions and regulate metabolic and/or behavior reactions connected with the activity of host natural microbiota [23] [24]. In 2020, Shenderov and colleagues dedicated a book to firmly stablish the term on beneficial compound group. Before that, the term has been used out-of-context about beneficial compounds. In such case, it is used as a synonymous of symbiotic effect in microbial consortium in foods (ripening of cheese and vegetables), animal (ruminant digestion system) and soil [25][26] [27][28][29][30][31][32][33].

Taverniti and Guglielmetti (2011) concepted the term 'paraprobiotic' (or 'ghost probiotics'), to be defined as 'non-viable microbial cells (intact or broken) or crude cell extracts (i.e. with complex chemical composition), which, when administered (orally or topically) in adequate amounts, confer a benefit on the human or animal consumer'. The authors stated that 'purified molecules of microbial origin or pure microbial cell products are omitted from the concept of

paraprobiotics, since their use should be included in conventional pharmaceutical methodologies. In addition, once a health benefit is demonstrated, the assignation of a product into the paraprobiotic category should not be influenced by the methods used for microbial cell inactivation, which may be achieved using physical or chemical strategies, including heat treatment, γ or UV ray deactivation, chemical or mechanical disruption, pressure, lyophilisation or acid deactivation' [34].

Tsilingiri (2012) introduced the term postbiotic as metabolic products, produced by probiotic strains for a healthier intestinal homeostasis, but also as therapeutic aids in inflammatory bowel disease (IBD) with, however, very little clinical benefit. This may be due to the lack of reliable preclinical models for testing the efficacy of different strains [35].

Dinan et al. (2013) define psychobiotic 'as a live organism that, when ingested in adequate amounts, produces a health benefit in patients suffering from psychiatric illness. As a class of probiotic, these bacteria are capable of producing and delivering neuroactive substances such as gamma-aminobutyric acid and serotonin, which act on the brain-gut axis'. Effects may be mediated via the vagus nerve, spinal cord, or neuroendocrine systems. The authors have not restricted the term to live probiotics, thus both probiotics or postbiotics can be assossiated to delevery psychobiotic effects [36].

Neef and Sanz (2013) introduced the concept of Next Generation of Probiotics based on evidence on microbiome-mediated effects by intervention with classical probiotics on humans is limited. Next generation of probiotics is linked with advances in next-generation sequencing methodologies and high-throughput sequence analysis. The discovered associations between microbiota members and human health and disease are leading to hypothesize that a collection of functionally distinct bacterial species may be more effective for specific conditions than single strains and classical probiotics, consisting of allochthonous species or species of low prevalence in the human gut. Thus, Clostridia clusters IV, XIVa and XVIII, *Faecalibacterium prausnitzii, Akkermansia muciniphila* and *Bacteroides uniformis* have also been included in preclinical trials with promising results for inflammatory and diet-related disorders [37].

It is also possible to note the use of terms related to specific targets. In this sense, the term PROPATRIA (PRObiotics in PAncreatitis TRIAl) has been

used to represent a double-blind, placebo-controlled randomised multicenter trial that aims to show a reduction in infectious complications by the enteral use of a multispecies probiotics preparation in patients with predicted severe acute pancreatitis [38]. [39]. In 2008, it was performed nationwide multicentre randomised, double-blind, placebo controlle PROPATRIA to assess the effects of probiotic pro phylaxis in patients with predicted severe acute pancreatitis. The methods/design are described in detail by Besselink et al (2008).

In TABLE 1 is showed advantages to the consumption of postbiotics instead probiotics.

TABLE 1 – BENEFICIAL ATTRIBUTED TO CONSUMPTION OF INACTIVATED PROBIOTICS.

Aspect	Advantages
Safety	No risk of translocation from gut lumen to blood, particularly in vulnerable individuals. No risk of acquisition and retransfer of antibiotic resistance genes. No risk of interference with normal colonization of gut microbiota in neonates.
Physiological effects	Release of active molecules from the disrupted inactivated cells, passing through the mucus layers and stimulating epithelial cells more directly. Loss of viability and cell lysis can produce further and more complex beneficial effects.
Pharmaceutical characteristics	Easier to standardize, transport, and store.

SOURCE: [41][42]

Postbiotic mechanism is still not totally elucidated, but several biological responses are reported after its administration as showed in TABLE 2 [43].

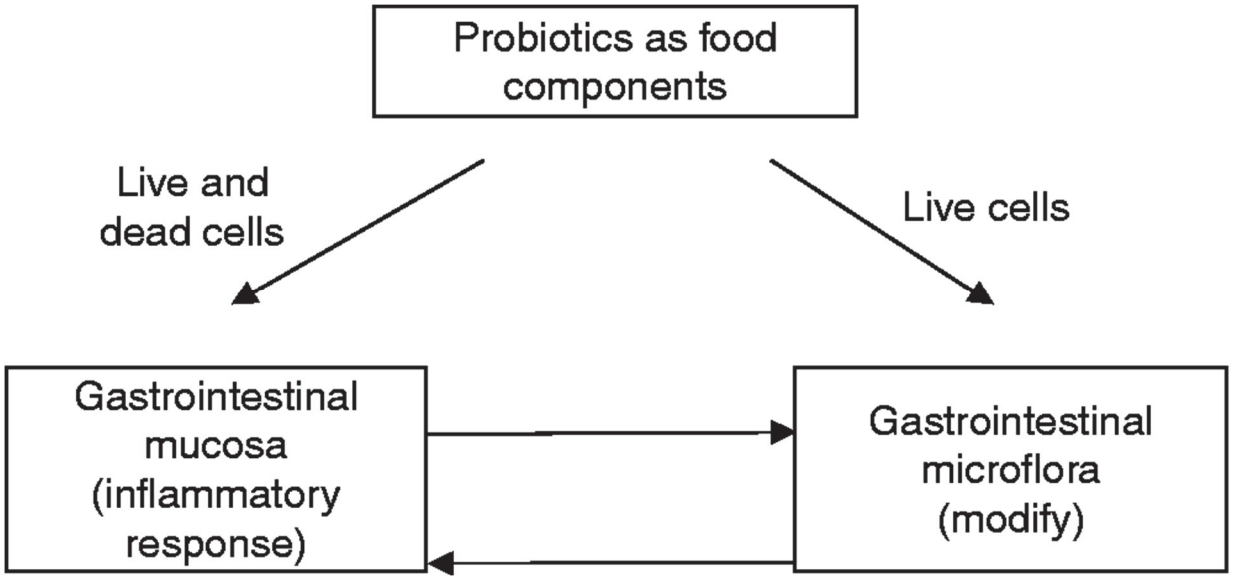
TABLE 2 – BIOLOGICAL RESPONSE IN THE HOST RELATED TO POSTBIOTIC COMPOUNDS.

Agent	Biological response
Live and heat-killed *L. rhamnosus* GG	To decrease in pro-inflammatory mediators induced by lipopolysaccharides and increase in anti-inflammatory mediators in gastrostomy-fed infant rats.
L. rhamnosus GG, *L. plantarum* L-137, *B. breve*, *Escherichia coli* Nissle 1917, *B. bifidum, L. acidophilus, L. helveticus, B. bifidum* and *L. casei*.	beneficial immunomodulatory responses, in vitro model
Enterococcus faecalis (EC-12)	To prevent vancomycin-resistant enterococci colonization in the cecum of newly hatched chicks
L. casei strain Shirota or *L. fermentum* MS15	To modulate inflammatory response by regulating Interleukin-10, human B-defensin and other pro-inflammatory cytokines
B. breve and *B. bifidum*	To increase the secretion of Interleukin-10, an anti-inflammatory cytokine
B. breve M-16V	Presented immune-modulating effects that suppressed pro-inflammatory cytokine production in spleen cells and affected intestinal metabolism

SOURCE: [43].

Adams (2010) reported that live and dead cells in probiotic products can generate beneficial biological responses. Live probiotic cells influence both the gastrointestinal microbiota and the immune response whilst the components of dead cells exert an anti-inflammatory response in the gastrointestinal tract (FIGURE 4) [44].

FIGURE 4 – PROPOSED MECHANISM TO BENEFICIAL BIOLOGICAL RESPONSES FROM LIVE (PROBIOTIC) AND DEAD (POSTBIOTIC) CELLS.



SOURCE: [44].

2.3 PERISHABILITY OF MEAT AND SHELF-LIFE VALIDATION

Foods require ‘use-by’ or ‘best-before’ date as parameter to validate their shelf-life. The term ‘use-by’ date indicates the date until which the food is considered safe for consumption. The term ‘best-before’ date corresponds to the date until which the food retains its specific properties (quality characteristics such as appearance, odor, texture, and flavor) when properly stored [45].

Microbial growth is the main cause of meat product spoilage during storage for sale in markets [45]. Microbial activity and autolysis lead to the breakdown of nutrients, which results in slime formation, discoloration, off-odors, and flavors, making meat products unacceptable for consumption [47]. The

growth of bacterial polysaccharide polymers is a common defect in meat products. Thus, the visual aspect created by the formation of ropy slime impacts the consumer appraisal and may cause the product rejection [46].

Numerous factors can influence microbial growth in food. Interactions involving food ingredients and microbes are complex to design [47]. Additionally, intrinsic parameters of foods can be affected by several factors such as the producing process and recipes, ingredients, water activity (aw), potential of hydrogen (pH), the natural presence or direct addition of preservatives, competitive microorganisms, temperature and time of storage, package atmosphere, redox potential, among others [48].

The food industry requires a relatively short time to obtain the information needed to determine the shelf-life of food products. For practical reasons, when the actual product shelf-life is long, the industry usually uses accelerated tests that considerably shorten the time spent to obtain relevant experimental data [49]. Moreover, predictive models have been extensively developed to shorten the testing time in food microbiology.

While some predictive methods allow to carry out a durability study or challenging tests at different temperatures, few methods permit the use of a dynamic temperature profile in the same test to achieve realistic temperature conditions based on the temperatures to which the products are exposed during storage for sale in markets [50]. Temperature variations caused by climatic and geographic factors and normal fluctuation over the day may affect microbial growth and impact the food shelf-life period.

The microbial growth predictor, named *MicroLab_shelfLife*, is a designed method to perform a durability study of meat products by predicting the microbial growth curve of their natural microbiota under a dynamic temperature profile. In brief, specific growth rates per hour (log cfu/g/h) at lower and higher temperatures are obtained by determining the angular coefficient of the exponential (log) phase in each growth curve (FIGURE 4). They were calculated to one unit of degree Celsius (log cfu/g/h/°C) by dividing the mean value of the angular coefficient by the difference between the higher and the lower temperature. This parameter is used to calculate the microbial growth per hour at each the temperature profile. Hourly microbial growth is obtained by multiplying the specific growth rate (log cfu/g/h/°C) by the temperature value during 1 hour. Daily growth is obtained by

the sum of all hourly growth. The method was designed to model the microbial growth in the deceleration phase based on the value of the exponential (log) phase. In a computational predictive modeling package, compiled using the Visual Basic Application – Excel 2016 (MicroSoft, Washington, USA), a borderline limit can be entered in order to preview ‘use-by’ or ‘best-before’ date of the test (FIGURE 5).

