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Synbiotic aerated dessert: diet product development and evaluation of the intake effects in individuals with metabolic syndrome

Douglas Xavier dos Santos

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Synbiotic aerated dessert: diet product development and evaluation of the intake effects in individuals with metabolic syndrome

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DEDICATION

This thesis is dedicated to everyone who believed in my ideals and, in special, to my mother Nádia, aunt Gecilene, and aunt Marilange (in memoriam).

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“De qualquer forma, eu aprendi uma coisa: só se conhece realmente uma pessoa depois de uma discussão. Então e então se pode avaliar o seu verdadeiro carater!”

“Because paper has more patience than people.”

“O papel tem mais paciência do que as pessoas.”

Anne Frank

ABSTRACT

XAVIER-SANTOS, D. **Synbiotic aerated dessert: diet product development and evaluation of the intake effects in individuals with metabolic syndrome**. 2017. 177p. Thesis (PhD). Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, 2017.

The objective of this work was to adapt a synbiotic aerated diet dessert, produced with the addition of a probiotic culture of *Lactobacillus acidophilus* La-5 and prebiotic ingredients (fructooligosaccharides and inulin), from the previously developed sucrose-containing formulation, and to evaluate the effects of its ingestion on adult volunteers with metabolic syndrome (MetS) during a period of 8 weeks of intervention. In addition, to improve the resistance of the probiotic to simulated gastrointestinal conditions, a microencapsulation process was optimized. For the development of the product, the formulations were produced in triplicates, in which probiotic culture survival, instrumental texture and sensory acceptability were evaluated up to 112 days of storage under freezing (-18 °C). Subsequently, a randomized, double-blind, placebo-controlled trial was carried out in which the product developed was administered to forty-five volunteers with MetS assigned into two groups, each receiving 40 g/day of: synbiotic diet mousse (SDM) (n=23) and placebo diet mousse (PDM) without pro- and prebiotics (n=22). Fasting blood samples were collected at the beginning and after 8 weeks of daily consumption of both mousses to determine the anthropometric, biochemical, haematological, inflammatory, and immunological parameters. Afterward, with the goal of improving the survival of *L. acidophilus* La-5 to *in vitro* simulated gastrointestinal conditions, the microencapsulation process conditions of the probiotic strain via spray drying were optimized using inulin as the encapsulating agent. The viability of *L. acidophilus* La-5 incorporated into SDM was above 7.8 log CFU/g and remained stable throughout storage. PDM showed lower acceptability (5.77-6.50) after storage than SDM (6.67-7.03). The texture was the most appreciated attribute and hardness of the SDM increased during storage, but remained stable for PDM. The clinical trial revealed significant reductions of total cholesterol and HDL-cholesterol, as well as of immunoglobulins (A and M), and interleukin-1β in both groups during the intervention period. However, regarding intergroup changes, there were not any significant differences for all parameters evaluated ($p>0.05$). After the optimization of the microencapsulation process of the probiotic culture (80 mL/min, 82% and 10%, respectively for feed flow, aspiration rate, and inulin concentration), the microencapsulated probiotic strain incorporated in the SDM mousse showed the highest *in vitro* gastrointestinal survival ($p<0.05$) in the different stages of the assay, as follows: after the gastric phase: 5.68 log CFU/g (83.3%), the enteral phase I: 5.61 log CFU/g (82.3%), the enteral phase II: 5.56 log CFU/g (81.4%). Therefore, these results suggest that the presence of probiotic and prebiotics in SDM did not provide an additional effect on the health of volunteers with MetS. Additionally, the results confirm the appropriateness of the spray drying process to microencapsulate *L. acidophilus* La-5 using inulin as coating agent, providing increased resistance to the microencapsulated probiotic strain under *in vitro* gastrointestinal stress.

Key-words: Probiotic; *Lactobacillus acidophilus*; Inulin; Fructooligosaccharides; Dairy dessert; Clinical trial; Microencapsulation; *In vitro* gastrointestinal resistance.

RESUMO

XAVIER-SANTOS, D. **Sobremesa aerada simbiótica: desenvolvimento do produto *diet* e avaliação dos efeitos da ingestão sobre indivíduos com síndrome metabólica**. 2017. 177p. Tese (Doutorado). Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, 2017.

O objetivo deste trabalho foi adaptar uma sobremesa aerada simbiótica *diet* do tipo musse, processada com a adição de uma cultura probiótica de *Lactobacillus acidophilus* La-5 e de ingredientes prebióticos (fruto-oligossacarídeos e inulina), a partir da formulação contendo sacarose desenvolvida anteriormente, e avaliar os efeitos de sua ingestão em voluntários adultos com síndrome metabólica (MetS) durante um período de 8 semanas de intervenção. Adicionalmente, para melhorar a resistência do probiótico frente às condições gastrintestinais simuladas, otimizou-se um processo de microencapsulação da cepa probiótica. Para o desenvolvimento do produto, as formulações foram produzidas em triplicata, em que se avaliou a sobrevivência da cultura probiótica, a textura instrumental e a aceitabilidade sensorial até 112 dias de armazenamento sob congelamento (-18 ^{o}C). Em seguida, foi realizado um estudo randomizado, duplo-cego e controlado por placebo, no qual o produto desenvolvido foi administrado a quarenta e cinco indivíduos com MetS divididos em dois grupos, cada um recebendo 40 g/dia de: mousse simbiótica *diet* (SDM) (n=23) e musse placebo *diet* (PDM) sem componentes pro- e prebióticos (n=22). As amostras sanguíneas foram coletadas em jejum no início e após 8 semanas de consumo diário de ambas as musses para a determinação dos parâmetros antropométricos, bioquímicos, hematológicos, inflamatórios e imunológicos. Posteriormente, com o intuito de melhorar a sobrevivência do *L. acidophilus* La-5 em condições gastrointestinais simuladas *in vitro*, as condições de processo de microencapsulação da cepa probiótica via *spray drying* foram otimizadas, utilizando inulina como agente encapsulante. A viabilidade de *L. acidophilus* La-5 incorporados na SDM foi superior a 7,8 log UFC/g e se manteve estável ao longo do armazenamento. A PDM mostrou menor aceitabilidade (5.77-6.50) após o armazenamento do que a SDM (6.67-7.03). A textura foi o atributo mais apreciado, sendo que a dureza da SDM apresentou elevação, enquanto a da PDM manteve-se estável. O ensaio clínico revelou reduções significativas de colesterol total, colesterol-HDL, imunoglobulinas (A e M) e interleucina1β em ambos os grupos durante o período de intervenção. Entretanto, no que se refere às mudanças intergrupos, não se observou diferenças significativas para todos os parâmetros avaliados (p>0,05). Após a otimização do processo de microencapsulação da cultura probiótica (80 mL/min, 82% e 10%, respectivamente para o fluxo de alimentação, taxa de aspiração e concentração de inulina), a cepa probiótica microencapsulada incorporada a amostra SDM apresentou a maior sobrevivência gastrointestinal *in vitro* (p<0,05) nas diferentes etapas do ensaio, a saber: após a fase gástrica: 5,68 log UFC/g (83,3%); fase entérica I: 5,61 log UFC/g (82,3%); fase entérica II: 5,56 log UFC/g (81,4%). Portanto, esses resultados sugerem que a presença de probiótico e prebiótico na SDM não apresentou efeitos adicionais na saúde dos voluntários com MetS. Adicionalmente, os resultados confirmaram a adequação do processo de *spray drying* para a microencapsulação de *L. acidophilus* La-5 utilizando inulina como agente de revestimento, proporcionando uma maior resistência da cepa probiótica microencapsulada às condições gastrintestinais simuladas *in vitro*.

Palavras-chave: Probiótico; *Lactobacillus acidophilus*; Inulina; Fruto-oligossacarídeos; Sobremesa láctea; Ensaio clínico; Microencapsulação; Resistência gastrointestinal *in vitro*.

RIASSUNTO

XAVIER-SANTOS, D. **Dessert aerato simbiotico: sviluppo del prodotto dietetico e valutazione degli effetti dell'ingestione su individui con sindrome metabolica**. 2017. 177p. Tesi (Dottorato). Facoltà di Scienza Farmaceutiche, Università di San Paolo, San Paolo, 2017.

L'obiettivo di questo lavoro è stato formulare un *dessert* aerato simbiotico dietetico tipo *mousse*, ottenuto con l'addizione di probiotici (*Lactobacillus acidophilus* La-5) e di prebiotici (frutto-oligosaccaridi e inulina), partendo da una formulazione contenente saccarosio sviluppata precedentemente. Inoltre è stato valutato l'effetto dell'assunzione di tale mousse da parte di volontari adulti con sindrome metabolica (MetS) per un periodo di 8 settimane. Inoltre, per migliorare la resistenza del probiotico alle condizioni gastrointestinali, è stato eseguito e ottimizzato un processo di microincapsulazione del ceppo probiotico. Per lo sviluppo del prodotto, le formulazioni sono state eseguite in triplicato, su queste sono state valutate: la sopravvivenza della cultura probiotica; la struttura e l'accettabilità sensoriale fino a 112 giorni di conservazione (-18 ° C). In seguito, è stato realizzato uno studio randomizzato, in doppio cieco con controllo placebo, somministrando a quarantacinque soggetti con MetS il prodotto sviluppato. I pazienti sono stati divisi in due gruppi, somministrando a ciscun gruppo 40 g/giorno sia la *mousse* simbiotica dietetica (SDM) (n=23) che la mousse placebo dietetica (PDM) senza pro- e prebiotici (n=22). Campioni di sangue sono stati raccolti a digiuno all'inizio e dopo 8 settimane di alimentazione con entrambe le mousse. Su questi sono stati determinati parametri antropometrici, biochimici, ematologici, immunologici e infiammatori. In seguito, con l'intento di migliorare la sopravvivenza di *L. acidophilus* La-5 alle condizioni gastrointestinali simulate *in vitro*, le condizioni di processo di microincapsulazione del ceppo probiótico via *spray drying* sono stati ottimizzate utilizzando inulina come agente incapsulante. La vitalità di *L. acidophilus* La-5 incorporato nell'SDM è stata di 7,8 log UFC/g e si è mantenuta stabile durante tutto il perido di stoccaggio. Il PDM ha mostrato minore resistenza (5,77-6,50) alla conservazione che il SDM (6,67-7,03). La struttura è stata il parametro più apprezzato, essendo aumentata la consistenza dell'SDM, mentre quella del PDM è rimasta stabile. Lo studio clinico ha rilevato riduzioni significative del colesterolo totale, del colesterolo-HDL, delle immunoglobuline (A e M) e dell'interleuchina-1β in entrambi i gruppi durante tutto il periodo di studio. Tuttavia, per quanto riguarda i cambiamenti intergruppi, non sono state osservate differenze significative per tutti i parametri (p>0,05) studiati. Dopo l'ottimizzazione del processo di microincapsulazione (parametri spray dryer: flusso di alimentazione 80 mL/min, aspirazione 82% e concentrazione di inulina 10%), il ceppo probiotico microincapsulato e incorporato nell'SDM ha presentato una maggiore sopravvivenza gastrointestinale in vitro ($p<0,05$) nelle differenti fasi studiate come segue: dopo nel fase gastrica: 5,68 log UFC/g (83,3%); fase enterica I: 5,61 log UFC/g (82,3%); fase enterica II: 5,56 log UFC/g (81,4%). Pertanto, questi risultati suggeriscono che la presenza di probiotico e prebiotici nell'SDM non ha portato ad effetti addizionali nella salute dei volontari con MetS. Inoltre, i risultati hanno confermato la fattibilità del processo di *spray drying* per la microincapsulazione di *L. acidophilus* La-5 usando inulina come agente di rivestimento, portando ad una maggiore resistenza del ceppo probiotico alle condizioni gastrointestinali simulate *in vitro*.

Parole-chiavi: Probiotico; *Lactobacillus acidophilus*; Inulina; Frutto-oligosaccaridi; Dessert a base di latte; Studio clinico; Microincapsulazione; Resistenza gastrointestinale in vitro.

ABREVIATIONS

AOAC - Association of Analytical Communities

ATP III - Adult Treatment Panel III

BC - Before Christ

BMI - Body mass index

CAAE - Certificate of Presentation for Ethical Consideration

CD 40 - cluster of differentiation 40

CFU - Colony Forming Units

CVD - Cardiovascular disease

DBP - Diastolic blood pressure

DP - Degree of polymerization

DVS type - Direct vat set

DWpart - Initial weight of microparticles (dry basis)

DWsup - Dry weight of the supernatant

FOS - Fructooligosaccharides

GALT - Gut-associated lymphoid tissue

GIT - Gastrointestinal tract

Group P - Group placebo

Group S - Group synbiotic

Hb - Hemoglobin

HCl - Chlorine acid

HDL-C - High-density lipoprotein cholesterol

Hg - Mercury

HOMA-IR - Homoeostasis model of assessment of insulin resistence

IgA - Immunoglobulin A

IgE - Immunoglobulin E

IgG - Immunoglobulin G

IgM - Immunoglobulin M

IL-10 - Interleukin 10

IL-12 - Interleukin 12

IL-1β - Interleukin 1β

IL-6 - Interleukin 6

IL-8 - Interleukin 8

LDL-C - Low-density lipoprotein cholesterol

LDL-C/HDL-C - LDL-cholesterol to HDL-cholesterol ratio

LPS - Lipopolysaccharide

M1 - Macrophage 1

M2 - Macrophage 1

MCP-1 - Monocyte chemoattractant protein-1

MetS - Metabolic syndrome

mRNA - messenger RNA

NCEP - National Cholesterol Education Program

NF-κB - Nuclear factor kappa B

NGF - Nerve growth factor

PDM - Non-synbiotic diet mousse

pH – Hydrogen-ionic potential

PW - Weight of pellet after centrifugation.

rpm - Revolutions per minute

SBP - Systolic blood pressure

SC - Swelling capacity

SCFA - Short chain fatty acids

SDM - Synbiotic diet mousse

T0 - Baseline

T8 - Week 8

TC/HDL-C - Total cholesterol to HDL-cholesterol ratio

TEV - Total energy value

TG - Triglycerides

TLR-5 - Toll-like Receptor-5

TNF-α - tumor necrosis factor alpha

TPA - Texture profile analysis

Tregs - regulatory T

UHT – Ultra Heat Temperature

VLDL-C - Very low density lipoprotein cholesterol

WAI - Water absorption index

WC - Waist circumference

WHO - World Health Organization

WPI - Whey protein isolate

WSI - Water solubility index

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SUMMARY

PRESENTATION

In 2,500 BC, the famous philosopher Hippocrates could not have been more precise in the prediction of food trends and preferences in the current world when he said: "Let the food be your medicine and the medicine be your food" (Khan et al., 2011). Thus, some authors emphasize that a functional food should promote beneficial effects in a satisfactory way to one or more target functions in the body, contributing to the improvement of health and reducing the risk of disease development, besides basic nutrition (Jiménez-Colmenero et al., 2010). Among the functional foods stand out probiotics, prebiotics, and synbiotics.

The feed ingredients present in a formulation might positively contribute to an interaction between the probiotic microorganism and its carbon source (prebiotic) through previous *in vivo* adaptation. In some cases, a competitive advantage in relation to the development of the probiotic strain in function of the synbiotic effect originated by the availability of prebiotic as a nutrient source was observed. Besides, this effect can be targeted to specific regions of the large and small intestine. The adequate consumption of products containing probiotics and prebiotics may further enhance the beneficial effects of both through a synergistic combination between a known strain and its substrate (Holzapfel & Schillinger 2002; Bielecka et al., 2002).

In the last years, several studies have been performed to evaluate the effect of probiotic microorganisms and prebiotic ingredients on the host's health. However, information about the beneficial effects promoted by these microorganisms on the parameters related to the metabolic syndrome (MetS) is still not clear. In addition, few studies have evaluated the effect of probiotic microorganisms on the health when incorporated in unconventional food products, such as aerated desserts like mousse, particularly when these products also contain prebiotic ingredients.

Thus, the idea of adapting a synbiotic product developed by our research group in order to produce a new dessert with no added sugar and reduced fat content and to test it on volunteers with MetS has become a promising research target.

The present thesis is organized in the form of scientific papers divided in the following chapters:

Chapter 1: "Probiotics, Prebiotics, and Metabolic Syndrome" - This section aims to report the beneficial effects of the supplementation of probiotics and/or prebiotics on parameters related to metabolic syndrome.

Chapter 2: "Texture profile and sensory acceptance of a synbiotic diet aerated mousse containing *Lactobacillus acidophilus* La-5, inulin, fructooligosaccharides, and sucralose" - This section aims to evaluate the effect of *Lactobacillus acidophilus* La-5, inulin, fructooligosaccharides, and sucralose on the instrumental texture profile and the sensory acceptance of a synbiotic diet mousse during 112 days of storage at -18 ℃ compared to a non-synbiotic diet mousse and another one containing sucrose used as a control.

Chapter 3: "Effect of the consumption of a synbiotic diet mousse containing *Lactobacillus acidophilus* La-5 by individuals with metabolic syndrome: a randomized controlled trial" - This section aims to assess the impact of a synbiotic diet dessert (mousse) containing *L. acidophilus* La-5 and the prebiotic ingredients inulin and fructooligosaccharides on anthropometric, biochemical, inflammatory, haematological, and immunological parameters of volunteers with metabolic syndrome.

Chapter 4: "Microencapsulation of *Lactobacillus acidophilus* La-5 using inulin as coating agent by spray drying and its survival under *in vitro* simulated gastrointestinal conditions" - This section aims to optimize the spray drying conditions for the microencapsulation of *Lactobacillus acidophilus* La-5, within a inulin concentrate matrix, applying it in a synbiotic diet mousse and evaluate his survival under simulated gastrointestinal conditions.

Chapter 1

Probiotics, Prebiotics, and Metabolic Syndrome

ABSTRACT

Several studies in the literature have contributed even more to the better understanding of bioactive compounds like the probiotic microorganisms and/or the prebiotic fibers on the modulation of the intestinal microbiota and subsequent positive effects on the host's health. Therefore, this review aimed to discuss the main benefits of the supplementation with probiotic and prebiotic and their effects on different risk factors for the development of metabolic syndrome (MetS). A better understanding of the daily supplementation of probiotics and prebiotics regarding the mechanisms involved on the modulation of the intestinal microbiota and the immune system of patients suffering from this metabolic disorder is necessary to establish the efficiency of possible biomarkers that could contribute for the clinical trials needed for an approved health claim. Although the results might be promising, the integrity of probiotics and prebiotics on the intestinal microbiota becomes critical for its modulation, contributing for the prevention and management of MetS components in clinical practice.

Keywords: Functional food; Bioactive compounds; Dysbiosis; Gut microbiota; Human health.

1. INTRODUCTION

Due to their diverse beneficial effects promoted to health, consumers are increasingly interested in incorporating bioactive compounds to their diets as a functional ingredient (Vo & Kim, 2012). Among these bioactive compounds, probiotics, prebiotics and synbiotics foods stand out as the most profitable in the functional food market (Cruz et al., 2010).

According to Hill et al. (2014), the International Scientific Association for Probiotics and Prebiotics (ISAPP) recently proposed a consensus statement on the proper use of the term probiotic: "live micro-organisms that, when administered in adequate amounts, confer a health benefit on the host". The recommendation for probiotic foods was established based on the daily portion of viable microorganisms that must be ingested, with the minimum stipulated from 10^8 to 10^9 Colony Forming Units/day (CFU/day), even though smaller concentrations may be accepted provided the manufacturer proves their effectiveness (ANVISA, 2016). A similar daily intake of viable probiotic cells per serving portion (10^9 CFU/day) is recommended by the public health agency of Canada (Health Canada, 2009) and the Italian Health Ministry (Ministero della Salute, 2013).

The health benefits attributed to the ingestion of probiotic cultures that mostly stand out are: control of the intestinal microbiota; stabilization of the intestinal microbiota after the use of antibiotics; promotion of the gastrointestinal endurance to colonization by pathogens; decrease in the count of pathogens resulting from the production of short-chain fatty acids, bacteriocins, and other antimicrobial compounds; modulation of the immune system; increased absorption of mineral salts and vitamin production; and constipation relief (Saad, 2006).

Prebiotic ingredients are also added to food formulations in order to develop products with functional claims that would attract consumers concerned about health (Hutkins et al., 2016). According to ISAPP, prebiotics are currently defined as "substrates that are

selectively used by host microorganisms, conferring a health benefit" (Gibson et al., 2017). The presence of prebiotic on the gastrointestinal tract induces the development and/or metabolic activation of beneficial microorganisms residing on the intestinal microbiota through the selectivity of the substrate (Martinez et al., 2015).