FIGURE 4 – DESIGN FOR SAMPLE INCUBATION IN MICROLAB_SHELF-LIFE METHOD

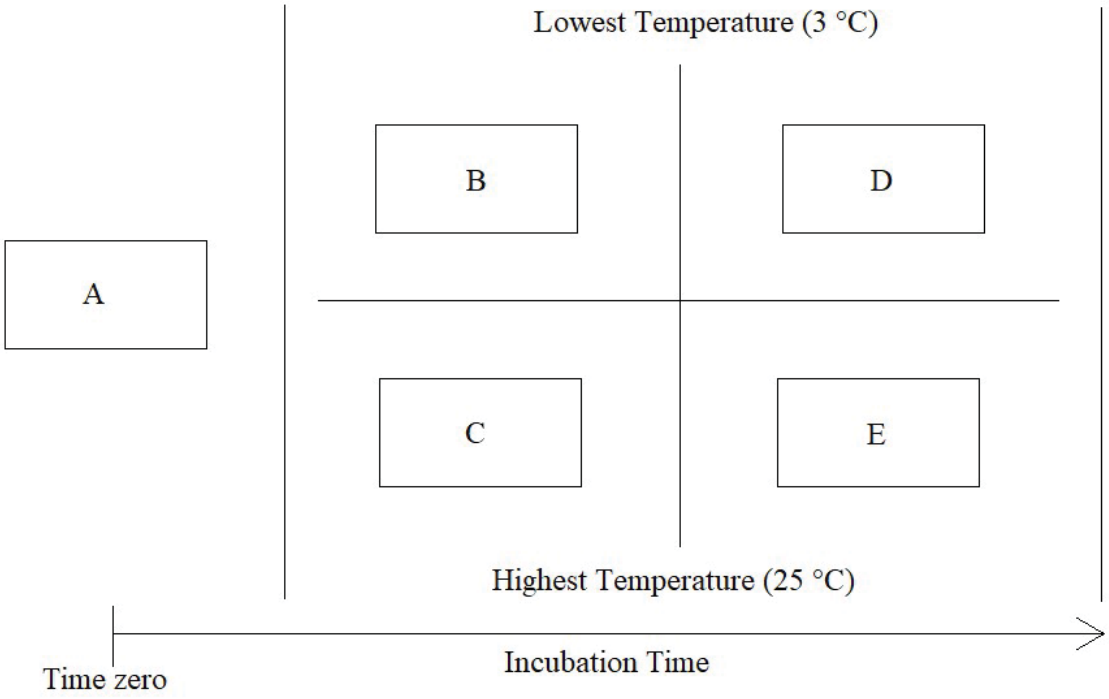
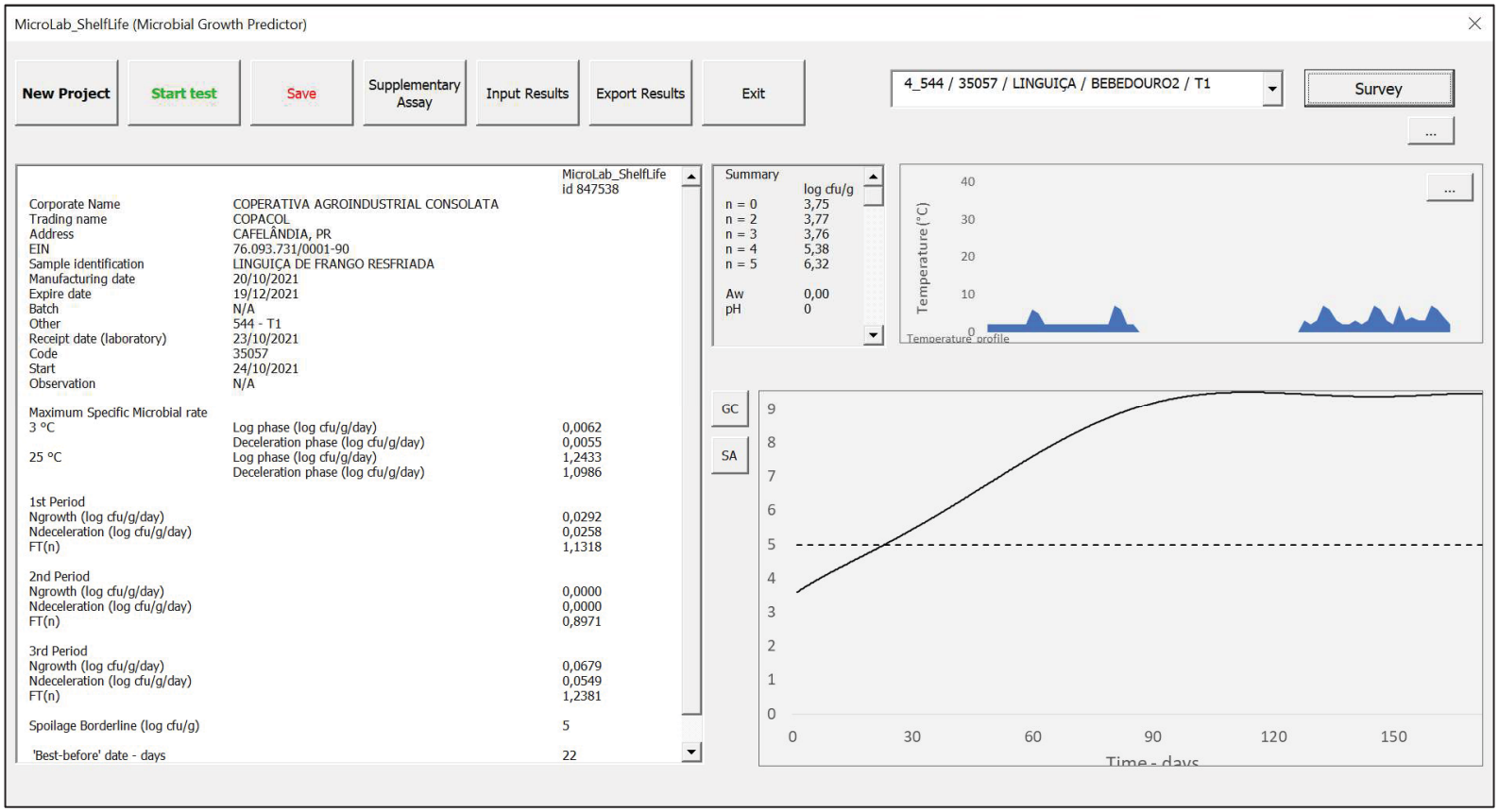


FIGURE 5 – MICROLAB_SHELFLIFE DASHBOARD



SUBTITLE: action and survey buttons positioned at the top left and right of the panel, respectively. Parameters of the test (left panel) composted by sample identification, microbial growth rate by counting plate at select incubation temperature, microbial growth rate at a chose temperature profile, spoilage borderline and predicted best-before date. Summary of plate counting (center panel). Temperature profile and typical microbial growth curve (up and down chart).

3 OBJECTIVES

3.1 GENERAL

Evaluate the addition of PPCP to extend the use-by date of raw chicken sausage and semi-finished chicken products.

3.2 SPECIFIC

1. To produce PPCP in a semi-culture fermentation system with *L. paracasei* DTA 83 and *S. boulardii* 17;
2. Obtain microorganisms associated with spoilage of chicken products;
3. Evaluate the doses of PPCP that achieve microbial susceptibility regarding spoilage microorganisms in an in vitro trial;
4. Add PPCP in chicken products to obtain an *in situ* partial inhibitory effects on spoilage microorganisms to extend the use-by date.

RESEARCH ARTICLE

POTENTIALLY POSTBIOTIC-CONTAINING PRESERVATIVE TO EXTEND USE-BY DATE OF RAW CHICKEN SAUSAGE AND SEMI-FINISHED CHICKEN PRODUCTS

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ABSTRACT

This study aimed to evaluate the use of potentially postbiotic-containing preservative (PPCP), produced in a semi-culture fermentation system with *Lacticaseibacillus paracasei* DTA 83 and *Saccharomyces cerevisiae* var. *boulardii* 17, to extend the use-by date of raw chicken sausages and semi-finished chicken products. Microorganisms associated with the spoilage of chicken products were stimulated to grow by pair incubation of the products at two different temperatures and with collection at different times. The turbidity method was performed to evaluate the microbial susceptibility to PPCP. PPCP was added in chicken products to obtain an *in situ* partial inhibitory effects on spoilage microorganisms to extend the use-by date. The *in vitro* trial showed total inhibition of the microbial growth by adding above 3.0% of PPCP. Although this concentration showed a remarkable inhibitory potential, its addition can severely impact the formulation cost. Thus, the application of doses with partial microbial inhibition may be a suitable strategy for the use of PPCP in chicken products. The results revealed that cold chain management and co-use of PPCP in chicken products extended the proposed use-by date, suggesting an alternative food preservation technology for the use of naturally derived compounds.

Keywords: Biopreservative. Biocontrol. Metabiotic. Beneficial. Compounds.

1 INTRODUCTION

Poultry meat is an indispensable source of animal protein for human growth and development, showing increasing global demand [4]. Currently, Brazil is the fourth largest poultry meat producer worldwide, about 14 363 thousand tonnes in carcass weight equivalent in 2020. Out of this production, approximately 68% remains in the domestic market; however, Brazil is the world's largest exporter of chicken meat. From 2020 to 2021, Brazilian chicken exports are estimated to increase 5%, totaling 4.05 million tons [1][2].

Due to the biological composition of raw meat, fresh poultry meat and poultry products, such as raw chicken sausages and semi-finished chicken products, are highly perishable foods. Therefore, the high consumption of poultry products leads to concerns about product safety, shelf life, quality, and desirable sensory characteristics [51]. Regulatory agencies prescribe for raw chicken or seasoned meats, cold or frozen stored' a plan class of n (number of samples) = 5, c (acceptable samples) = 2, m (minimum microbiological limit) = 5 log cfu/g, and M (maximum microbiological limit) = 6 log cfu/g to indicate the use-by date [52][53][54]. Thus, the theory of barriers, as the use of preservatives to slowing microbial growth, is often applied to extend shelf-life period.

Over the past decades, consumer awareness of the impact of food on health has grown, emerging alternative technologies for food preservation based on naturally derived compounds. *Lacticaseibacillus paracasei* DTA 83 and *Saccharomyces cerevisiae* var. *boulardii* 17 have been reported as candidate strains to deliver probiotics in food matrices. Moreover, probiotic strains have been extensively used in the meat poultry chain, increasing consumer's interest in functional foods. The high capacity of probiotics to provide beneficial health effects in the host has attracted scientific and commercial interests, highlighting the microbial administration as a health-promoting strategy.

Some rigorous processes and analyses precede the commercialization of a probiotic-containing functional food to guarantee their safety for consumption [18]. However, there are also many restrictions related to the consumption of live microbes: systemic infections due to translocation, particularly in vulnerable patients like pregnant and pediatric and geriatric populations; acquisition of

antibiotic resistance genes; interference with gut colonization in neonates [34][43]. Therefore, delivery probiotics to health-impaired individuals or when medical institutions are considered is still a matter of discussion. Indeed, the postbiotic effect derived from G.R.A.S microorganisms can be safe in all circumstances. As a result, the production of products containing non-viable microorganisms or microbial cells extracts to provide beneficial effect in the host, like probiotics, has received considerable attention in recent years [55].

In this background, this study aimed to use potentially postbiotic-containing preservative (PPCP), produced in a semi-culture fermentation system with *L. paracasei* DTA 83 and *S. boulardii* 17, to extend the use-by date of raw chicken sausage and semi-finished chicken products. In addition, three logistic distribution routes (R) were drafted to evaluate the impact of the cold chain management on the use-by date of the products.

2 MATERIALS AND METHODS

2.1 METHOD DESIGN

The products, raw chicken sausages and semi-finished chicken products were manufactured in an industrial scale at meat industry located in the state of Paraná, Brazil. The study was performed in two phases During Phase 1, microorganisms associated with the spoilage of chicken products were collected after pair incubation of the products at lower (3 °C) and higher (25 °C) temperatures, with collection within 4 days. Different culture media were obtained from HiMedia (Mumbai, India). Brain-heart infusion (BHI), casoy, deMan, Rogosa, and Sharp (MRS), and yeast-peptone-dextrose extract (YPD) broth were used to collect major group of microorganisms, including Gram-positive and negative bacteria, and yeasts. The susceptibility of the target microbiota to PPCP was assayed by turbidity method. During Phase 2, PPCP has produced in a pilot-industrial scale semi-cultured fermentation system and tested *in situ* in a controlled blind design, adding 1.0 and 1.5 % of PPCP. The standard formulation of each group was produced under the same conditions to serve as a control. Five packages of each sample group (totaling 75 packages) were addressed to the laboratory for shelf-life validation. A durability study was performed by a

microbial growth predictor (*MicroLab_ShelfLife*®) under a realistic temperature profile recorded by an electronic device in three logistic distribution routes, totaling 45 simulations. Borderline of 5 log cfu/g was entered in the predictive modeling to indicate the use-by date of the products, according to regulatory agencies [52][53][54].

2.2 MICROBIAL COLLECTION

L. paracasei DTA 83 was isolated from stools of infants aged 2 weeks old at Rio de Janeiro (Brazil) in selective modified MRS agar medium (Lawvab) [57]. A protocol of Fernandes Figueira Institute (FIOCRUZ) was rigorously applied to collect and transport the samples. The strain was firstly identified by sequencing of the 16S rDNA. Then, the complete genome was drafted and deposited in GenBank under the accession number QRBH00000000, https://www.ncbi.nlm.nih.gov/nuccore/ QRBH00000000 [56,57]. *L. paracasei* DTA 83 has been classified as a candidate probiotic by in vitro and in vivo trials [72-58]. This strain has been considered to carry out food bioprocesses as reported by Guerra et al., Silva et al., and Oliveira et al. [57,73-74]. *S. boulardii* 17 (FLORATIL-200, Merck, France) was acquired as freeze-dried culture sachets.

The cultures were registered in the self-declared system of the Brazilian genetic heritage (SISGEN): *L. paracasei* DTA 83 and *S. boulardii* 17 (FLORATIL-200, Merck, France)

2.3 PPCP PRODUCTION

PPCP was produced in a semi-cultured fermentation system at BRC Ingredientes Ltda, located in the state of São Paulo, Brazil. *L. paracasei* DTA 83 and *S. boulardii* 17 cultures were thawed at 7 °C for approximately 4 hours, and centrifuged at 6000*xg* for 5 minutes (2K15, Sigma Laborzentrifugen, Germany) for pellet separation. The liquid fraction (culture medium and glycerol) was discarded; then, the remaining cell pellet was reconstituted with MRS or YPD, followed by overnight incubation at 36 and 30 °C for the growth of *L. paracasei* DTA 83 and *S. boulardii* 17, respectively. To obtain sufficient biomass to produce

PPCP in a pilot-industrial scale, the cultures were scaled up 1/10 (vol/vol) in an axenic cultivation in sterile culture medium with 0.05 M soy protein, 0.1 M glucose and 0.005 M phosphate. A cylindrical bioreactor (300 L), made of stainless steel equipped with a stirring system and with a domed top and bottom, were used to produce PPCP. About 70% of the nominal capacity of the vessel was loaded with culture medium under a slight agitation (about 84 rpm) performed axially using a mechanical stirrer with a 4-blade propeller (50 × 15 mm, length × width) and a 45° pitch, coupled to the bioreactor. The heat treatment at (75 °C/2 hours) was carried out by the electrical activation of three resistors (3 kw), equidistant installed around the circumference of the vessel and positioned at ¼ the height of the tank bottom. After that, the temperature was reduced to 36 °C by adding 20 kg of drinking ice. Semi-culture fermentation system was performed by inoculating of *L. paracasei* DTA 83 to obtain a final cell concentration of *ca.* 7 log cfu/ml. After 30 h, the pH decreased to around 4.8 and the temperature of the medium was reduced to 30 °C, at a rate of 0.5 °C/min.Then, *S. boulardii* 17 was inoculated to obtain a final cell concentration of *ca.* 6.0 log cfu/ml. After 3 days of fermentation coupled with pH decay to around 4.0, the product was heat treated at 90 °C for 10 minutes (heating rate of 1.2 °C for minute) to obtain PPCP. Variables, such as pH and temperature, were continuously monitored over the process by a portable digital pH meter (AK40, Akrom, Brazil) equipped with an automatic temperature compensation. *L. paracasei* DTA 83 was enumerated on MRS agar medium (HiMedia, Mumbai, India) supplemented with 0.2 mM of natamycin to prevent the yeast growth [60]. *S. boulardii* 17 were enumerated on WL agar medium (Oxoid, Basingstoke, UnitedKingdom). The drop-plate technique was applied for both enumerations. A 12-well plastic microtiter plate was used to seed the drops (25 µL). Before seeding, 1 mL of melted agar medium kept at 50^{o}C was added to the wells using a micropipette and sterile tips. After complete solidification, each dilution was seeded into two different wells of the plate. Each well was seeded with only one drop. Decimal dilutions (up to 8th level) were performed by serial aliquot transfers (100 µL) to Eppendorf tubes containing 900 µL of 0.1% sterile peptone water. To check any possible contamination during the process, sample aliquots were regularly removed from the bioreactor and observed in the Neubauer chamber, every 24 h. Equal parts (1:1) of the

diluted cell suspension were mixed with methylene blue solution (0.1% w/v), and the solution was observed using an optical microscope (Biofocus - R, USA) at 1200*x* (100*x* objective). PPCP was hot bottled in polypropylene containers of 20 L. The presence of remaining cell of *L. paracasei* DTA 83 and *S. boulardii* 17 in PPCP after heat treatment was assessed by plate counting on MRS and WL agar medium as previously described. Plates was examined for the presence of typical colonies of each culture.

2.4 DETERMINATION OF KINETIC FERMENTATION PARAMETERS

Initial (X_0), maximum (X_{max}) and viable cell concentration (log10 cell/mL) during the time (t) (X), specific maximum growth rate (μ_{max}) and Lag phase period (λ) were normalized according to modified Gompertz's mathematical model [61][62] (Equation 1 and 2), or [63] model (Equation 3 and 4). To evaluate the adequacy of mathematical models, coefficient of determination (R^2) obtained by DMFit software, version 3.5 (Institute of Food Research, Norwich, UK), root mean square error (RMSE), bias factor (Bf) and accuracy factor (Af) were determined, Equation 6 to 8 [61][62][64].

$$y(t) = X_{max} \cdot exp\left(-exp\left((\mu_{max} \cdot e/X_{max}) \cdot (\lambda - t)\right) + 1\right) \quad \text{(Equation 1)}$$

$$y(t) = \ln(X/X_0) \quad \text{(Equation 2)}$$

$$y(t) = y_0 + \mu_{máx} \cdot X_{max}(t) - (1/m) \cdot \ln\left(1\left(\left(e^{(m \cdot \mu_{max} \cdot X_{max}(t))} - 1\right)/e^{(m \cdot (y_{max} - y_0))}\right)\right) \quad \text{(Equation 3)}$$

$$X_{max}(t) = t + (1/\mu_{max}) \cdot \ln\left(e^{(-\mu_{máx} \cdot t)} + e^{(-h_0)} - e^{\left((-\mu_{máx} \cdot t) - h_0\right)}\right) \quad \text{(Equation 4)}$$

$$h_0 = -\ln \alpha_0 = \ln\left(1 + (1/q_0)\right) = \mu_{máx} \cdot \lambda \quad \text{(Equation 5)}$$

$$RMSE = \sqrt{\sum\left(value_{predicted} - value_{observed}\right)^2/n} \quad \text{(Equation 6)}$$

$$A_f = 10^{((\sum log|value_{predicted}/value_{observed}|)/n)} \quad \text{(Equation 7)}$$

$$B_f = 10^{((\sum log(value_{predicted}/value_{observed}))/n)} \quad \text{(Equation 8)}$$

where, X – viable cell concentration (cell/mL) on the time (t), X_0 – initial viable cell concentration (cell/mL), X_{max} – maximum viable cell population (ln cell/mL), y(t) – viable cell concentration (ln cell/mL) on the time (t), y_0 – initial viable cell concentration (ln cell/mL), y_{max} – maximum viable cell concentration (ln cell/mL), m – parameter related to the curving profile between the log and stationary phase, n – number of experimental points taken over the experiment.

2.5 *IN VITRO* TRIAL

2.5.1 SPOILAGE MICROBIAL OBTENTION AND INOCULUM PREPARATION

Potentially food spoilage microorganisms were obtained from raw chicken sausage and semi-finished chicken products (seasoned chicken slit back, thigh, wing drumettes, and middle wings). Five package of each sample group was produced under the same conditions and addressed to the laboratory. A package per group was analyzed immediately after receiving the samples in the laboratory. In orthers microorganisms were stimulated to grow by pair incubation of the products at 3 °C and 25 °C, according to *MicroLab_ShelfLife*® method. Biological oxygen demand (BOD) incubators were used to incubate the samples with withdrawal on days 2 and 4 (3 °C), 1 and 3 (25 °C). A decimal suspension (1/10) was prepared by weighting 25 g of the product into 225 mL of PB. An aliquot (100 µL) was transferred with a micropipette and sterile tip to screw-cap tubes with enrichment culture broth medium (BHI, casoy, MRS, and YPD) for growth of Gram-positive and negative bacteria and yeasts (all media were obtained from HiMedia, Mumbai, India). The tubes were incubated at 30 °C for 24 h. Then,those with expressive microbial growth, with an absorbance value above 0.2 at 620 nm (Biospectro, SP-2000UV, Brazil), were used to prepare the inoculum. Tubes absent of growth were incubated for more 24 h and re-evaluated. Remaining the absence, the tubes were eliminated of the test.

An aliquot (1 mL) from each tube with expressive microbial growth, grouped per culture medium, were transferred to an empty sterile screw-cap tube. Washed out biomass cell pellet was obtained by centrifugation (2K15, Sigma

Laborzentrifugen, Germany) at 6000×*g* for 6 minutes, pellet separation from the liquid fraction, and pellet washing with PB. This procedure was repeated twice to obtain cells free of toxic cellular compounds produced during microbial growth. The turbidity of the inoculum tube was adjusted to achieve 0.5 McFarland standard, *ca.* 8.0 log cfu/mL of *L. paracasei* DTA 83 and *ca.* 6.5 log cfu/mL of *S. boulardii* 17. To perform this step accurately, a spectrophotometric device (Biospectro, SP-2000UV, Brazil) was used to compare the inoculum turbidity and the 0.5 McFarland standard. The microbial suspension was used within 30 minutes.

2.5.2 MICROBIAL SUSCEPTIBILITY TO PPCP

The turbidity method was performed to evaluate PPCP doses that achieve microbial susceptibility regarding spoilage microorganisms. Thus, PPCP was randomly outlined ranging the concentration from 0.5 to 3.5 % in BHI broth medium, raising up 0.5 % from tube to tube. The inoculum was prepared as described in the section 2.2. Tubes absent of PPCP and absent of inoculum were included as control and blank, respectively. The tubes were incubated at 30 °C in a stirred thermostatically water bath and at a regular 6-hour time intervals the turbidity was measured in a spectrophotometer device (Spectrum SP-2000UV/2000UVPC, Shanghai, China). The external surface of the tubes was dried with a paper tower and the absorbance value was directly measured in the tubes, dispensing the use of a cuvette. The blank tube was used to calibrate the photometer device before measurements. Potentially dosage to achieve microbial susceptibility was expressed into considering three categories: *i)* totally inhibit, a category that implies absence of growth above that dosage (absorbance value very close to the blank); *ii)* partially inhibit, a category that implies reduction in the growth with that dosage (with visible growth lesser than the positive control); *iii)* not inhibit, a category that implies a normal growth below that dosage (with visible growth equal to the positive control).

2.6 *IN SITU* TRIAL

2.6.1 POULTRY PRODUCTS PROCESSING

Broiler chickens of about 7 weeks of breeding were obtained from the meat industry suppliers, located in the state of Paraná (Brazil), and used to manufacture raw chicken sausages and semi-finished chicken products in a pilot-industrial scale. Birds were transferred into holding cages or modular bins, specifically designed for transport, to the processing plant to ensure that birds did not hurt themselves or other birds, and that air was able to circulate. At the processing plant, birds were stunned (rendered unconscious and unaware of pain) and then slaughtered with a quick and single cut to the throat. Trained workers ensured that each bird was properly slaughtered before feather removal, evisceration, and cleaning. Carcasses were prepared for further production by removal of feathers, internal organs, and feet. Then, they were thoroughly washed and chilled to 4 °C within 4 hours to reduce any possible foodborne pathogen growth. Carcasses was trussed after chilling in a leg dressing machine (Linco Food Systems, Denmark). Dorsal-blade part of poultry carcasses, breast, legs, wing which was divided into drumette, middle wing (mid-section) and tips, were mechanical separately in a portion cutting equipment (Linco Food Systems, Denmark) to shape desired end-products. All the parts were passed by the seasoning stage with spices into a spinning drum (Incomaf, Brazil) for 15 min (slit back, thigh, and wing drumettes) or 25 min (middle wings). Three batches of each part were prepared: control (no addition of PPCP), T1 (1.0% of PPCP), and T2 (1.5 % of PPCP), as showed in TABLE 1. A stainless-steel digital thermometer was used to monitor the temperature of the batter to maintain the temperature below 7 °C throughout the process. After tumbling, the products were cold storage in a cold chamber (Gelopar, Brazil) to achieve temperatures below 4 °C.

Valuable poultry meat remaining in carcasses were separated in a meat harvesting machine (607-513, Baader, UK) and further used to prepare raw chicken sausages, according to the standard formulation showed in TABLE 2. Chicken meat was minced in an electric grinding machine (CPG119, Cozzini, EUA) by using a stainless-steel plate disc knife with 10 mm hole diameter and mixed in an automatic mixer (MJ35, Jamar, Brazil) for 90 s. Then, the other

ingredients were added and the mixture was mixed for more 90 s. Three batches of sausage were prepared including control (no addition of PPCP), T1 (1.0% of PPCP), and T2 (1.5 % of PPCP). After batter preparation, the meat batter was stuffed into collagen casings (1.0 m of length, 26 mm of gauge) using an automatic stuffer (VF 610 E8, Handtmann, Germany) and manually twisted to shape segments of about 10 cm of length.

Table 1 – Semi-finished chicken products formulations with or without PPCP.