Synbiotic is a nutritional supplementation composed by a simultaneous addition of probiotics and of prebiotics in a food matrix which might lead to a synergic activity (Vrese & Schrezenmeir, 2008; Wu et al., 2016). This interaction *in vivo* might be favoured by an adaptation of the probiotic to prebiotic before the consumption, which in some cases can result in a competitive advantage for the microorganism (Saad et al., 2011). According to Kolida and Gibson (2011), a synergistic action occurs when the prebiotic aims to improve survival and growth of the probiotic in the host. On the other hand, a complementary action occurs when the prebiotic used selectively increases the concentrations of the beneficial components of the microbiota.

Much is known about the benefits of probiotic microorganisms and/or the prebiotic fibres on the human body. However, information regarding the beneficial effects promoted by these microorganisms and fibres on the parameters that characterize the metabolic syndrome (MetS) are still not clear. Thus, the aim of this review was to report studies on the beneficial effects of the supplementation of probiotics and/or prebiotics to improve the parameters related to MetS.

2. METABOLIC SYNDROME

MetS is a term suggested by the World Health Organization (WHO) in 1998 to universally relate factors that favour a set of metabolic abnormalities associated with the development of coronary heart disease, stroke and cardiovascular mortality (Afsana et al.

2010a). Medical disorders stemming from the prevalence of MetS increased in the late 20th century, becoming significant issues worldwide (Chou & Fang, 2010). Some researchers reported that it reaches 1 in 5 adults and is considered a new millennium epidemic that will affect the lives of millions of people around the world (Bhatnagar et al., 2011). Many factors can be considered in MetS development process as a consequence of the multi-process lifestyle, perinatal programming and (epi-) genetic pathway (Graf & Ferrari, 2016). Although some therapies have been reported, changes in dietary habits and lifestyle are, undoubtedly, the most important non-pharmacological factors for the prevention and treatment of this syndrome (Kim et al., 2016; Scavuzzi et al., 2015).

According to the National Cholesterol Education Program, Adult Treatment Panel III (NCEP/ATP III) (Grundy et al., 2005), MetS is characterized by the occurrence of at least three of the following five factors: 1) abdominal obesity (waist circumference of ≥ 88 cm for women and ≥ 102 cm for men); 2) high triglycerides (≥ 150 mg/dL); 3) reduced high-density lipoprotein cholesterol (HDL-C) (< 50 mg/dL for women and < 40 mg/dL for men); 4) high blood pressure (systolic ≥ 130 mmHg and diastolic ≥ 85 mmHg); 5) high fasting glucose (≥ 100 mg/dL).

Factors related to MetS assist in developing preventive approaches when metabolic disorders are detected before the onset of chronic diseases (Martin et al., 2016). It may be induced through a non-healthy diet with high fat that results in dyslipidemia, high blood pressure, hyperglycaemia, as well as insulin resistance (Mostafa et al., 2016; Robberecht et al., 2017), increasing the incidence of chronic non-communicable diseases (Bitzur et al., 2016; Monroy-Muñoz et al., 2017). In addition, determination of these parameters is necessary for a treatment that aims to reduce cardiovascular morbidity as a prevention method (Eckel et al., 2010; Westerink et al., 2016).

Obesity, a component of MetS, has been considered its major driving force, leading to both cardiometabolic risk and insulin resistance (Giugliano et al., 2008; Westerink et al., 2016). Moreover, according to Org et al. (2015), atherosclerosis risk factors are associated to levels of insulin resistance, bile acid metabolism, and inflammatory processes. These researches reported also that the metabolites derived from the intestinal microbiota contribute to the development of atherosclerosis and cholesterol metabolism through alternative metabolic pathways. According to Hand et al. (2016), the dyslipidaemia process and the cellular composition of the adipose tissue can also be influenced by the immune system of a metabolically active microbiota.

Through the increased load of free fatty acids coupled with insulin stimulation of hepatic lipogenesis, the synthesis of hepatic triglycerides, very low density lipoprotein cholesterol (VLDL-C) and steatosis becomes modulated by liver (Boden, 2008).

MetS is characterized by increased renal clearance and hepatic uptake of HDL-C, influencing low levels of HDL-C and increased levels of triglycerides (Gallagher et al., 2011). Although there are considerable differences in the mechanisms of excessive distribution of abdominal adipose tissue, the clinical diagnosis of MetS does not distinguish between increased amounts of subcutaneous and visceral fat (Eckel et al., 2005).

Inappropriate activation of the renin-angiotensin system due to the insulin resistance process may induce excess aldosterone and glomerular hypertension (Chou & Fang, 2010). In addition, many researchers associate insulin resistance with the development of metabolic diseases, while cardiologists relate it to cardiometabolic morbidity and mortality in individual patients (Genser et al., 2016). Insulin resistance could also be attributed to problems in specific substrate receptors and tyrosine phosphorylation in the liver of rats fed a high-fat diet (Eckel et al., 2005). In addition, insulin resistance is also related to the accumulation of lipids in insulin-sensitive tissues, so-called ectopic fat deposition (Karpe et al., 2011; Yki-Järvinen,

2002), mediated by modulation of the function/expression of the transporter proteins (Karpe et al., 2011; Holloway et al., 2008). Among these risk factors associated with MetS, an experiment conducted with 499 American non-diabetic Eastern American volunteers suggested that mechanisms related to hyperglycaemia and hypertension are independent of central adiposity or insulin resistance (Boyko et al., 2010). The composition of the intestinal microbiota exerts a major influence on the loss of function of the toll-like receptor-5 (TLR-5) (Hartstra et al., 2016). It has been identified that insulin resistance also induces a nonsense mutation in TLR-5 that induces the development of type 2 diabetes mellitus (Al-Daghri et al., 2013; Hartstra et al., 2016).

The process of hyperglycaemia may induce the generation of reactive oxygen that will result in lipid peroxidation that will further aggravate the Type 2 diabetes mellitus process (Vangaveti et al., 2016). It is one of the leading global causes of premature mortality due to a range of vision problems, renal dysfunction, disability, coronary heart disease, vascular disease, and physical and cognitive impairment (Noale et al., 2012). Physiologically it is observed that pancreatic islet B cells maintain glucose tolerance by their ability to overcome insulin resistance. However, this phenomenon does not occur in people with type 2 diabetes mellitus (Genser et al., 2016; Kahn, 2001).

Oxidative stress originated from metabolic overload (high caloric intake) can result in cardiovascular risk and low-grade of inflammation (Robberecht et al., 2016). Adipose cell enlargement leads to serial proinflammatory response on cells with reduced levels of adiponectin and increased levels of many cytokines and chemokines such as interleukins (IL) IL-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1) (Gustafson et al., 2007).

3. THE INTESTINAL MICROBIOTA

The intestinal microbiota is a set of microorganisms that particularly colonize the gastrointestinal tract in a greater number than cells of the human body (Breban, 2016). That microbiota is directly associated with the host's health as well as the aggravation of diseases, resultant of the great diversity of microorganisms, which makes it the most important environmental agent (Thakur et al., 2016). Although, cross-sectional studies and short-term intervention experiments have brought important information about the relationship of the intestinal microbiota and parameters that characterize the MetS, some approaches still are needed to evaluate certain parameters that are relatively important such as the interaction between host genetics, diet, and microbiota in the regulation of metabolism (Ussar et al., 2015).

According to Rosenbaum et al. (2015), some specific phyla that make up the intestinal microbiota might represent about 97 % of the population of microorganisms such as Bacteroidetes (~0 to 25 %), Firmicutes (~60 to 65 %), Proteobacteria (~5 to 10 %), and Actinobacteria (~3 %).

However, according to Li et al. (2016), the process of colonization and establishment of microorganisms is complex and a function of the microorganism-microorganism and the microorganism-host relations. These researches stated that this process of colonization is so dynamic that not all bacteria are able to colonize an intestinal microbiota permanently.

Moreover, it is known that an unbalance of the microbiota (dysbiosis) may be a consequence of changes in the nitrogen cycle that would compromise its diversity and amount (Briskey et al., 2016). The dysbiosis process establishes a new proportion between the two phylum Firmicutes to Bacteroidetes on the intestinal microbiota of obese individuals (Ley et al., 2005; Martinez et al., 2016). The dysbiosis process is related to many chronic syndromes through the loss of normal functions provided by a commensal microbiota that would

attenuate the disease state (Frank et al., 2011). Thus, a diet rich in fat further influences the dysbiosis process, favouring increased serum hepatic lipids, increased circulating lipopolysaccharide (LPS), and intestinal barrier dysfunction (Norris et al., 2016). On the another hand, dysbiosis aggravates even further the pathogenesis of chronic inflammatory disease that remains unexplained to date (Bredan, 2016). Due to interactions between genetic and environmental factors, the gut microbiota also contributes to the incidence of obesity, diabetes, and MetS (Ussar et al., 2015).

According to He et al. (2016), the development of MetS is a direct association between an interaction of the innate immune system and the intestinal microbiota. Recent approaches are aimed at establishing intestinal homeostasis through a specific diet that can restore the underlying immune system or possible changes in the microbiota (Thakur et al., 2016). In addition, it was evidenced in experimental models that strain specificity on the gut microbiota is important to attenuation of certain immune responses related to chronic inflammation (Kang et al., 2016). Although the intestinal microbiota of adults presents stability, it was observed that possible changes can occur due to diet, genotypic/epigenetic composition, and immuno-metabolic function (Ling et al., 2016). As reported by Moran and Shanahan (2014), different signalling pathways are used as a mean of communication between the microbiota and host involving different classes of effector ligands required to modulate the immune system.

Inflammatory biomarker presented in oxidative and endoplasmic stress induced by diabetes aggravate the synthesis of β-cells influencing the levels of insulin sensitivity and glucose homeostasis (Hasnain et al., 2014; He et al., 2016). It is important to emphasize that the more invasive and inflammatory the composition of the intestinal microbiota is, the greater are the changes in the immune environment adipose compartment from M2 to M1 macrophages that may contribute to the development of the MetS (Burcelin et al., 2012; Hand

et al., 2016). Besides, according to Breban (2016), it exerts anti-inflammatory effects on intestinal cells both by reducing nuclear factor kappa B (NF-κB) levels and synthesis of pro-inflammatory cytokines by the microbiota.

In addition, the diet may change the composition of the gut microbiota, influencing the physiopathology of nutritional disorders such as obesity, severe acute malnutrition, and anorexia nervosa (Alou et al., 2016). Intake of specific nutritional supplements contributes to the modification of the microbiota composition (Hussey & Bergman, 2014). The presence of prebiotics in the diet improves the growth of beneficial species, modifying their composition in a way that can promote beneficial effects on the host's health (Alou et al., 2016). Interest of the consumer market in supplementing food with probiotic ingredients as an alternative medicine is growing, because it claims to induce a homeostasis of the inflammatory response as a result of the presence of these beneficial microorganisms in the gastrointestinal tract (Penga et al., 2014).