	Treatments		
Ingredients	Control (%)	T1(%)	T2(%)
Chicken parts (drumette, middle wing, thigh and part of carcasses)	92 - 93	91 - 92	90,5 – 91,5
Water	2 - 5	2 – 5	2 - 5
Seasoning	2.1	2.1	2.1
Phosphate	0.50	0.50	0.50
Sodium erythorbate	0.6	0.6	0.6
Annatto dye	0.02	0.02	0.02
Sodium lactate	0 - 2	0 - 2	0 - 2
PPCP		1.0	1.5

PPCP – Potentially postbiotic-containing preservative. Control (no addition of PPCP), T1 (addition of 1.0 % PPCP), T2 (addition of 1.5 % PPCP).

Table 2 – Raw chicken sausage formulations with or without PPCP.

	Treatments		
Ingredients	Control	T1	T2
Minced chicken meat	86.34	85.34	84.84
Water	8.0	8.0	8.0
Seasoning	2.87	2.87	2.87
TSP	2.5	2.5	2.5
Phosphate	0.25	0.25	0.25
Curing salt	0.12	0.12	0.12

Cochineal carmine dye	0.02	0.02	0.02
PPCP	0	1.0	1.5

PPCP – Potentially postbiotic-containing preservative. Control (no addition of PPCP), T1 (addition of 1.0 % PPCP), T2 (addition of 1.5 % PPCP).

Five packages of each sample group were packaged in polyethylene bags and sealed in a heat sealer. Freezing tunnel (Recrusul, Brazil) was used to freeze the products at -12 °C. The sample group were blind coded and shipped to the laboratory in isothermal box with ice-brick ice. Codes was unblinded only after performing a durability study.

2.7 DURABILITY STUDY

A predictive microbial method, named *MicroLab_ShelfLife*®, was used to perform a durability study in raw chicken sausage and semi-finished chicken products. It was carried out considering a realistic temperature profile in three logistic distribution routes (R), including distribution centers (DC) and sale disposal of the products in the markets (M).

One package per group was analyzed soon after being received in the laboratory to count the initial microbial load (time zero). Microbial growth was stimulated to grow by pair incubation at lower (3 °C) and higher (25 °C) temperatures. The method ISO 4833 (2013), with a few modifications, was used for enumeration of microorganisms in samples, with counts at intervals on days 2 and 4 (lower temperature) and on days 1 and 3 (higher temperature) of incubation [65]. BOD incubators were used for precise temperature control. The doores were maintained closed, except during sample withdrawals. Briefly, initial decimal suspension (1/10) was prepared by weighing 25 g of the product in 225 mL in 0,1 % sterile peptone water. Further decimal dilutions (up to 9th level) were performed by serial aliquot transfers (100 µL) to Eppendorf tubes containing 900 µL of peptone water. A 12-well plastic microtiter plate was used to seed the drops (25 µL). Before seeding, 1 mL of melted Plate Count agar medium (HiMedia, Mumbai, India) kept at 50 °C was added to the wells using a micropipette and sterile tips. After complete solidification, each dilution was seeded into two different wells of the plate, followed by incubation at 30 °C for 48 hours. Each well

was seeded with only one drop to avoid droplets crossing. Colonies were counted using a colony counter (Interscience, Saint Nom, France), and results related to the colony counting were entered in the *MicroLab_ShelfLife*® computational package. The *MicroLab_ShelfLife*® was compiled to calculate results by using at least two successive dilution levels, according to Equation 9 [66], and to obtain information about the parameters of the method and the microbial growth curve at a chosen dynamic temperature profile.

$$N = \frac{\sum \mathrm{C}}{V\,[\mathrm{n1}+0.1\mathrm{n2})\,d} \qquad \text{(Equation 9)}$$

Where, ∑c - sum of the colonies counted on the two plates retained from two successive dilutions (at least one of which contains a minimum of 10 colonies), V - volume of inoculum placed in each well (mL), n1 and n2 - number of wells selected in the first dilution and number of wells selected in the second dilution, respectively, and d - level of the first dilution retained.

The regulatory agencies prescribe the standard minimum (m) limit for ‘raw chicken or seasoned meats, cold or frozen stored’ as a plan class of n = 5, c = 2, m = 5 log cfu/g, and M = 6 log cfu/g. Thus, the values were inserted in the predictive modeling package as borderline to indicate the ‘use-by date’ of the products. Where, n – number of sample unit, c – suitable acceptance number, m and M – minimum and maximum microbiological limits, respectively.

2.8 TEMPERATURE PROFILE OF THE TEST

An electronic device (QII343, XpressPDF Logger, Emerson, USA), with a temperature range from -40°C to 85°C (±0.5°C accuracy), was used to elucidate the temperatures to which the products have been exposed during transport and sale. The equipment was adjusted as follows: sensor reaction time of 5 minutes, a sampling frequency of 1 hour to 10 days, the data storage capacity of 8000 readings. At the end of the acquisition period, the logger was recovered and data were downloaded into a computer. Data were grouped for hourly mean over one day to fit the data in the *MicroLab_ShelfLife*®. Three logistic distribution routes

(R1, R2, and R3) were strategically included in the study, encompassing three distribution centers (DC) and three markets (M). Briefly, DC1, which is located in the city of Penha, state of Paraná (Brazil), is a common and mandatory route to other routes. In R1, from DC1 the product is shipped to DC2, located in the city of Bebedouro, state of São Paulo (Brazil), after to DC3 (Bebedouro, São Paulo, Brazil) and finally to the M1(Bebedouro, São Paulo, Brazil). In R2, from DC1 the product is shipped to M2, located in the city of Cafelândia, state of Paraná (Brazil). In R3, from DC1 the product is shipped to DC2 and to the M3 (Bebedouro, São Paulo, Brazil), as depicted in Figure 1 and 2.

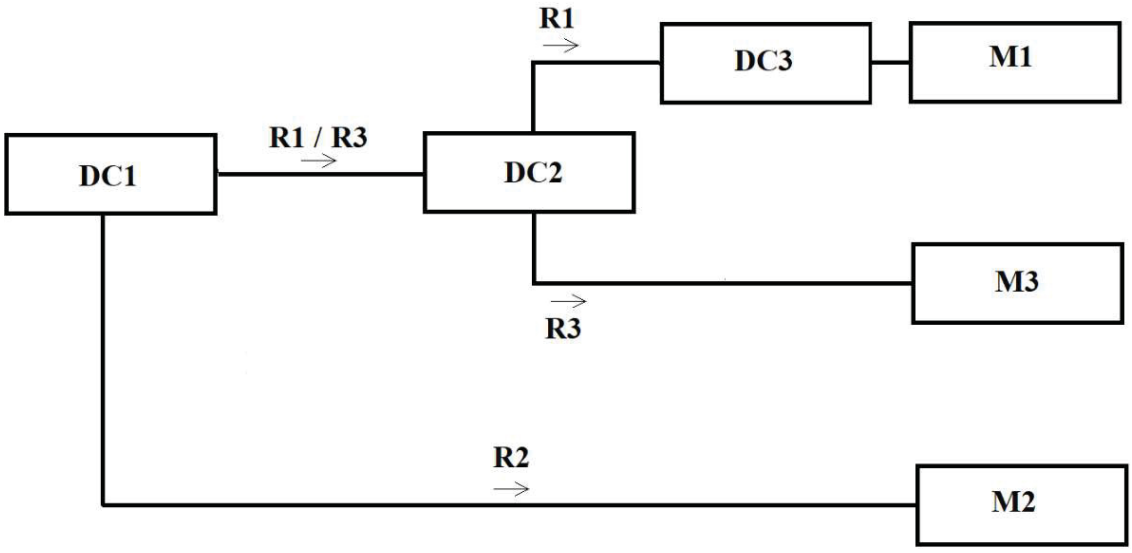

Figure 1 – Logistic distribution routes. Route (R1) – From the distribution center DC1 (Penha, Paraná, Brazil) to the distribution center DC2 (Bebedouro, São Paulo, Brazil), to another distribution center DC3 (Bebedouro, São Paulo, Brazil), and to the market M1 (Bebedouro, São Paulo, Brazil). Route (R2) – From the distribution center DC1 (Penha, Paraná, Brazil) to the market M2 (Cafelândia, Paraná, Brazil). Route (R3) – From the distribution center DC1 (Penha, Paraná, Brazil) to the distribution center DC2 (Bebedouro, São Paulo, Brazil), and to the market M3 (Bebedouro, São Paulo, Brazil). Retention time at DC was included in the modeling (2 days), according to information from meat industry.

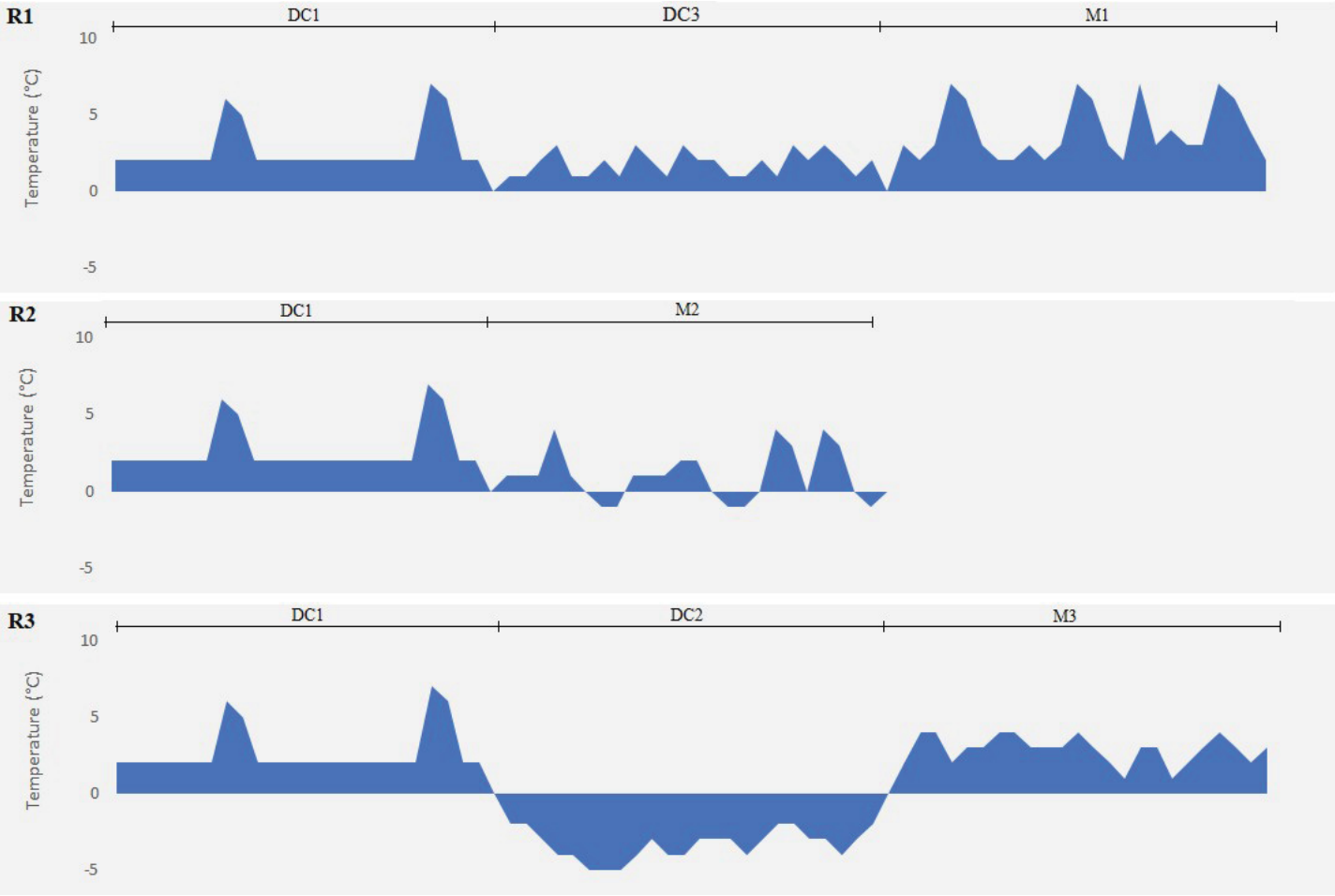

Figure 2 - Temperature profile based on hourly variation during a 1-day period to represent distribution centers (DC1, DC2, and DC3) and markets (M1, M2, and M3) in three logistic distribution routes (R1, R2, and R3). DC2 was eliminated of R1 due to the insignificancy to microbial growth once products have been stored at a temperature profile constantly below zero, keeping frozen storage temperatures.

2.9 STATISTICAL ANALYSIS

Results were presented as Mean ± Standard Error (SE) from replicates. The assumption of normal data distribution was assessed with the Shapiro-Wilk test. Grubbs and Tietjen-Moore tests were used for detecting a single or more than one outlier. Data were evaluated by analysis of variance (ANOVA) followed by Fisher's LSD test ($p < 0.05$).

Confidence interval for the mean and prediction interval for the sample of linear regression were estimated according to Equation 10 ($\dot{Y} \pm t\alpha/2 * SE * \sqrt{hi}$), Equation 11 ($\dot{Y} \pm t\alpha/2 * SE * \sqrt{1 + hi}$), and Equation 12 ($hi = 1/n + (xi - x)^2/\sum(xi - x)^2$), respectively; where, $\dot{Y}$ - value of estimative, tα/2 - value of Student's t distribution, n – number of observations, xi – value of sample, x – mean.

3 RESULTS

L. paracasei DTA 83 convert glucose into acids and produce PPCP. In addition, *L. paracasei* DTA 83 showed an amensal interaction, without prejudice any strain (Figure 3).

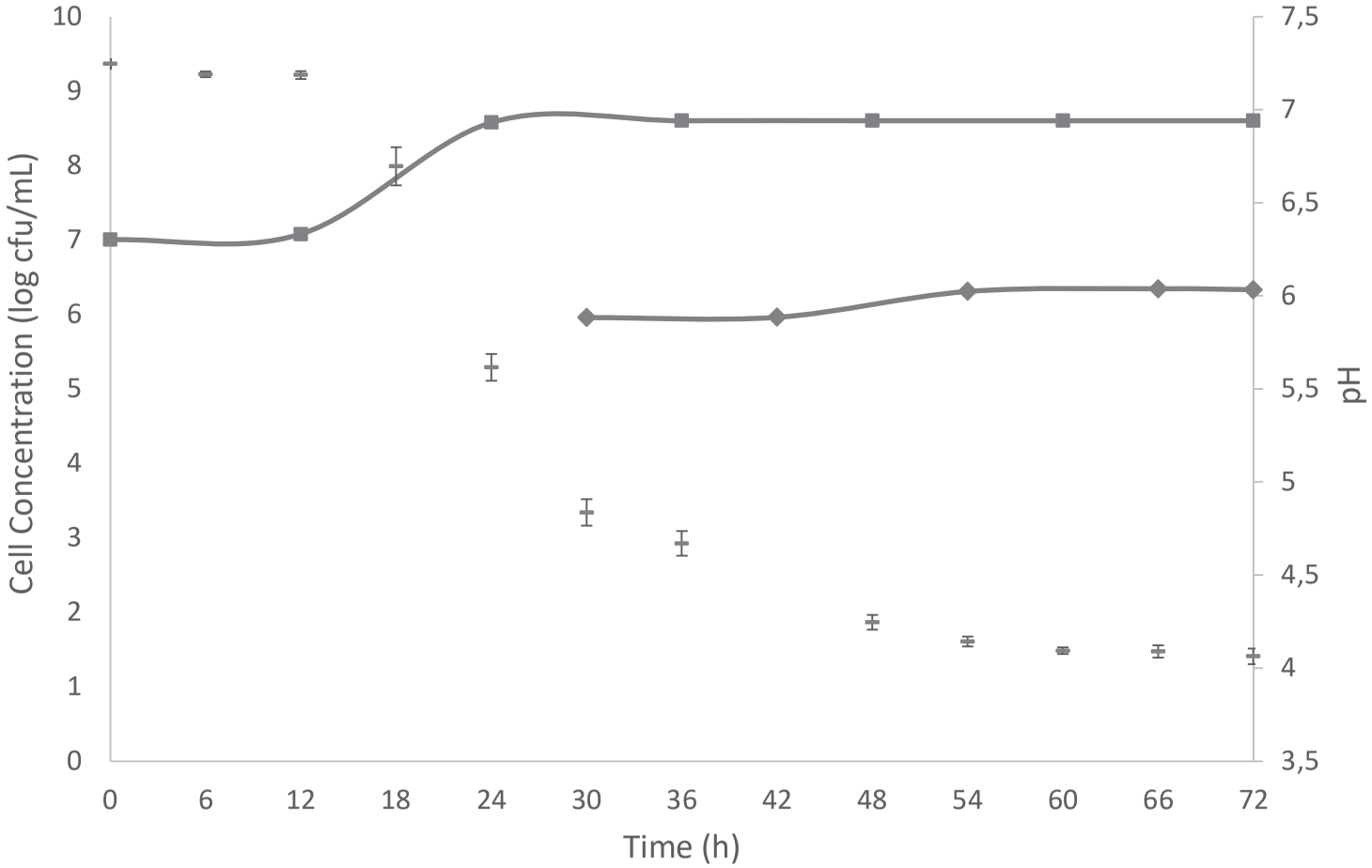

Figure 3 – *L. paracasei* DTA 83 (—■—), *S. boulardii* 17 (—♦—) viability, and pH measurement (unconnected points) during potentially postbiotic-containing preservative (PPCP) production. (—) Experimental data adjusted to the Baranyi's model using the DMFit software version 3.5 (Institute of Food Research, Norwich). (⌶) Standard Error.

PPCP may be more effective as a preservative than organic acid, since semi-cultured is an adequate fermentation system for *L. paracasei* DTA 83 and *S. boulardii* 17 to produce lactic and acetic acid, respectively. Moreover, other preservatives, as biocides, may be produced by these strains during fermentation. While the concentration of produced lactic acid and acetic or biocides were not measured in the present study, the stressful effect to chicken-related contaminants were designed and demonstrated in Figure 4. Linear regression parameters of the microbial growth of chicken-related contaminants at different concentrations of PPCP are showed in Figure 5 and Table 3.

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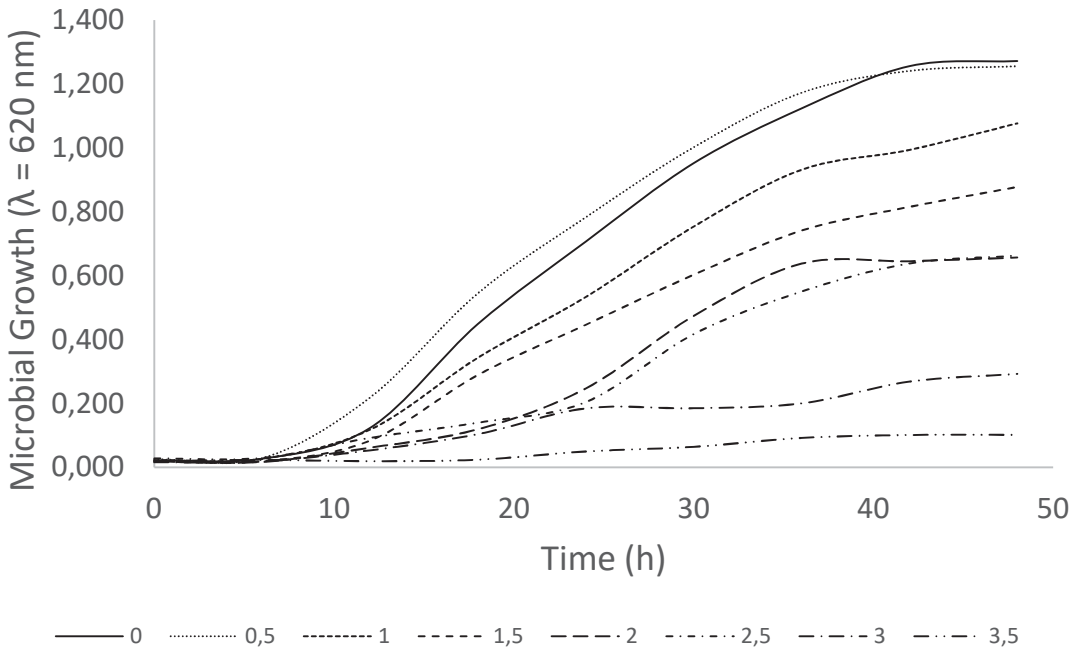

Figure 4 - Susceptibility of spoiling chicken product-related microorganisms to potentially postbiotic-containing preservative (PPCP).

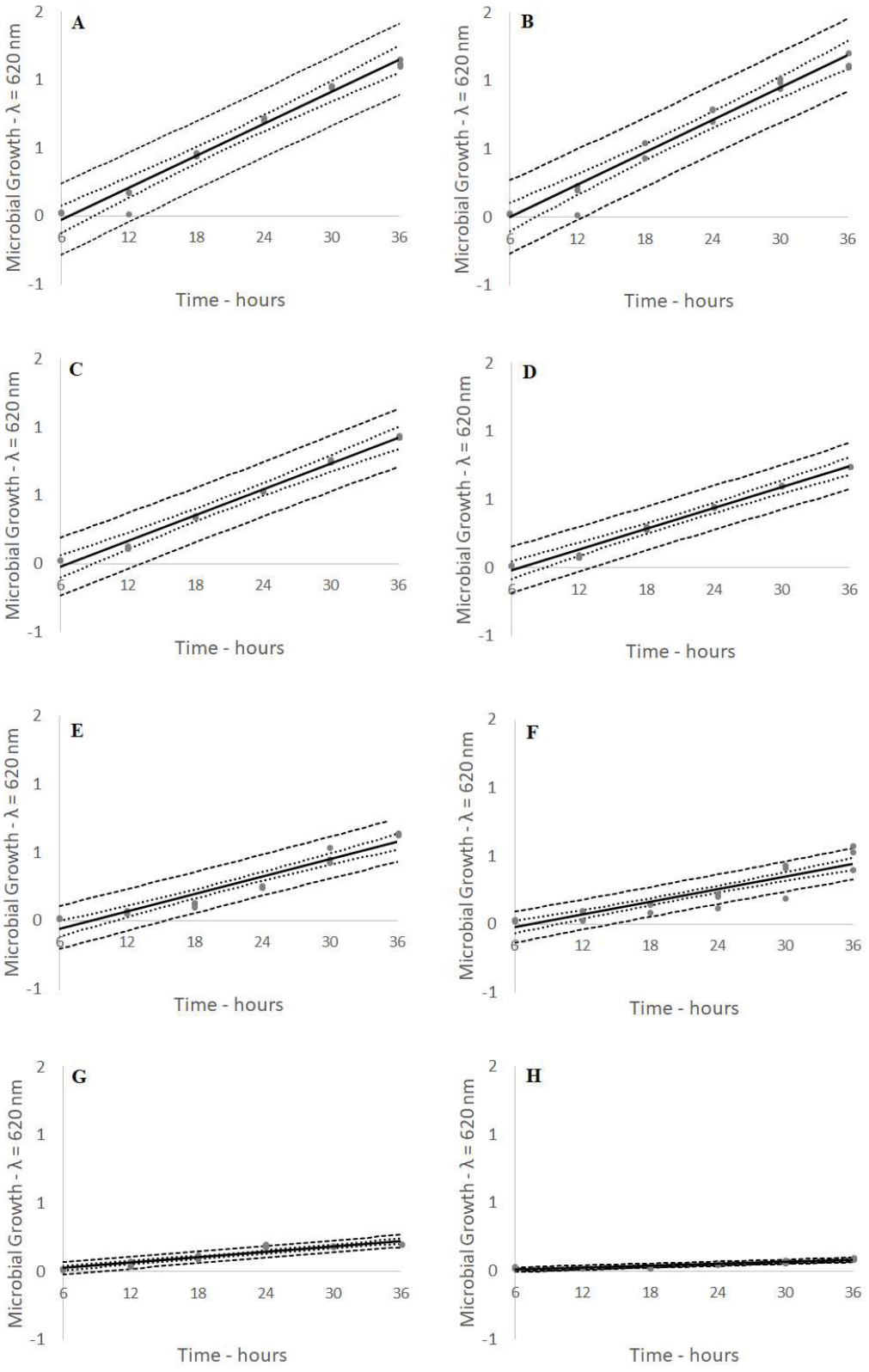

Figure 5 – Linear regression (—) parameters of the microbial growth of chicken-related contaminants at different concentrations of PPCP (**(a)** 0.0%; **(b)** 0.5 %; **(c)** 1.0 %; **(d)** 1.5 %; **(e)** 2.0 %; **(f)** 2.5 %; **(g)** 3.0 %; **(h)** 3.5 %). Confidence interval for the mean (.....) and prediction interval for the sample (-------) were estimated from the Equation 9 (˙Y ± t¸ /2*SE*√hi), Equation 10 (˙Y ± t¸ /2*SE*√1 + hi), and Equation 11 (hi = 1n+ (xi−x)2 - (xi−x)2), respectively. PPCP – Potentially Postbiotic-Containing Biopreservative.

Table 3 - Linear regression parameters of the microbial growth of chicken-related contaminants at different concentrations of potentially postbiotic-containing preservative (PPCP).