4. INFLUENCE OF PREBIOTICS AND/OR PROBIOTICS ON RELATED METABOLIC SYNDROME PARAMETERS

Scientific literature has pointed out, among nutritional therapies to prevent MetS, the consumption of products containing probiotics, prebiotics, and synbiotics (Scavuzzi et al., 2015). Some beneficial effects present in this review towards specific pathological conditions described in the scientific literature resulting from the supplementation with probiotics and/or prebiotics are shown in Table 1.

Table 1. Main effects described for the supplementation of probiotics and/or prebiotics on the human health.

Clinical conditions	Study design	N (completed)	Intake vehicle	Dose and consumption period	Effect	Reference
Abdominal pain	Randomized double-blind placebo-controlled trial	93	Tablets	*Lactobacillus reuteri* DSM 17938 (10^8 CFU/g) for 4 weeks	Decrease of abdominal pain	Weizman et al., 2016
Dyslipidaemia	Randomized double-blind placebo-controlled crossover trial	37	Capsules	*Bifidobacterium animalis* subsp. *lactis* MB 2409 (DSM 23733), *Bifidobacterium* MB 109 (DSM 23731), and *Bifidobacterium longum* subsp. *longum* BL04 (DSM 23233), each at the dosage of 10^9 CFU for 12 weeks	Decrease of lipid profile	Guardamagna et al., 2014
Gestational diabetes mellitus	Randomized double-blind placebo-controlled trial	60	Capsules	*Lactobacillus acidophilus* (2×10^9 CFU/g), *Lactobacillus* casei ($2x10^9$ CFU/g) and *Bifidobacterium bifidum* ($2x10^9$ CFU/g) for 6 weeks	Decrease glycemia levels, triglycerides, and very low-density lipoprotein cholesterol	Karamali et al., 2016
Healthy	Randomized double-blind placebo-controlled trial	110	Sachet	*Lactobacillus plantarum* LP01 and *Bifidobacterium breve* BR03 ($2.5x10^9$ CFU/g), *Bifidobacterium animalis* subsp. *lactis* BS01 ($5x10^9$ CFU/g) for 30 days	Relief at the evacuation	Del Piano et al., 2010
Postmenopausal women with metabolic syndrome	Randomized double-blind placebo-controlled trial	24	Fermented milk	*Lactobacillus plantarum* Lp-115 ($1.25x10^7$ CFU/g) for 12 weeks	Decrease glucose levels and homocysteine	Barreto et al., 2014
Healthy	Randomized double-blind placebo-controlled trial	21	Yoghurt and tablet	*Bifidobacterium animalis* DN-173 010 ($2x10^9$ CFU/g), *Lactobacillus reuteri* DSM 17938 (10^9 CFU/g), and *Lactobacillus plantarum* 299v (10^8 CFU/g) for 2 weeks	Glycemic homeostasis	Nilsson et al. 2016
Patients with metabolic syndrome	Randomized double-blind placebo-controlled trial	51	Fermented milk	*Bifidobacterium animalis* ssp. lactis HN019 ($3.4x10^8$ CFU/g) for 6 weeks	Decrease in body mass index measure, total cholesterol levels and low-density lipoprotein cholesterol	Bernini et al. 2016
Irritable bowel syndrome	Randomized double-blind, placebo-controlled multicentric trial	30	Fermented milk	*Lactobacillus acidophilus* La-5 ($1.8x10^7$ CFU/g) and *Bifidobacterium animalis* ssp. *lactis* BB-12 ($2.5x10^7$ CFU/g) and 2% dietary fiber Beneo Orafti Synergy1 (90% inulin, 10% oligofructose) for 7 weeks	Decrease of symptoms of irritable bowel syndrome	Matijašić et al., 2016
Major depressive disorder	Randomized double-blind placebo-controlled trial	40	Capsules	*Lactobacillus acidophilus* YAB ($2x10^9$ CFU/g), *Lactobacillus casei* TD_2 ($2x10^9$ CFU/g), and *Bifidobacterium bifidum* B12 ($2x10^9$ CFU/g) for 8 weeks	Decrease serum insulin levels and homeostasis model of assessment of insulin resistance	Akkasheh et al. 2016
Hypercholesterolemic	Randomized double-blind placebo-controlled trial	32	Capsules	*Lactobacillus acidophilus* CHO-220 (10^9 CFU/g) and 0.2 g inulin for 12 weeks	Decrease of total cholesterol levels, high-density lipoprotein cholesterol, and very low-density lipoprotein cholesterol	Ooi et al. 2010
Type 2 diabetes mellitus	Randomized double-blind placebo-controlled trial	49	Sachet	10 g chicory inulin enriched with fructooligosaccharides for 8 weeks	Glycemic homeostasis	Farhangi et al., 2016
Type 2 diabetes mellitus	Randomized double-blind placebo-controlled trial	49	Sachet	Daily dose of 10 g of fructooligosaccharides enriched inulin for 8 weeks	Improvements in immune system with increasing IL-4 (anti-inflammatory cytokine); reduction in IL-12 and IFNlevels (inflammatory cytokines).	Dehghan et al. 2016
Type 2 diabetes mellitus	Randomized double-blind placebo-controlled crossover trial	102	Packages	*Lactobacillus sporogenes* (10^7 CFU/g), 0.1 g inulin and 0.05 g beta-carotene for 6 weeks	Decrease of serum insulin levels, homeostasis model of assessment of insulin resistance, triglycerides, very low-density lipoprotein cholesterol, and total cholesterol/high-density lipoprotein cholesterol	Asemi et al., 2016

High levels of triglycerides and low levels of HDL-cholesterol contribute to the accumulation of fatty acids as visceral adiposity in subjects with MetS (Welty et al., 2016). Consequently, this clinical picture contributes to the aggravation of cardiovascular diseases (Tselios et al., 2016) and cerebrovascular accident (Shin et al., 2015). Song et al. (2014) correlated triglyceride and HDL-cholesterol levels with immunoglobulin M (IgM) synthesis in individuals with MetS. The blood test data from 10,015 participants, including immunological tests, showed increased triglycerides levels and decreased HDL-C levels correlating with the reduction of IgM levels in individuals with MetS.

Jones et al. (2012b) evaluated the influence of the daily intake of two capsules containing $2.9x10^9$ CFU of *L. reuteri* NCIMB 30242 in a randomized, multi-centre, placebo-controlled design. The authors reported that the probiotic strain did not influence the glycaemia levels of 124 hypercholesterolemic volunteers during 9 weeks of intervention. On the other hand, Ejtahed et al. (2012) evaluated the glycaemia levels of 64 diabetic patients who consumed 300 g/day of probiotic yogurt containing $1.8x10^6$ CFU/g of *Bifidobacterium animalis* subsp. *lactis* BB-12 and $1.9x10^6$ CFU/g of *Lactobacillus acidophilus* La-5 for 6 weeks of supplementation. According to these researchers, the changes in the intestinal microbiota related to the presence of probiotic microorganisms possibly contributed to a reduction in glucose levels throughout the intervention period.

It has been shown that the supplementation of two prebiotics, inulin and fructooligosaccharides (FOS), in the diet of rats possibly contributed positively to the prevention of some metabolic disorders, such as arterial hypertension, hypertriglyceridemia, in addition to renal damage on the animals evaluated (Rault-Nania et al., 2008). As a result of their secondary metabolism, these microorganisms release short-chain fatty acids (SCFA), among them acetate and propionate, through complex metabolic pathways present in

numerous species belonging to the phyla Actinobcteria, Firmicutes, Bacteroidetes and others (Miremadi et al. 2016).

5. PREBIOTICS' AND PROBIOTICS' MECHANISMS OF ACTION ON THE HOST

The mechanism of action of probiotics on the host is not yet clearly elucidated. However, the literature has several studies with *in vivo* and *in vitro* models which support some hypotheses (Daliri & Lee, 2015). Although some mechanisms of action of probiotics, prebiotics, and synbiotics to reduce cholesterol levels are reported in the scientific literature (Bedani et al., 2015; Miremadi et al., 2014; Zhang et al., 2017), there is a consensus about the importance of the strain specificity in relation to its possible mechanisms of action for probiotic effects (Higashikawa et al., 2010; Ooi & Liong, 2010).

According to the literature, the main mechanism of action of prebiotics is their ability to reduce lipid levels in the bloodstream by the presence of SFCA synthesized in the intestinal microbiota (Miremadi et al., 2016). As a source of energy, these fibres stimulate the development of microorganisms in the microbiota that release bioactive compounds such as acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, and caproate (Breban, 2016; Ooi & Ling, 2010; Fernández et al., 2016; Wang et al., 2012b). Thus, these SCFA can inhibit the synthesis of hepatic cholesterol and/or assist in the redistribution of cholesterol from the plasma to the liver (Al-Sheraji et al., 2012).

The modulation of hepatic cholesterol biosynthesis in the body begins by absorption of the SCFA in the gastrointestinal tract, where they are metabolised by the liver cells (Fernández et al., 2016; Wong et al., 2006). In turn, propionate and butyrate are also involved in the control of hepatic cholesterol synthesis that can lead to the reduction of plasma cholesterol levels (Trautwein et al., 1998). Thus, this can inhibit hepatic cholesterol synthesis

and/or assist in the redistribution of cholesterol from plasma to liver (Al-Sheraji et al., 2012). Metabolism of cholesterol in the liver is a result of hepatic modulation that increases the levels of bile acids excretion, according to the presence of endogenous cholesterol (Ditscheid et al., 2005). The deconjugated bile salts are less absorbed along the intestinal lumen and excreted through the faeces after the hydrolysis process, resulting in free bile acids (Jones et al., 2012a; Kumar et al., 2012; Wang et al., 2012a). This process increases the demand for cholesterol molecules by the body as precursors for the synthesis of new bile salts (Begley et al., 2006). It is not entirely clear whether SCFA actually promotes beneficial effects to the host's health by modulating the immune system by regulatory T (Tregs) and innate immune cells or by regulating other metabolic aspects (Hand et al., 2016).