Coefficients	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*
xi	0,0392 [a]	0,0396 [a]	0,0315 [b]	0,0254 [c]	0,0213 [d]	0,0155 [d]	0,0066 [e]	0,0024 [f]
yi	-0,2583	-0,2358	-0,2108	-0,1684	-0,1870	-0,1149	-0,0137	-0,0048
R^2	0,9802	0,9684	0,9909	0,9894	0,9219	0,8121	0,9035	0,8320
SE	0,0983	0,1000	0,0787	0,0634	0,0551	0,0428	0,0172	0,0066
SQ	1890	1890	1890	1890	1890	1890	1890	1890
n	18	18	18	18	18	18	18	18
DF (n - 2)	16	16	16	16	16	16	16	16
tα/2	2,4729	2,4729	2,4729	2,4729	2,4729	2,4729	2,4729	2,4729
Confidence Interval	0,95	0,95	0,95	0,95	0,95	0,95	0,95	0,95

xi – Angular coefficient; yi – Linear coefficient; R2 – Coefficient of determination; SE – Standard Error; SQ – Sum of Squares; n – Number of observations; DF – Degrees of Freedom; tα/2 - Value of Student's t distribution corresponding. A (control, 0 %); B (0,5 %); C (1,0 %); D (1,5 %); E (2,0 %); F (2,5 %); G (3,0 %); H (3,5 %) of PPCP. Different lowercase letters indicate a significant difference by Fisher's test ($p > 0.95$).

In vitro trial showed that microbial susceptibility of chicken-related contaminants was directly proportional to the added concentration of PPCP. When 0.5 % of PPCP was added in raw chicken sausages or semi-finished chicken products, the susceptibility of chicken-related contaminants was not observed. Partial inhibition was obtained by adding 1.0 to 2.5 % of PPCP and total inhibition was obtained by adding above 3.0 % ($p > 0.95$).

Concentrations of 1.0 and 1.5 % of PPCP was chosen to be studied in raw chicken sausage and semi-finished chicken products to obtain an *in situ* partial inhibitory effects on spoilage microorganisms to extend the use-by date. Although concentrations of PPCP above 3.0 % showed a remarkable inhibitory potential, its addition can severely impact the formulation cost.

All three logistic distribution routes included in the study began in DC1, located in Bebedouro, state of Paraná (Brazil). As expected, *Ngrowth* and *Ndeceleration* parameters of the model were equal in the same sample group (control or T1 or T2) in R1, R2, and R3. However, significative difference was observed when different sample groups were compared (control > T1 > T2), indicating the potential effect of PPCP to control spoilage growth in raw chicken

sausage and semi-finished chicken products. These results were in line with those observed in 2nd and 3rd period (Table 4 to 8).

Table 4 – Durability study of raw chicken sausage (control, 1.0 % (T1), and 1.5 % (T2) of added PPCP) under a dynamic temperature profile in distribution routes (R).

	Sample incubation		Treatments								
	Temperature (°C)	Time (days)	Control			T1			T2		
Laboratorial data (log cfu/g)		0	3.80			3.75			3.72		
	3	2	3.83			3.77			3.72		
		4	3.84			3.76			3.74		
	25	1	5.53			5.38			5.37		
		3	9.30			6.32			6.11		
Specific maximum growth rate (log cfu/g/day)	3	L phase	0.0125			0.0062			0.0025		
		D phase	0.0110			0.0055			0.0022		
	25	L phase	1.7817			1.2433			1.2233		
		D phase	1.5742			1.0986			1.0809		
			R1	R2	R3	R1	R2	R3	R1	R2	R3
1st period	*Ngrowth (log cfu/g/day)*		0.0423	0.0423	0.0423	0.0292	0.0292	0.0292	0.0282	0.0282	0.0282
	Ndeceleration (log cfu/g/day)		0.0374	0.0374	0.0374	0.0258	0.0258	0.0258	0.0249	0.0249	0.0249
	Ft(n)		1.1318	1.1318	1.1318	1.1318	1.1318	1.1318	1.1318	1.1318	1.1318
2nd period	*Ngrowth (log cfu/g/day)*		0.0026	0.0127		0.0013	0.0083		0.0005	0.0075	
	Ndeceleration (log cfu/g/day)		0.0025	0.0126		0.0012	0.0083		0.0005	0.0074	
	Ft(n)		1.0548	1.0034		1.0548	1.0034		1.0548	1.0034	
3rd period	*Ngrowth (log cfu/g/day)*		0.0998	0.0127	0.0290	0.0679	0.0083	0.0185	0.0643	0.0075	0.0156
	Ndeceleration (log cfu/g/day)		0.0806	0.0141	0.0252	0.0549	0.0093	0.0161	0.0519	0.0083	0.0136
	Ft(n)		1.2381	0.8971	1.1501	1.2381	0.8971	1.1501	1.2381	0.8971	1.1501
Use-by date - days			16	91	43	22	146	69	24	167	83

'Use-by' date was used to indicate the period that a food remain safe to the human consumption [67], according to regulatory agencies [52][53][54]. *Ngrowth* – daily microbial population growth (log cfu/g) in the L phase; *Ndeceleration* daily microbial population growth (log cfu/g) in the D phase; *FT(n)* – correlation variable factor that describes specific growth rates between L and D phases at a chosen dynamic temperature profile based on hourly variation according to the measurements *in loco* (Figure 2).; L – microbial growth (log) phase; D – microbial deceleration phase. PPCP – Potentially postbiotic-containing preservative.

Table 5 – Durability study of seasoned chicken slit back (control, 1.0 % (T1), and 1.5 % (T2) of added PPCP) under a dynamic temperature profile in distribution routes (R).

	Sample incubation		Treatments								
	Temperature (°C)	Time (days)	Control			T1			T2		
Laboratorial data (log cfu/g)	3	0	3.90			3.92			4.01		
		2	4.08			4.07			4.01		
		4	4.03			4.01			4.05		
	25	1	7.11			5.32			5.25		
		3	9.40			6.91			7.34		
Specific maximum growth rate (log cfu/g/day)	3	L phase	0.0613			0.0488			0.0050		
		D phase	0.0541			0.0431			0.0044		
	25	L phase	2.5217			1.1983			1.1750		
		D phase	2.2281			1.0588			1.0382		
			R1	R2	R3	R1	R2	R3	R1	R2	R3
1st period	*Ngrowth (log cfu/g/day)*		0.0661	0.0661	0.0661	0.0343	0.0343	0.0343	0.0274	0.0274	0.0274
	Ndeceleration (log cfu/g/day)		0.0584	0.0584	0.0584	0.0303	0.0303	0.0303	0.0242	0.0242	0.0242
	Ft(n)		1.1318	1.1318	1.1318	1.1318	1.1318	1.1318	1.1318	1.1318	1.1318
2nd period	*Ngrowth (log cfu/g/day)*		0.0128	0.0267		0.0102	0.0167		0.0010	0.0077	
	Ndeceleration (log cfu/g/day)		0.0121	0.0266		0.0096	0.0166		0.0010	0.0077	
	Ft(n)		1.0548	1.0034		1.0548	1.0034		1.0548	1.0034	
3rd period	*Ngrowth (log cfu/g/day)*		0.1718	0.0267	0.0713	0.0953	0.0167	0.0476	0.0636	0.0077	0.0168
	Ndeceleration (log cfu/g/day)		0.1387	0.0298	0.0620	0.0770	0.0186	0.0414	0.0514	0.0086	0.0146
	Ft(n)		1.2381	0.8971	1.1501	1.2381	0.8971	1.1501	1.2381	0.8971	1.1501
Use-by date - days			10	39	18	15	63	26	19	124	60

'Use-by' date was used to indicate the period that a food remain safe to the human consumption [67], according to regulatory agencies [52][53][54]. *Ngrowth* – daily microbial population growth (log cfu/g) in the L phase; *Ndeceleration* daily microbial population growth (log cfu/g) in the D phase; *FT(n)* – correlation variable factor that describes specific growth rates between L and D phases at a chosen dynamic temperature profile based on hourly variation according to the measurements *in loco* (Figure 2).; L – microbial growth (log) phase; D – microbial deceleration phase. PPCP – Potentially postbiotic-containing preservative.

Table 6 – Durability study of seasoned chicken thigh (control, 1.0 % (T1), and 1.5 % (T2) of added PPCP) under a dynamic temperature profile in distribution routes (R).

	Sample incubation		Treatments								
	Temperature (°C)	Time (days)	Control			T1			T2		
Laboratorial data (log cfu/g)		0	3.91			3.82			3.79		
	3	2	4.00			383			3.84		
		4	4.03			3.92			3.93		
	25	1	6.78			6.29			4.41		
		3	9.40			8.01			5.83		
Specific maximum growth rate (log cfu/g/day)	3	L phase	0.0375			0.0150			0.0300		
		D phase	0.0331			0.0133			0.0265		
	25	L phase	2.3500			1.9333			0.6500		
		D phase	2.0764			1.7082			0.5743		
			R1	R2	R3	R1	R2	R3	R1	R2	R3
1st period	*Ngrowth (log cfu/g/day)*		0.0588	0.0588	0.0588	0.0461	0.0461	0.0461	0.0191	0.0191	0.0191
	Ndeceleration (log cfu/g/day)		0.0520	0.0520	0.0520	0.0407	0.0407	0.0407	0.0169	0.0169	0.0169
	Ft(n)		1.1318	1.1318	1.1318	1.1318	1.1318	1.1318	1.1318	1.1318	1.1318
2nd period	*Ngrowth (log cfu/g/day)*		0.0062	0.0210		0.0140	0.0140		0.0078	0.0098	
	Ndeceleration (log cfu/g/day)		0.0059	0.0209		0.0140	0.0140		0.0074	0.0097	
	Ft(n)		1.0548	1.0034		1.0548	1.0034		1.0548	1.0034	
3rd period	*Ngrowth (log cfu/g/day)*		0.1464	0.0210	0.0528	0.1093	0.0140	0.0324	0.0542	0.0098	0.0283
	Ndeceleration (log cfu/g/day)		0.1182	0.0234	0.0459	0.0883	0.0156	0.0282	0.0438	0.0109	0.0246
	Ft(n)		1.2381	0.8971	1.1501	1.2381	0.8971	1.1501	1.2381	0.8971	1.1501
Use-by date - days			11	49	23	14	80	38	26	122	46

'Use-by' date was used to indicate the period that a food remain safe to the human consumption [67], according to regulatory agencies [52][53][54]. *Ngrowth* – daily microbial population growth (log cfu/g) in the L phase; *Ndeceleration* daily microbial population growth (log cfu/g) in the D phase; *FT(n)* – correlation variable factor that describes specific growth rates between L and D phases at a chosen dynamic temperature profile based on hourly variation according to the measurements *in loco* (Figure 2).; L – microbial growth (log) phase; D – microbial deceleration phase. PPCP – Potentially postbiotic-containing preservative.

Table 7 – Durability study of seasoned chicken wing drumette (control, 1.0 % (T1), and 1.5 % (T2) of added PPCP) under a dynamic temperature profile in distribution routes (R).

	Sample incubation		Treatments								
	Temperature (°C)	Time (days)	Control			T1			T2		
Laboratorial data (log cfu/g)		0	3.80			3.82			3.80		
	3	2	3.98			3.83			3.82		
		4	3.97			3.92			3.90		
	25	1	6.74			6.29			4.98		
		3	9.05			8.01			6.05		
Specific maximum growth rate (log cfu/g/day)	3	L phase	0.0663			0.0150			0.0175		
		D phase	0.0585			0.0133			0.0155		
	25	L phase	2.3450			1.9333			0.9650		
		D phase	2.0720			1.7082			0.8526		
			R1	R2	R3	R1	R2	R3	R1	R2	R3
1st period	*Ngrowth (log cfu/g/day)*		0.0628	0.0628	0.0628	0.0461	0.0287	0.0287	0.0245	0.0245	0.0245
	Ndeceleration (log cfu/g/day)		0.0555	0.0555	0.0555	0.0407	0.0253	0.0253	0.0216	0.0216	0.0216
	Ft(n)		1.1318	1.1318	1.1318	1.1318	1.1318	1.1318	1.1318	1.1318	1.1318
2nd period	*Ngrowth (log cfu/g/day)*		0.0138	0.0267		0.0047	0.0109		0.0036	0.0090	
	Ndeceleration (log cfu/g/day)		0.0131	0.0267		0.0044	0.0109		0.0035	0.0090	
	Ft(n)		1.0548	1.0034		1.0548	1.0034		1.0548	1.0034	
3rd period	*Ngrowth (log cfu/g/day)*		0.1662	0.0267	0.0728	0.1093	0.0109	0.0284	0.0616	0.0090	0.0232
	Ndeceleration (log cfu/g/day)		0.1342	0.0298	0.0633	0.0883	0.0122	0.0247	0.0497	0.0101	0.0201
	Ft(n)		1.2381	0.8971	1.1501	1.2381	0.8971	1.1501	1.2381	0.8971	1.1501
Use-by date - days			11	43	19	14	101	42	23	130	54

'Use-by' date was used to indicate the period that a food remain safe to the human consumption [67], according to regulatory agencies [52][53][54]. *Ngrowth* – daily microbial population growth (log cfu/g) in the L phase; *Ndeceleration* daily microbial population growth (log cfu/g) in the D phase; *FT(n)* – correlation variable factor that describes specific growth rates between L and D phases at a chosen dynamic temperature profile based on hourly variation according to the measurements *in loco* (Figure 2).; L – microbial growth (log) phase; D – microbial deceleration phase. PPCP – Potentially postbiotic-containing preservative.