Among the mechanisms responsible for the hypocholesterolemic effect, it has often been attributed to the assimilation and/or incorporation of the cholesterol molecule into the bacteria cell membrane during the microbial growth phase (Alhaj et al., 2010; Kumar et al., 2012). The binding of cholesterol to the cell surface can occur, independently of the physiological state of the cell (living or dead) (Kimoto et al., 2002). Nevertheless, it was clear in this research that some microorganisms in the growth phase can remove higher levels of cholesterol compared to the dead cell. Choi and Chang (2015) have demonstrated in an *in vitro* study that *L. plantarum* EM possesses a great ability to bind the cholesterol molecule to its cell surface, regardless of its viability. Thus, this mechanism of action reduces its absorption in the gastrointestinal tract after the assimilation of this lipid molecule on the cell surface (Lye et al., 2010).

Some studies indicate that the daily supplementation of *L. plantarum* and *L. casei* strains showed a potential anti-hypertensive effect in hypertensive volunteers (Naruszewicz et al., 2002; Nakajima et al., 1995). As observed by Lollo et al. (2015), probiotics have contributed to the reduction of blood pressure through the degradation of proteins from the

food matrix, mainly milk protein, releasing peptides with a hypotensive effect that act on the renin-angiotensin system.

Mechanisms of action of probiotics on the modulation of the immune system still require more investigation in order to carry out hypotheses that lead to a conclusion (Reid et al., 2016). A possible mechanism suggested by Tejada-Simon et al. (1999) is the influence of some components of the bacterial cell on the immunomodulatory activity in the lymphoid tissue. According to these researchers, cell membrane components such as peptideoglycans and endotoxic lipopolysaccharides (LPS) would be responsible for the signalling and translocation of antigens by the intestinal mucosal barrier. Hence, activation of an immune response by a non-pathogenic strain contributes to homeostasis, favouring an immunomodulatory reaction by the host's immune system (Kotzamanidis et al., 2010). Activation of certain immune defence cells such as dendritic cells and T-helper 1 lymphocytes have been modulated by *L. acidophilus* by the toll-like and proteoglycan receptor recognition of enterocytes (Daliri & Lee, 2015). Thus, the adhesion of probiotic strains on the intestinal epithelia in function of the hydrophobic affinity and autoaggregation of the cell surface can stimulate the immune responses in the gut-associated lymphoid tissue (GALT) through the strain antigen (Kotzamanidis et al., 2010). However, some interactions such as hydrophobic and autoaggregation of the cell surface can be impaired by exposure to the bile salts resulting from the digestive process (Burns et al., 2011). As observed by Thakur et al. (2016), among *Lactobacillus* strains, only *Lactobacillus casei* Lbs (MTCC5953) showed the ability to reduce the secretion of tumour necrosis factor alpha (TNF-α) and IL-6 after induction by the presence of LPS in an *in vivo* assay with rats. Besides, *L. rhamnosus* GG has the ability to modulate the immune system through the reduction in IL-8 levels induced by TNF-α (Zhang et al., 2005). Consequently, in addition to inhibiting the secretion of IL-8, this strain stimulates increased levels of nerve growth factor (NGF) (Ma et al., 2004).

6. CONCLUSIONS

In this review article, we have described that numerous non-transmissible chronic diseases result from the process of intestinal dysbiosis, thus, evidencing the importance of the function of the intestinal microbiota on the health of the host through an organized system. Besides, beneficial association between modulation of the intestinal microbiota and prevention of the components related with MetS through a daily diet composed of foods containing probiotic strains and prebiotic fibres are showing promising results. A better understanding of the mode of action of probiotics ingredients and/or prebiotics in clinical trials are required for their application, aiming at beneficial effects of their supplementation on the human health. Therefore, studies have shown that a regular diet with these bioactive compounds promotes beneficial effects on the MetS-related parameters.

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Chapter 2

Texture profile and sensory acceptance of a synbiotic diet aerated mousse containing *Lactobacillus acidophilus* La-5, inulin, fructooligosaccharides, and sucralose

ABSTRACT

This study aimed at evaluating the effects of *Lactobacillus acidophilus* La-5, inulin, fructooligosaccharides (FOS), sucralose, and time of storage on pH variation, and instrumental texture profile of a synbiotic diet mousse (SDM) during 112-day storage at -18 °C, compared to a non-synbiotic diet mousse (PDM) and another formulation of synbiotic non-diet mouse (CSM) on sensory acceptance. SDM pH throughout the storage period was slightly lower than that of PDM. SDM showed a total energy value about 20% lower than PDM. Hardness and gumminess of SDM increased and cohesiveness decreased throughout storage, while adhesiveness and springiness kept relatively stable. PDM showed lower acceptability after storage than CSM and SDM, probably due to its higher powdered milk content and absence of inulin and FOS. These results suggest that the presence of pro- and prebiotics, as well as the storage time, although causing significant changes in SDM instrumental texture profile, did not exert any appreciable influence on its sensory acceptability, and that sucralose could be a good sucrose substitute in mousses.

Keywords: Instrumental texture profile; Sensory performance; Probiotic; Prebiotic; Mousse.

1. INTRODUCTION

The modern lifestyle is often taken into account in developing new foods. Therefore, the search for attractive and healthier products has become a challenge for the food industry (Komatsu, Buriti, & Saad, 2008). Thus, research efforts aim at modifying the technological properties of food macromolecules to develop products able to promote improvements in the quality of consumer's life.

According to Hill et al. (2014), a consensus declaration on the scope and the proper use of the term 'probiotics' has recently given by the International Scientific Association for Probiotics and Prebiotics (ISAPP) as "live micro-organisms that, when administered in adequate amounts, confer a health benefit on the host". Probiotics have in fact attracted special attention because of their nutritional and functional properties, and a number of studies have been developed aiming to clarify the mechanisms of action of these strains in the human body (Daliri & Lee, 2015; Kemgang, Kapila, Shanmugam, Reddi, & Kapila, 2016; Reid, 2016; Shah, 2007; Vasiljevic & Shah, 2008). As a result, similar daily intake of viable probiotic cells per serving portion (10^9 CFU/day) is recommended by the Canadian Public Health Agency (Health Canada, 2009) and the Italian Health Ministry (Ministero della Salute, 2013).

The incorporation of probiotic cultures into frozen desserts has been performed in different studies with the aim of diversifying the types of probiotic foods on the market (Cruz, Antunes, Souza, Faria, & Saad, 2009). Dairy desserts are widely consumed worldwide by several groups of consumers, including children and elderly, in different meals, environments, and occasions (Buriti & Saad, 2014), and such a large consumption is mainly influenced by the nutritional and sensory characteristics of these products (Tárrega & Costell, 2007; Ares, Giménez, & Gámbaro, 2009; Ferraz et al., 2012).

Lactobacillus are lactic acid bacteria in rod form and many species are present on the mucosal surfaces of animals (Hymes, Johnson, Barrangou & Klaenhammer, 2016). These microorganisms are widely use in the development of dairy products (Gebara, Ribeiro, Chaves, Gandara, & Gigante, 2015). *Lactobacillus acidophilus* La-5 stands out for playing an important role in the modulation of the intestinal microbiota, suppression of harmful bacteria, hypocholesterolemic effect, and immune tolerance (Anderson & Gilliland, 1999; Medellin-Penã & Griffiths, 2009; Zhao et al., 2015).

Prebiotic ingredients are also added to food formulations in order to develop products with functional claims that would attract consumers concerned about health (Hutkins et al., 2016). According to ISAPP, prebiotics are currently defined as "substrates that are selectively used by the host micro-organisms, conferring a health benefit" (Gibson et al., 2017). According to Wang (2009), these ingredients contribute significantly to the improved sensory characteristics of products, such as taste and flavour, as well as their structural stability during the processing period, in terms of heat, pH, and conditions favouring the Maillard reaction.

Inulin is being increasingly used in the food industry owing to its technological characteristics, especially as a fat substitute, as well as to increase the food fibre content (Barclay, Ginic-Markovic, Cooper, Petrovsky, & 2010; Bitzios, Fraser, & Haddock-Fraser, 2011; Gunnarsson, Svensson, Johansson, Karakashev, & Angelidaki, 2014; Khuenpeta et al., 2017). Moreover, the bifidogenic effect of inulin can be prolonged by an increase either in its solubility or the degree of polymerization of its chain (Pompei et al., 2008). FOS is a fairly soluble fibre, which is marketed either as a viscous syrup (containing 75% of total solids) or as a powder (up to 95% purity). In its pure form, it has a sweetness of about 30-35% compared to sucrose, combining well with delicate flavour and reducing sweetener aftertaste (Franck, 2008).

Synbiotics are nutritional supplements composed of both probiotics and prebiotics (Moumita et al., 2017; Vrese & Schrezenmeir, 2008; Wu, Liu, Liang, Hu, & Huang, 2016). According to Kolida and Gibson (2011), a synergistic activity occurs in a food matrix when the prebiotic aims to improve survival and growth of the probiotic in the host, whereas a complementary action occurs when the prebiotic selectively increases the concentrations of beneficial components of the microbiota.

It is known that many commercialized desserts contain high levels of sucrose, a sugar which is readily metabolized by the body. Its consumption has been associated with many diseases; therefore, its low intake or substitution by non-caloric sweeteners is recommended (Morais, Lima, Morais, & Bolini, 2015).

Sucralose is a sweetener derived from sucrose throught the selective substitution of three hydroxyl groups by chlorine atoms, and therefore non-caloric and widely used by the food industry (Magnuson, Roberts, & Nestmann, 2017). This sweetener has a sweetening power of approximately 600 times greater then sucrose (Rodriguez Furlán, Baracco, Zaritzky, & Campderrós, 2016).

Finally, the freezing process may contribute to maintain cell vitality along the period of the product storage, reducing the probiotic mortality rate when compared to storage above 0 °C (Magariños, Selaive, Costa, Flores, & Pizarro, 2007).

The objective of this study was to evaluate the effect of *Lactobacillus acidophilus* La-5, inulin, FOS, sucralose, and time of storage on instrumental texture profile and sensory acceptance of a synbiotic diet mousse (SDM) during 112-days of storage at -18 °C, compared to a non-synbiotic diet mousse (PDM) and another containing sucrose used as a control (CSM).

2. MATERIALS AND METHODS

2.1. Production of synbiotic diet mousse

The aerated SDM was prepared as an adaptation of a low-calorie formulation developed by Buriti, Castro, and Saad (2010a,b) and characterized by Komatsu et al. (2013), which will be referred here as CSM, only used to evaluate sensory acceptability (Table 1).

Table 1. Ingredients employed in the production of synbiotic diet mousse (SDM), and non-synbiotic diet mousse (PDM), and control synbiotic mousse (CSM).

Ingredients (g/100 g)	**SDM**	**PDM**	***CSM**
Skimmed milk[1]	61.7	61.7	59.3
Skimmed milk powder[2]	4.0	14.0	4.0
Sucrose[3]	-	-	11.0
Sucralose[4]	1.1	1.1	-
FOS[5]	6.0	-	6.0
Inulin[6]	4.0	-	4.0
Pasteurized and frozen guava pulp[7]	20.0	20.0	12.5
Stabilizer/emulsifier[8]	2.8	2.8	2.8
Lactic acid[9]	0.4	0.4	0.4
Lactobacillus acidophilus La-5[10]	0.05	-	0.05
Total	100.0	100.0	100.0

[1]Paulista (Danone, Guaratinguetá, SP, Brazil); [2]Molico (Nestlé, Araçatuba, SP, Brazil); [3]União (Cosan, Limeira, SP, Brazil); [4]Sucralose (Línea Sucralose, São Paulo, SP, Brazil); [5]Beneo P95 (Orafti, Oreye, Belgium); [6]Beneo HP (Orafti); [7]Icefruit-Maisa (Icefruit Comércio de Alimentos, Tatuí, SP, Brazil); [8]Cremodan Mousse 30 (Danisco, Cotia, SP, Brazil); [9]Purac (Purac Sínteses, Rio de Janeiro, RJ, Brazil; 85g/100g food-grade solution); [10]Strain La-5 (Christian Hansen, Hoersholm, Denmark). *Previously developed non-diet sucrose-containing formulation (Buriti et al., 2010a,b; Komatsu et al., 2013), which was only used to evaluate sensory acceptability.

For the preparation of both SDM and CSM, a commercial freeze-dried direct-to-vat probiotic culture of *L. acidophilus* La-5 was used, which was stored frozen (-18±2 °C). Powdered skimmed milk and FOS were dissolved in ultra-high temperature (UHT) skimmed

milk one day before product preparation in order to make the dissolution of these ingredients easier. The resulting pre-mixture was stored under refrigeration at 4 °C until the addition of the remaining ingredients. One portion (40 mL) of this pre-mixture was sterilized at 121 ºC/15 min and employed, the next day, for the activation of the probiotic culture for 120 min at 37 °C (Komatsu et al., 2013).

The other ingredients listed in Table 1 were added and mixed until complete mass uniformity in a 6 kg-mixer, model UMMSK-12 (Geiger, Pinhais, PR, Brazil). The resulting mixture was pasteurized in the same mixer at 85 °C for 5 min, allowed cooling to 40 °C and supplemented with milk containing the activated probiotic culture. Then, the mixture was kept in refrigerator (5 ºC) for subsequent aeration at a temperature between 10 and 15 °C in a 20 L-planetary mixer, model 20 (Irmãos Amadio Ltda., São Paulo, SP, Brazil), during which its volume increased by 80-85%. Afterwards, the mousse was transferred to a manual filler, model IQ81-A (Intelimaq Máquinas Inteligentes, São Paulo, SP, Brazil), and then packed in polypropylene plastic pots for food with 75 mm diameter, 42 mm height and 100 mL capacity (Tries Aditivos Plásticos, São Paulo, SP, Brazil), which were sealed with aluminium cover in a sealer, model 1968 (Delgo Metalúrgica, Cotia, SP, Brazil). Figure 1 schematically illustrates the main steps of SDM manufacture.

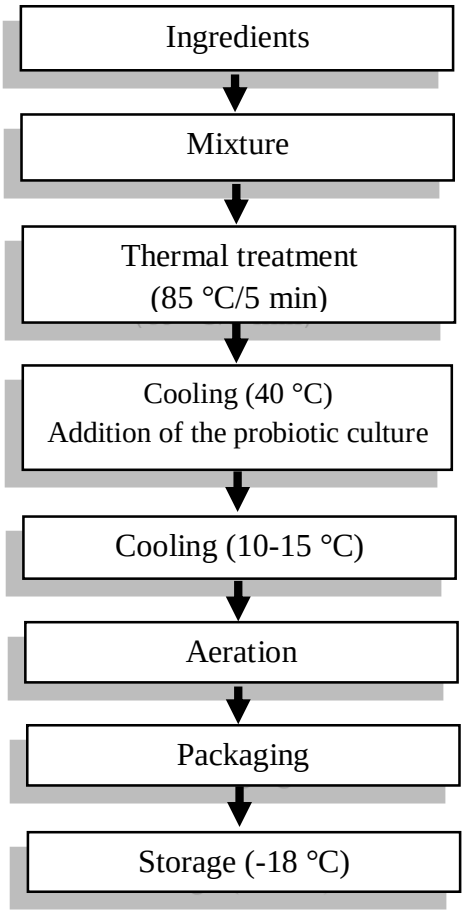


Figure 1. The main steps of synbiotic diet mousse production.

2.2. Determination of pH and microbiological parameters

The pH values were determined on quadruplicate samples (four different pots of the same trial) using a pH meter Orion Model Three Stars (Thermofisher Scientific, Waltham, USA) equipped with a penetration electrode type for solid foods and semi-solid.

The counts of *L. acidophilus* La-5 were monitored during the production process and along the storage frozen period. For this purpose, 25 g portions of quadruplicate mousses samples (four different pots of the same trial) were collected in aseptic condition and added to 225 mL of 0.5% NaCl solution, using a Bag Mixer 400 (Interscience, St. Nom, France).

Samples were serially diluted in 0.1 peptone water solution, and seeded in MRS agar, modified by the addition of a maltose solution (50% w/v) using the pour plate method and with incubation at 37 °C for 48 h, as proposed by the International Dairy Federation (IDF, 1995).

Aliquots of 1 mL of each sample dilution were transferred to Petrifilm™ EC (3M Microbiology) and Petrifilm™ YM (3M Microbiology) plates for counting of coliforms and *Escherichia coli*, molds and yeasts, all according to the manufacturer's instructions. The Petrifilm™ EC plates were incubated at 35-37 °C for 24 h, while Petrifilm™ YM plates were incubated at 20-25 °C for 3 to 5 days.

2.3. Chemical composition and total energy value of mousses

Portions of SDM and PDM previously submitted to the whole storage period were also analysed in triplicate for their chemical composition and total energy value (TEV). For this purpose, the solid contents of 5.0 g mousse samples were determined by drying at 70 °C in a vacuum oven equipment, model 440/A (Nova Ética, Vargem Grande Paulista, SP, Brazil), according to Instituto Adolfo Lutz (2005). The protein content was determined by measuring the nitrogen content of mousses through the micro Kjeldahl method and multiplying by a conversion factor of 6.38, according to the AOAC official methods 690.52 (AOAC, 2003). The lipid content was quantified by the method of Soxhlet, and that of ash, or fixed mineral residue, was determined gravimetrically by incineration of 2.0 g of sample at 550 °C (Instituto Adolfo Lutz, 2005). Finally, the percentage of carbohydrates (excluding total dietary fibre) was calculated as the difference to obtain 100 % of the total composition (ANVISA, 2003).

The contents of these macronutrients were converted into TEV (kJ 100/g) through the Atwater factors and energy from all components (16.74 kJ/g × *x* g proteins/100 g + 16.74

kJ/g × *y* g carbohydrates/100 g + 6.28 kJ/g × *z* g fructans/100 g + 37.66 kJ/g × *t* g total lipids/100 g) (Roberfroid, 1999, 2005; ANVISA, 2003; FAO, 2003).

2.4. Instrumental texture profile

Texture profile analysis (TPA) was carried out in samples collected during a storage period of 112 days by double compression test at room temperature, using an aluminium cylinder with 25 mm diameter (P25) fixed to a texture analyser, model TA-XT2 (Stable Micro Systems, Haslemere, UK), and employing a distance of 10 mm and a penetration speed of 1 mm/s. To avoid any interference of freezing with hardness analysis (Muse & Hartel, 2004), diet mousses were transferred from the freezer to a refrigerator at 4±1 °C where they remained for 6 h before testing. The following texture parameters were determined: hardness, cohesiveness, adhesiveness, springiness, and gumminess. Data were collected and analysed through the Texture Expert for Windows software, version 1.20 (Stable Micro Systems).

Samples of mousses stored at -18±2 °C were collected in quintuplicate, thawed at 4 °C and analysed for instrumental texture parameters one day after their manufacture and after 7, 35, 56, 84, and 112 days.

2.5. Sensory evaluation

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki. The protocol followed for sensory analysis of mousses was approved by the Research Ethics Committees of the School of Pharmaceutical Sciences of the University of São Paulo, São Paulo, SP, Brazil (CAAE 30539214.6.0000.0067) and of the University Hospital, São Paulo, SP, Brazil (Protocol Number 663.138). The sensorial evaluation was conducted on samples of the three mousse formulations stored at -18 °C for 7,

35, 56, 84, and 112 days and thawed at 4 °C, 2 h before the start of sensory sections. Samples were codified with 3 random digits and distributed among participants for their individual evaluation.

Sensory acceptability tests were performed by voluntary consumers, using a structured 9-point hedonic scale (1 = dislike extremely; 9 = like extremely) for overall acceptability (Hough, 2010), and allowing the judge to indicate what was the sensory characteristics that he liked most or least.

Thirty untrained adults participated in each of the five sensory analysis sections, giving a total of 150 consumers, of which 50.0% were female and 50.0% male, with ages between 18 and 60 years (mean age of 24.4±7.4 years). Healthy volunteers were mostly undergraduate and graduate students and University of São Paulo employees. Criteria of exclusion included people with history of allergic manifestation, food intolerance or chronic diseases such as diabetes, hypothyroidism, hyperthyroidism, hypertension or others, flu or indisposed people, people making medical treatment or having a cold, and people that were in contact with strong smelling materials, foods or cosmetics less than 1 h before.

In particular, texture, appearance, odour, and taste were evaluated, which are the main sensory attributes chosen by consumers to assess quality and characteristics of a food (Afoakwa, Paterson, Fowler, & Viera, 2009).

2.6. Statistical analyses

Variance homogeneity for each set of data was verified, using the Hartley, Cochran, and Bartlett tests. The Student *t* test was used to determine statistically significant differences ($p<0.05$) between two means when a homogeneous variance was observed. Results were compared by the analysis of variance (ANOVA) using the Tukey's test, considering a

significance level of $p<0.05$. When normal distribution was not found, we employed the nonparametric Kruskal-Wallis test followed by the Dunn's test.