Table 8 – Durability study of seasoned chicken middle wings (control, 1.0 % (T1), and 1.5 % (T2) of added PPCP) under a dynamic temperature profile in distribution routes (R).

	Sample incubation		Treatments					
	Temperature (°C)	Time (days)	Control			T1		
Laboratorial data (log cfu/g)	3	0	3.86			3.70		
		2	3.87			3.75		
		4	3.91			3.73		
	25	1	5.60			5.05		
		3	8.34			5.87		
Specific maximum growth rate (log cfu/g/day)	3	L phase	0.0088			0.0162		
		D phase	0.0077			0.0144		
	25	L phase	1.6167			1.0367		
		D phase	1.4284			0.9160		
			R1	R2	R3	R1	R2	R3
1st period	*Ngrowth (log cfu/g/day)*		0.0380	0.0380	0.0380	0.0259	0.0259	0.0259
	Ndeceleration (log cfu/g/day)		0.0336	0.0336	0.0336	0.0229	0.0229	0.0229
	Ft(n)		1.1318	1.1318	1.1318	1.1318	1.1318	1.1318
2nd period	*Ngrowth (log cfu/g/day)*		0.0018	0.0110		0.0034	0.0092	
	Ndeceleration (log cfu/g/day)		0.0017	0.0109		0.0032	0.0092	
	Ft(n)		1.0548	1.0034		1.0548	1.0034	
3rd period	*Ngrowth (log cfu/g/day)*		0.0888	0.0110	0.0245	0.0644	0.0092	0.0231
	Ndeceleration (log cfu/g/day)		0.0717	0.0122	0.0213	0.0520	0.0102	0.0201
	Ft(n)		1.2381	0.8971	1.1501	1.2381	0.8971	1.1501
Use-by date - days			16	100	48	24	138	59

'Use-by' date was used to indicate the period that a food remain safe to the human consumption [67], according to regulatory agencies [52][53][54]. *Ngrowth* – daily microbial population growth (log cfu/g) in the L phase; *Ndeceleration* daily microbial population growth (log cfu/g) in the D phase; *FT(n)* – correlation variable factor that describes specific growth rates between L and D phases at a chosen dynamic temperature profile based on hourly variation according to the measurements *in loco* (Figure 2).; L – microbial growth (log) phase; D – microbial deceleration phase. PPCP – Potentially postbiotic-containing preservative.

4 DISCUSSION

Gompertz's modified and Baranyi models indicated greater growth of *L. paracasei* DTA 83 (*ca.* 1.6 log cfu/g) than *S. boulardii* 17 (*ca.* 0.65 log cfu/g) during fermentation for PPCP production. Latency in Lag phase (λ) was longer for *S. boulardii* 17, showing that the metabolites produced by *L. paracasei* DTA 83, especially lactic acid, may be tress factor for the growth of *S. boulardii* 17. Although this point may seems to be negative, it was strategically designed once *S. boulardii* 17 can produce acetic acid in stressful condition, as reported by [62].

S. boulardii 17 performance to assimilate sugars in acid conditions was previously demonstrated , indicating its suitability to be associate with acid lactic bacteria in a culture system [68]. Moradi et al. (2018) reported that *S. boulardii* is more tolerant of acidic pH and temperature variation than other *S. cerevisiae* strains and can survive at pH values as low as 2.0 [69]. Otherwise, *L. paracasei* DTA 81, which is a close genetic strain to the *L. paracasei* DTA 83 used in the present study [56], showed sensitivity to the metabolites produced by *S. boulardii*, suggesting that the semi-cultured system is an adequate choice to culture the strains in fermentation processes [68].

Stanojević-Nikolić et al. (2016) reported antimicrobial activity of lactic acid against pathogen and spoilage microorganisms, including *Escherichia coli*, *Proteus mirabilis*, *Salmonella enteritidis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Bacillus cereus*, *Bacillus megaterium, Rhodotorula* sp., *S. cerevisiae* and *Candida albicans*. Lactic acid minimal inhibitory concentration for bacteria was about ten times lesser than to inhibit yeasts. Most of microorganisms studied by Stanojević-Nikolić and colleagues are chicken-related contaminants, indicating that acid lactic can be a potential preservative used in chicken products to prevent food-born pathogenic and spoilage microorganisms. The inhibitory effect against spoilage microbial growth may be optimized using PPCP when bacteriocins may be produced during lactic acid fermentation [70].

In addition, acetic acid has been shown to have good antibacterial activity against various microorganisms, such as bacteria, yeasts, and molds. Halstead et al. (2015) reported the effect of acetic acid against *Pseudomonas aeruginosa*, *Acinetobacter baumannii, Escherichia coli, Staphylococcus aureus,* and

Klebsiella pneumoniae. Minimum inhibitory concentration of 0.16–0.31% for all isolates was reported to prevent formation of biofilms [71].

In R1, the addition of 1.0 % or 1.5 % of PPCP in raw chicken sausage increased the use-by date from 16 (control) to 22 (T1) and 24 (T2) days, respectively. However, it was not sufficient to guarantee aerobic mesophilic counts below 5 log cfu/g during 60 days of storage. For this reason, concentrations of PPCP above 1.5 % should be studied, according to the inhibition potential observed in Figures 4 and 5.

Additionally, proper management of the cold chain throughout distribution is a suitable strategy to achieve greater use-by date in this route. As showed in Figure 2, R1 was the route with the highest temperature profile. In R2, aerobic mesophilic counts below 5 log cfu/g (m) were achieved for more than 60 days only by cold chain management, dispensing the addition of any added preservative in the product. However, by adding PPCP in the sausages remarkable increases of 55 days (T1) and 76 (T2) days were achieved. In R3, it was possible to note the importance of adding PPCP in sausages. The use-by date increased from 43 days (control) to 69 (T1) and 83 (T2) days, respectively, ensuring aerobic mesophilic counts below 5 log cfu/g (m) during 60 days of storage (Table 4).

In semi-finished chicken products, excepting seasoned chicken middle wings in R2, all sample group showed use-by date below 60 days, regardless of route. These results indicate that the positive effect on shelf-life due to cold chain management may be enhanced by using PPCP. In this sense, the addition of 1.0 % of PPCP in seasoned chicken slit back increased the use-by date from 39 to 63 and 124 days in R2. Only with the addition PPCP at 1.5 % of PPCP, the use-by date was increased to 124 and 60 days in R2 and R3, respectively.

In seasoned chicken thigh and chicken wing drumettes, aerobic mesophilic counts below 5 log cfu/g were only achieved in R2 with the addition of PPCP at 1.0 % (80 and 101 days) and 1.5 % (122 and 130 days) (Table 8). These results reinforce the importance of PPCP to extend the use-by date in semi-finished chicken products; however, temperature profile in the logistic distribution routes is the crucial factor for product shelf-life extension (Figure 6, Table 6 and 7). This trend would also be observed in chicken middle wings, but the

laboratorial results was detected as an outlier by Grubbs' test, thus they were removed from the study.

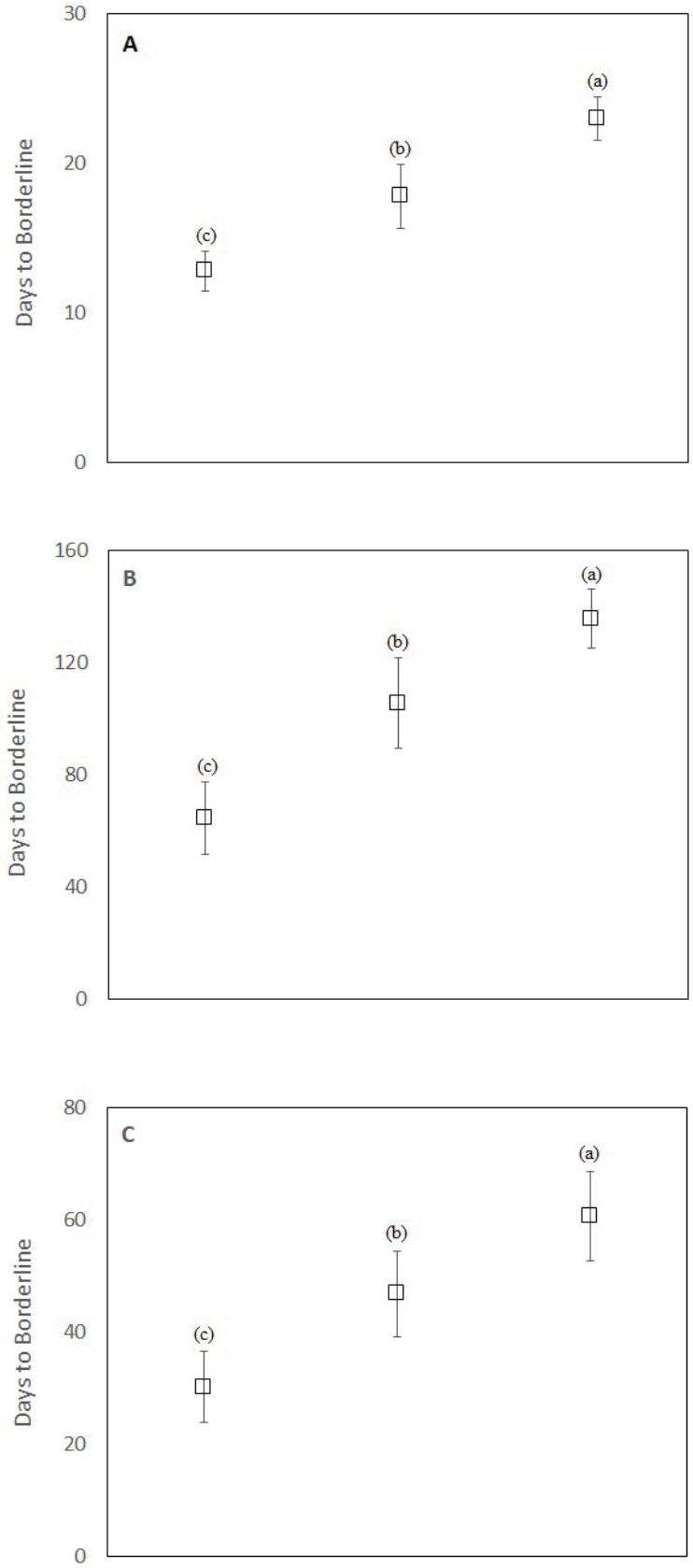

Figure 6 – Impact of temperature profile by the routes R1 (A), R2 (B), and R3 (C) in the use-by date of chicken products. Squares represent Mean and bars represent Standard Error. Different letters in the same box indicate a significative different by Fisher's LSD test at 0.95 of reliability.

Figure 7 is showing the percentage of chicken products (control, T1, and T2) that complies the parameter of 5 log cfu/g prescribed by the regulatory agencies as a minimum marginal limit in 3-class plan.

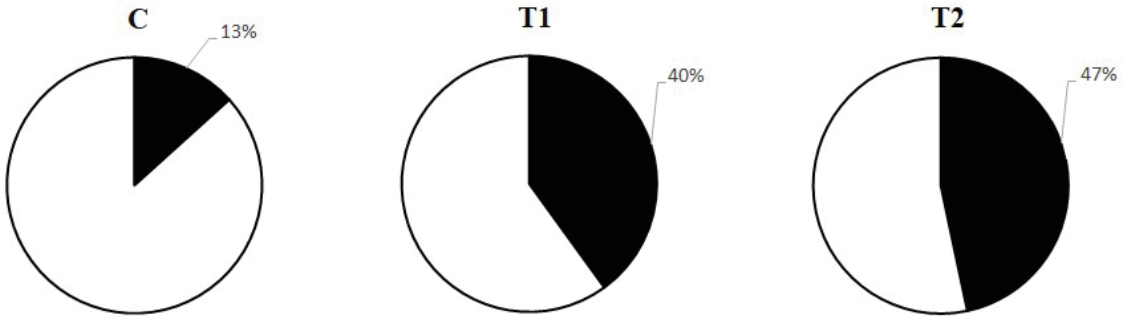

Figure 7 - Percentage of chicken products (raw chicken sausage and semi-finished chicken products) in compliance with minimum limit prescribed by regulatory agencies.