3. RESULTS AND DISCUSSION

3.1. Viability of the probiotic microorganism

Some studies emphasized the importance of previously testing the compatibility between probiotic microorganism and prebiotic ingredient, in order to provide a positive interaction that could contribute to increased microbial viability throughout storage (Alves et al., 2013; Peredo, Beristain, Pascual, Azuara, & Jimenez, 2016; Sathyabama, Ranjith, Bruntha, Vijayabharathi, & Brindha, 2014). *L. acidophilus* La-5 population in the diet synbiotic mousse (SDM) remained above 7.8 log CFU/g (results not shown) during the 112 day-long storage at -18±2 °C, with no significant differences ($p>0.05$) in its viability.

Such *L. acidophilus* La-5 viability in SDM was higher than that previously observed in a sucrose-based synbiotic mousse either after 14 days of refrigerated storage (6 log CFU/g) or after 112 days of frozen storage (>7 log CFU/g) (Buriti, Castro, & Saad, 2010b). The post-acidification process, attributed to a fermentation process of the probiotic strain at refrigerated storage, reduces the pH value due of the generation of organic acids, which could impair the viability of the strain, therefore reducing of its population (Settachaimongkon et al., 2016; Shah, 2000). On the other hand, Moura et al. (2016) reported higher counts of the same probiotic (8.62-8.92 log CFU/g) in a sucrose-based dairy dessert after 15 days of refrigerated storage. In that study, the effect of post-acidification may be minimized by the absence of inulin in the formulation of this probiotic dessert, since some *Lactobacillus* strains have genes for the activity of β-fructofuranosidase responsible for the fermentation of inulin-type fructans (Bielecka, Biedrzycka, & Majkowska, 2002; Hopkins, Cummings, & Macfarlane, 1998;

Makras, Van Acker, & De Vuyst, 2005). In the present study, the frozen storage preserved the *L. acidophilus* La-5 populations above 7.8 log CFU/g during 112 days.

No microbial contaminants were detected in frozen mousses during storage, likely due to the good manufacturing practices employed during production and storage of mousses (data not shown).

3.2. pH variation

The mean pH values of both diet mousses are listed in Table 2. It can be seen that the pH of SDM was significantly lower ($p<0.05$) than that of PDM throughout the whole storage period. Besides, the addition of skimmed milk powder in both formulations may contribute to the buffering effect due to the presence of proteins and phosphates (Antunes, Cazetto, & Bolini, 2005; Buriti, Castro, & Saad, 2010a). This fact may explain the higher pH values in PDM.

Table 2. Mean pH values (standard deviation) of non-synbiotic diet mousse (PDM) and synbiotic diet mousse (SDM) stored at -18±2 °C for up to 112 days.

Storage (days)	PDM	SDM
1	6.40 (0.01) ^{Aa}	5.85 (0.02) ^{Ba}
7	6.37 (0.02) ^{Aab}	5.82 (0.01) ^{Bab}
35	6.28 (0.03) ^{Abc}	5.80 (0.03) ^{Babc}
56	6.26 (0.01) ^{Acd}	5.71 (0.03) ^{Bbcd}
84	6.22 (0.02) ^{Acd}	5.68 (0.09) ^{Bcd}
112	6.18 (0.02) ^{Ad}	5.66 (0.02) ^{Bd}

Different uppercase letters in the same line indicate statistically significant differences ($p<0.05$) between the two diet mousse formulations after the same storage period. Different lowercase letters in the same column indicate statistically significant differences ($p<0.05$) between different storage periods.

3.3. Chemical composition and total energy value

The chemical composition, the energy contribution of macronutrients, and the TEV of both diet mousses formulations are listed in Table 3.

Table 3. Chemical composition, energy contribution of macronutrients, and total energy values (TEV) of synbiotic diet mousse (SDM) and non-synbiotic diet mousse (PDM) referred to 100 g of mousses (dry weight).

	PDM	**SDM**
Composition (g/ 100 g)		
Ash	1.42 (0.17) ^{B}	0.90 (0.06) ^{A}
Proteins	8.55 (0.33) ^{B}	6.77 (0.37) ^{A}
Simple carbohydrates	17.53 (1.01) ^{B}	10.24 (0.97) ^{A}
Fructans	0.00 ^{B}	9.63 ^{A,*}
Lipids	0.12 (0.04) ^{A}	0.22 (0.05) ^{A}
Moisture	72.38 (1.84) ^{A}	72.24 (1.59) ^{A}
Total	100.00	100.00
Energetic value (kJ/ 100 g)		
Proteins	143.09 (5.52)	113.30 (6.67)
Lipids	4.52 (2.26)	8.28 (2.07)
Simple carbohydrates	293.24 (16.90)	171.38 (31.07)
Fructans	0.00	60.46
TEV	440.85 (11.42)	353.42 (27.91)

Values expressed as averages (standard deviation). TEV: Total Energy Value. *Estimate based on information given by the supplier (Orafti) for the prebiotic ingredients (Beneo P95 and Beneo HP). Different uppercase letters in the same line indicate statistically significant differences ($p<0.05$) between the two diet formulations.

There were no significant differences (p>0.05) between lipids, and moisture contents of formulations, while those of ash, carbohydrates, and proteins were significantly higher (p<0.05) in PDM, possibly due to its higher percentage (14% w/w) of powdered skimmed milk (Table 1). Similar results were observed by other researchers. To provide just a few examples, Morais, Lima, Morais, and Bolini (2015) observed protein levels between 6.7 to 7.1% for milk chocolate desserts containing different sweeteners (sucrose, sucralose, aspartame, neotame or stevia), but higher ash content (from 1.7 to 2.1%), while Komatsu et al. (2013) reported protein and ash contents in the ranges 4.4-8.0% and 0.8-1.0%, respectively, for milk guava mousses using inulin as fat replacer and/or whey as a food supplement.

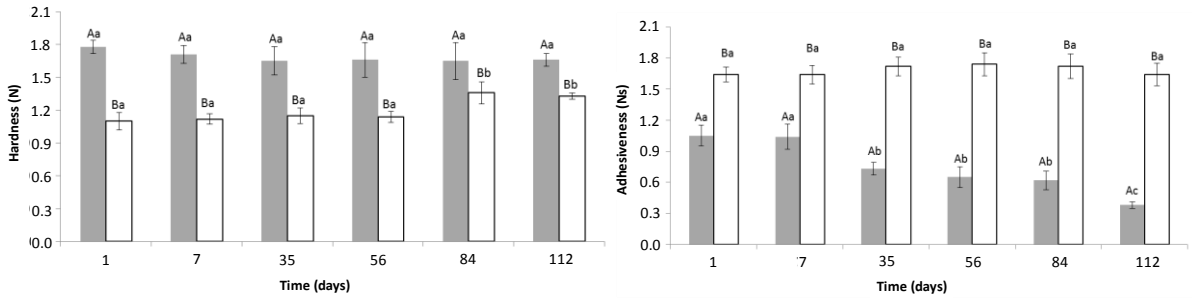
TEVs of PDM (440.85 kJ/100g) and SDM (353.42 kJ/100g) were 10.8 and 28.5% lower, respectively, than that of CSM (494.0 kJ/100g), due to the high sucrose content of the control mousse (11%). Therefore, according to the Brazilian legislation, both non-synbiotic and synbiotic formulations proposed in this study can be considered not only diet, for they contained less than 0.5 g of free sugars like sucrose, fructose, and glucose (dextrose) and/or fats per 100 g of product, but also as “zero”, with TEVs < 400 kcal/100 g (1674 kJ/100 g) (ANVISA, 1998).

3.4. Texture profile analysis

The texture profiles of both the synbiotic and non-synbiotic mousses stored at -18 °C are illustrated in Figure 2. One can see that hardness and gumminess of SDM increased and cohesiveness decreased significantly throughout storage (p<0.05), while adhesiveness and springiness kept almost the same (p>0.05) until 112 and 84 days, respectively. On the other hand, PDM hardness did not vary significantly during storage (p>0.05), while gumminess, cohesiveness, springiness, and adhesiveness gradually decreased with time (p<0.05).

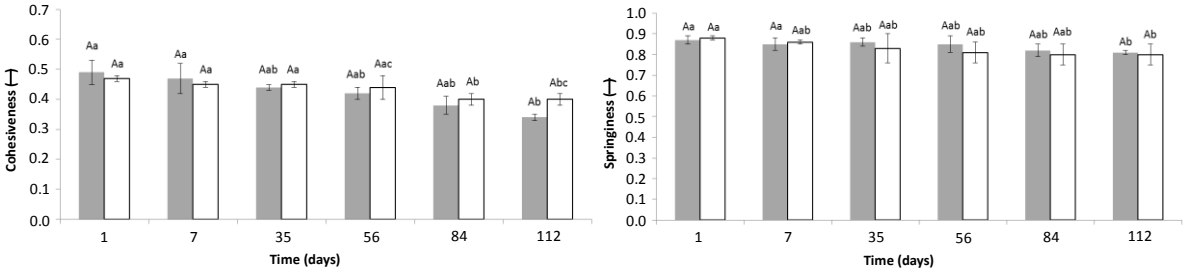
(a) Hardness

(b) Adhesiveness*



(c) Cohesiveness

(d) Springiness



(e) Gumminess

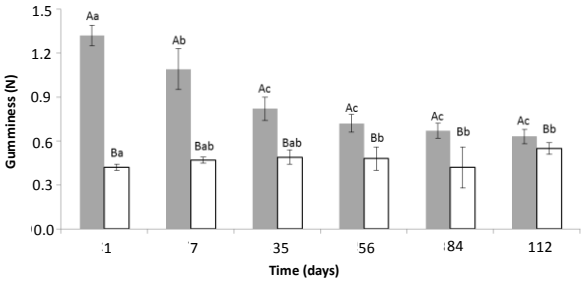


Figure 2. Instrumental texture profiles of mousse formulations: (■) non-synbiotic diet mousse; (□) synbiotic diet mousse. Different uppercase letters indicate statistically significant differences (p<0.05) between the two *diet* mousse formulations for the same storage time. Different lowercase letters indicate statistically significant differences (p<0.05) among different storage times for the same mousse formulation. *Absolute values of adhesiveness in module.

In addition, hardness of PDM was relatively stable along the whole storage period (p>0.05), while that of SDM increased significantly (p<0.05) after 84-days storage. Opposite to what was observed for the diet mousses was reported by Borreani, Llorca, Quiles, and Hernando (2017). According to these researchers, occorred a general decrease in the hardness of formulations of dairy desserts containing skimmed milk powder either enriched with liquid cream. It is noteworthy to take in mind, in this regard, that a relatively constant hardness along storage, like that observed for PDM, is a desirable property in any food, because it suggests that the stored product preserved the main features of the original formulation (Maruyama, Cardarelli, Buriti, & Saad, 2006).