In the control group, that is the current industry formulation, only 13 % of the chicken products complied with the minimum limit (m) prescribed by regulatory agencies [52][53]. By adding 1.0 % or 1.5 % of PPCP, the percentage increased to 40 and 47 %, respectively. This finding demonstrates that besides delivering postbiotic compounds, PPCP may act as a natural preservative in raw chicken sausages and semi-finished chicken products to control aerobic mesophilic below 5 log cfu/g during 60 days of cold storage (Figure 7).

Interactions concerning food ingredients and microbes are complex to design in a durability study to estimate use-by dates [47]. Moreover, temperature variations caused by external aspects as climatic and geographic factors, and normal fluctuation over the day may affect microbial growth and impact the food shelf-life period. While some predictive methods allow to carry out a durability study at different temperatures, few methods permit the use of a dynamic temperature profile in the same test to achieve realistic temperature conditions based on the temperatures to which the products are exposed during storage for sale in markets [50]. In the present study, the microbial growth predictor, named *MicroLab_shelfLife*, was designed to perform a durability study of meat products by predicting the microbial growth curve of their natural microbiota under a

dynamic temperature profile. In brief, specific growth rates per hour (log cfu/g/h) at lower and higher temperatures are obtained by determining the angular coefficient of the exponential (log) phase in each growth curve.

A realistic temperature profile collected in DC throughout the logistic routers were considered in this study. In addition, a method to perform a durability study of raw chicken sausage and semi-finished chicken products by predicting the microbial growth curve of their natural microbiota under a dynamic temperature profile was used.

5 CONCLUSION

PPCP produced by a semi-separated co-culture system with *L. paracasei* DTA 83 and *S. boulardii* 17 may be a functional natural alternative to extend the use-by date in raw chicken sausage and semi-finished chicken products. However, cold chain management throughout logistic is the crucial factor to avoid product spoilage. The present study reveals the impact of the temperature profile on chicken products spoilage and may be useful for guiding the responsible use of preservatives. Food operators should support the use of preservatives regarding the logistic routes to where the product will pass by. Additionally, the beneficial immunomodulatory responses of PPCP in the host must be further studied in an *in vivo* model. For the preservative effect, a robust study should be designed to draft the temperature profile in DC in order to verify failures in cold chain management that may impact the use-by date of the products.

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APPENDIX 1

instructions for Authors – Sustainability (MDPI ACS Journals)

MANUSCRIPT PREPARATION

General Considerations

Research manuscripts should comprise:

Front matter: Title, Author list, Affiliations, Abstract, Keywords

Research manuscript sections: Introduction, Materials and Methods, Results, Discussion, Conclusions (optional).

Back matter: Supplementary Materials, Acknowledgments, Author Contributions, Conflicts of Interest, References.

Review manuscripts should comprise the front matter, literature review sections and the back matter. The template file can also be used to prepare the front and back matter of your review manuscript. It is not necessary to follow the remaining structure. Structured reviews and meta-analyses should use the same structure as research articles and ensure they conform to the PRISMA guidelines.

Graphical Abstract:

A graphical abstract (GA) is an image that appears alongside the text abstract in the Table of Contents. In addition to summarizing the content, it should represent the topic of the article in an attention-grabbing way. Moreover, it should not be exactly the same as the Figure in the paper or just a simple superposition of several subfigures. Note that the GA must be original and unpublished artwork. Any postage stamps, currency from any country, or trademarked items should not be included in it.

The GA should be a high-quality illustration or diagram in any of the following formats: PNG, JPEG, TIFF, or SVG. Written text in a GA should be clear and easy to read, using one of the following fonts: Times, Arial, Courier, Helvetica, Ubuntu or Calibri.

The minimum required size for the GA is 560 × 1100 pixels (height × width). The size should be of high quality in order to reproduce well.

Acronyms/Abbreviations/Initialisms should be defined the first time they appear in each of three sections: the abstract; the main text; the first figure or table. When defined for the first time, the acronym/abbreviation/initialism should be added in parentheses after the written-out form.

SI Units (International System of Units) should be used. Imperial, US customary and other units should be converted to SI units whenever possible.

Accession numbers of RNA, DNA and protein sequences used in the manuscript should be provided in the Materials and Methods section. Also see the section on Deposition of Sequences and of Expression Data.

Equations: If you are using Word, please use either the Microsoft Equation Editor or the MathType add-on. Equations should be editable by the editorial office and not appear in a picture format.

Research Data and supplementary materials: Note that publication of your manuscript implies that you must make all materials, data, and protocols associated with the publication available to readers. Disclose at the submission stage any restrictions on the availability of materials or information. Read the information about Supplementary Materials and Data Deposit for additional guidelines.

Preregistration: Where authors have preregistered studies or analysis plans, links to the preregistration must be provided in the manuscript.

Guidelines and standards: MDPI follows standards and guidelines for certain types of research. See https://www.mdpi.com/editorial_process for further information.

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Front Matter

These sections should appear in all manuscript types

Title: The title of your manuscript should be concise, specific and relevant. It should identify if the study reports (human or animal) trial data, or is a systematic review, meta-analysis or replication study. When gene or protein names are included, the abbreviated name rather than full name should be used.

Author List and Affiliations: Authors' full first and last names must be provided. The initials of any middle names can be added. The PubMed/MEDLINE standard format is used for affiliations: complete address information including city, zip code, state/province, and country. At least one author should be designated as corresponding author, and his or her email address and other details should be included at the end of the affiliation section. Please read the criteria to qualify for authorship.

Abstract: The abstract should be a total of about 200 words maximum. The abstract should be a single paragraph and should follow the style of structured abstracts, but without headings: 1) Background: Place the question addressed in a broad context and highlight the purpose of the study; 2) Methods: Describe briefly the main methods or treatments applied. Include any relevant preregistration numbers, and species and strains of any animals used. 3) Results: Summarize the article's main findings; and 4) Conclusion: Indicate the main conclusions or interpretations. The abstract should be an objective representation of the article: it must not contain results which are not presented and substantiated in the main text and should not exaggerate the main conclusions.

Keywords: Three to ten pertinent keywords need to be added after the abstract. We recommend that the keywords are specific to the article, yet reasonably common within the subject discipline.

Research Manuscript Sections

Introduction: The introduction should briefly place the study in a broad context and highlight why it is important. It should define the purpose of the work and its

significance, including specific hypotheses being tested. The current state of the research field should be reviewed carefully and key publications cited. Please highlight controversial and diverging hypotheses when necessary. Finally, briefly mention the main aim of the work and highlight the main conclusions. Keep the introduction comprehensible to scientists working outside the topic of the paper.

Materials and Methods: They should be described with sufficient detail to allow others to replicate and build on published results. New methods and protocols should be described in detail while well-established methods can be briefly described and appropriately cited. Give the name and version of any software used and make clear whether computer code used is available. Include any pre-registration codes.

Results: Provide a concise and precise description of the experimental results, their interpretation as well as the experimental conclusions that can be drawn.

Discussion: Authors should discuss the results and how they can be interpreted in perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible and limitations of the work highlighted. Future research directions may also be mentioned. This section may be combined with Results.

Conclusions: This section is not mandatory, but can be added to the manuscript if the discussion is unusually long or complex.

Patents: This section is not mandatory, but may be added if there are patents resulting from the work reported in this manuscript.

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Supplementary Materials: Describe any supplementary material published online alongside the manuscript (figure, tables, video, spreadsheets, etc.). Please indicate the name and title of each element as follows Figure S1: title, Table S1: title, etc.

Funding: All sources of funding of the study should be disclosed. Clearly indicate grants that you have received in support of your research work and if you received funds to cover publication costs. Note that some funders will not refund article processing charges (APC) if the funder and grant number are not clearly and correctly identified in the paper. Funding information can be entered separately into the submission system by the authors during submission of their manuscript. Such funding information, if available, will be deposited to FundRef if the manuscript is finally published.
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Acknowledgments: In this section you can acknowledge any support given which is not covered by the author contribution or funding sections. This may include administrative and technical support, or donations in kind (e.g., materials used for experiments).

Author Contributions: Each author is expected to have made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work; or have drafted the work or substantively revised it; AND has approved the submitted version (and version substantially edited by journal staff that involves the author’s contribution to the study); AND agrees to be personally accountable for the author’s own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and documented in the literature.
For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, X.X. and Y.Y.; Methodology, X.X.; Software, X.X.; Validation, X.X., Y.Y. and Z.Z.; Formal Analysis, X.X.; Investigation, X.X.; Resources, X.X.; Data Curation, X.X.; Writing – Original Draft Preparation, X.X.; Writing – Review & Editing, X.X.; Visualization, X.X.; Supervision, X.X.; Project

Administration, X.X.; Funding Acquisition, Y.Y.", please turn to the CRediT taxonomy for the term explanation. For more background on CRediT, see here. "**Authorship must include and be limited to those who have contributed substantially to the work. Please read the section concerning the** criteria to qualify for authorship **carefully**".

Data Availability Statement: In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Please refer to suggested Data Availability Statements in section "MDPI Research Data Policies". You might choose to exclude this statement if the study did not report any data.

Conflicts of Interest: Authors must identify and declare any personal circumstances or interest that may be perceived as influencing the representation or interpretation of reported research results. If there is no conflict of interest, please state "The authors declare no conflict of interest." Any role of the funding sponsors in the choice of research project; design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results must be declared in this section. *Sustainability* does not publish studies funded partially or fully by the tobacco industry. Any projects funded by industry must pay special attention to the full declaration of funder involvement. If there is no role, please state "The sponsors had no role in the design, execution, interpretation, or writing of the study". For more details please see Conflict of Interest.

References: References must be numbered in order of appearance in the text (including table captions and figure legends) and listed individually at the end of the manuscript. We recommend preparing the references with a bibliography software package, such as EndNote, ReferenceManager or Zotero to avoid typing mistakes and duplicated references. We encourage citations to data, computer code and other citable research material. If available online, you may use reference style 9. below.

Citations and References in Supplementary files are permitted provided that they also appear in the main text and in the reference list.

In the text, reference numbers should be placed in square brackets [ ], and placed before the punctuation; for example [1], [1–3] or [1,3]. For embedded citations in the text with pagination, use both parentheses and brackets to indicate the reference number and page numbers; for example [5] (p. 10). or [6] (pp. 101–105).

The reference list should include the full title, as recommended by the ACS style guide. Style files for Endnote and Zotero are available.

References should be described as follows, depending on the type of work:

 Journal Articles:

1. Author 1, A.B.; Author 2, C.D. Title of the article. *Abbreviated Journal Name* **Year**, *Volume*, page range.

 Books and Book Chapters:

2. Author 1, A.; Author 2, B. *Book Title*, 3rd ed.; Publisher: Publisher Location, Country, Year; pp. 154–196.

3. Author 1, A.; Author 2, B. Title of the chapter. In *Book Title*, 2nd ed.; Editor 1, A., Editor 2, B., Eds.; Publisher: Publisher Location, Country, Year; Volume 3, pp. 154–196.

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 Conference Proceedings:

7. Author 1, A.B.; Author 2, C.D.; Author 3, E.F. Title of Presentation. In *Title of the Collected Work* (if available), Proceedings of the Name of the Conference,

Location of Conference, Country, Date of Conference; Editor 1, Editor 2, Eds. (if available); Publisher: City, Country, Year (if available); Abstract Number (optional), Pagination (optional).

- Thesis:

8. Author 1, A.B. Title of Thesis. Level of Thesis, Degree-Granting University, Location of University, Date of Completion.

- Websites:

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Unlike published works, websites may change over time or disappear, so we encourage you create an archive of the cited website using a service such as WebCite. Archived websites should be cited using the link provided as follows:

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See the Reference List and Citations Guide for more detailed information.

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Preparing Figures, Schemes and Tables

File for Figures and Schemes must be provided during submission in a single zip archive and at a sufficiently high resolution (minimum 1000 pixels width/height, or a resolution of 300 dpi or higher). Common formats are accepted, however, TIFF, JPEG, EPS and PDF are preferred.

Sustainability can publish multimedia files in articles or as supplementary materials. Please contact the editorial office for further information.

All Figures, Schemes and Tables should be inserted into the main text close to their first citation and must be numbered following their number of appearance (Figure 1, Scheme I, Figure 2, Scheme II, Table 1, *etc.*).

All Figures, Schemes and Tables should have a short explanatory title and caption.

All table columns should have an explanatory heading. To facilitate the copy-editing of larger tables, smaller fonts may be used, but no less than 8 pt. in size. Authors should use the Table option of Microsoft Word to create tables.

Authors are encouraged to prepare figures and schemes in color (RGB at 8-bit per channel). There is no additional cost for publishing full color graphics.