According to Cardarelli, Aragon-Alegro, Alegro, Castro, and Saad (2008), mousse particles take a long time to settle stably at low temperature, owing to the low mobility of air bubbles in the intrinsic structure. It has been suggested that the hardness of inulin-containing foods can be promoted by the ability of this prebiotic to interact with water molecules and milk protein fraction (Gokavi, Zhang, Huang, Zhao, & Guo, 2005), thus forming larger aggregates (Tárrega & Costel, 2006). Moreover, prebiotic incorporation in different products improved their hardness (Oliveira, Perego, Oliveira, & Converti, 2011; Shoaib et al., 2016). Thus, the higher hardness observed in the present study for PDM lacking in inulin compared with SDM may be ascribed to its significantly higher content of powdered skimmed milk (Table 1).

SDM adhesiveness was significantly higher (p<0.05) than that of PDM, probably due to the presence of FOS, which are more hygroscopic than inulin (Tonon et al., 2009; Franck, 2002). For the same reason, while PDM adhesiveness showed a statistically significant decrease at the end of storage, it remained almost unchanged for SDM (p>0.05) (Figure 2). Such a stability in SDM adhesiveness is consistent with the one observed by Buriti, Castro, and Saad (2010b) for a FOS-containing synbiotic guava mousse stored in the same way. On

the contrary, a progressive increase in this parameter was observed either in synbiotic chocolate mousses stored for only 28 days (Cardarelli, Aragon-Alegro, Alegro, Castro, & Saad, 2008) or in different formulations of probiotic dessert after 28 days of storage (Frederico et al., 2016).

Springiness and cohesiveness along storage were always similar in both mousses ($p>0.05$), and their values at the end of storage were significantly lower than at the start ($p<0.05$). These results suggest that neither FOS nor inulin significantly contributed to these properties. It is surprising that the presence of inulin did not significantly influence SDM cohesiveness, taking into account that the addition of this prebiotic to whey protein suspensions (Herceg, Režec, Lelas, Krešić, & Franetović, 2007) and dairy desserts (Lobato, Grossmann, & Benassi, 2009) led to a reduction of this parameter, as a possible consequence of inulin ability to form hydrogen bonds with proteins and to reduce surface tension and stability of these systems.

The well-known aggregating effect not only of inulin but also of FOS is evident in the behaviour of gumminess along storage, which decreased significantly ($p<0.05$) in PDM, whereas increased ($p<0.05$) in SDM that contained both. Finally, the significantly higher gumminess of PDM compared with SDM can be ascribed to its higher content of powdered skimmed milk.

3.5. Sensory analysis

According to Table 4, which summarizes the results of sensory analysis, there was no statistically significant difference ($p>0.05$) between the average scores attributed by consumers to SDM and CSM throughout the whole storage period at low temperature.

Table 4. Mean scores of sensory acceptability (standard deviation) attributed by consumers to non-synbiotic diet mousse (PDM), synbiotic diet mousse (SDM), and control synbiotic mousse (CSM) stored at -18±2 °C for up to 112 days.

Storage (days)	PDM	SDM	CSM
7	5.9 (1.4) ^{A}	6.9 (1.1) ^{B}	7.5 (0.9) ^{B}
35	6.0 (1.5) ^{A}	7.0 (1.4) ^{B}	7.7 (1.0) ^{B}
56	6.4 (1.5) ^{A}	6.8 (1.6) ^{AB}	7.6 (1.3) ^{B}
84	6.5 (1.3) ^{A}	6.7 (1.3) ^{A}	6.8 (1.6) ^{A}
112	5.8 (2.2) ^{A}	6.7 (1.5) ^{AB}	7.5 (0.9) ^{B}

Different uppercase letters indicate statistically significant differences (p<0.05) among the three *diet* mousse formulations for the same storage time.

The low powdered skimmed milk content (4%) and the presence of both probiotic and prebiotics (inulin and FOS) in SDM may have been responsible for its low hardness, low gumminess and high adhesiveness (Figure 2), resulting in a better acceptability (p<0.05) in comparison with PDM (Table 4) during the entire storage period. Some ingredients such as inulin and protein concentrates are often used in the development of milk desserts not only to substitute fat, but also to provide special functional and nutritional properties to products (Bayarri, González-Tomás, Hernando, Lluch, & Costell, 2011; Morais et al., 2016). In this regard, Cardarelli, Aragon-Alegro, Alegro, Castro, and Saad (2008) reported that the simultaneous addition of *L. paracasei* subsp. *paracasei* LBC 82 and inulin directly improved the main sensory characteristics of chocolate mousses, i.e., texture, colour and flavour, when compared to a control lacking in these ingredients.

The satisfatory acceptability of acceptability of both mousses suggests that sucralose may be considered a good substitute for sucrose in dairy products, thus confirming previous observations (Brito & Bolini, 2010). This result is consistent with the observation of very

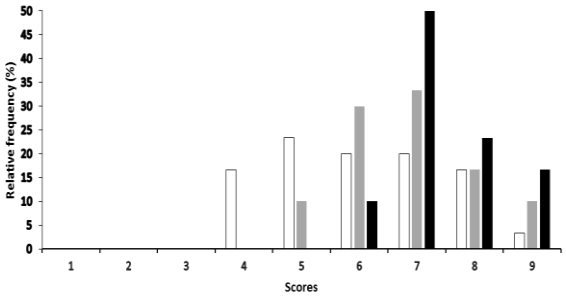
similar characteristics of dairy desserts supplemented with sucralose or other sweeteners such as aspartame, neotame, and stevia (Morais, Lima, Morais, and Bolini, 2015).

The mean scores attributed to CSM, ranging from 6.8 to 7.7, were lower than those reported by Buriti, Castro, and Saad (2010b) for the same guava mousse (7.6 to 8.0) stored exactly in the same way, but having lower contents inulin (2.0%), which suggests some influence of these contents in the taste of the final product.

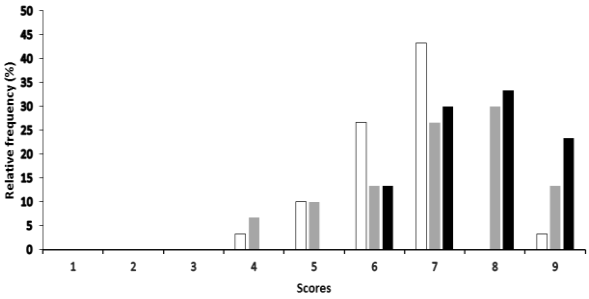
Among the criteria used for assigning scores, texture was the most appreciated attribute among consumers for both PDM and SDM, while flavour was the one that stood out for CSM (results not shown). However, odour was the least rated attribute for the three formulations.

The relative frequencies of scores assigned to mousses after all storage times (7, 35, 56, 84, and 112 days) are illustrated in Figure 3.

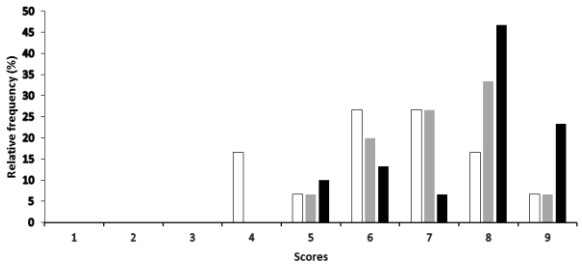
(a) Day 7



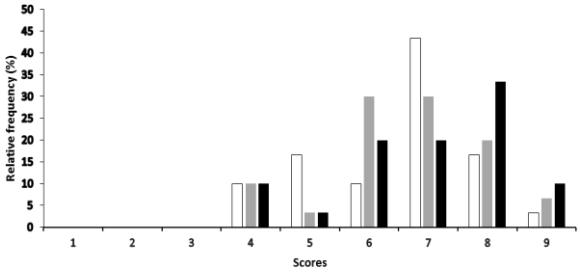
(b) Day 35



(c) Day 56



(d) Day 84



(e) Day 112

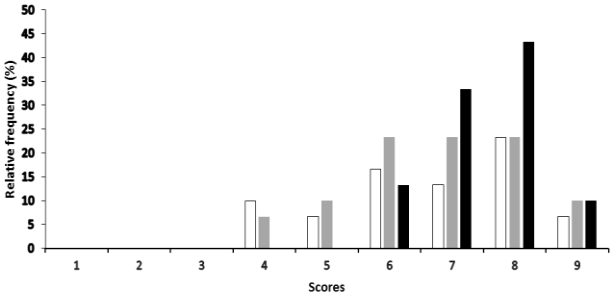


Figure 3. Relative frequencies of scores assigned to mousses in all storage times. 1, dislike extremely; 2, dislike very much; 3, dislike moderately; 4, dislike slightly; 5, neither like or dislike; 6, like slightly; 7, like moderately; 8, like very much; 9, like extremely. (□) Non-synbiotic diet mousse; (▒) Synbiotic diet mousse; (■) Control synbiotic mousse.

PDM received scores between 4 and 9 after all storage times, and the score 7 was the one attribute with the highest frequency (around 45%), whereas scores 4, 5 and 9 those with the lowest one (around 5%). SDM received scores between 5 and 9 after 7 and 56 days of storage, but the score 8 was by far the most frequent one (around 35%) after all storage periods. CSM showed slightly higher scores (between 6 and 9) after 7, 35 and 112 days of storage, and the score 7 was the most frequent one (around 50%), whereas scores 6 and 9 the less frequent ones (both approximately 10%). In general, it can be concluded that CSM was the mousse that had, on average, the best scores throughout the whole storage period, followed by SDM, whereas PDM was the one that consumers liked less.

4. CONCLUSIONS

The present study evaluated the effects of *Lactobacillus acidophilus* La-5, inulin, fructooligosaccharides (FOS), sucralose, and time of storage on pH variation, and instrumental texture profile of a synbiotic diet mousse (SDM), when compared to a non-synbiotic diet mousse (PDM) and to a sucrose-containing control synbiotic mousse (CSM) on sensory acceptance. The dietary symbiotic mousse stored at -18 °C showed a *L. acidophilus* La-5 population greater than 7.5 log CFU/g over a 112 day-long storage. SDM showed lower pH values than PDM. Regarding the instrumental texture, SDM hardness and gumminess increased and cohesiveness decreased throughout storage ($p<0.05$), while adhesiveness and springiness remained almost stable. On the other hand, PDM hardness did not vary significantly, while gumminess, cohesiveness, springiness and adhesiveness gradually decreased along storage. In addition, sensory acceptability of all formulations was satisfactory throughout storage, with average scores from 6.7 to 7.0 for SDM, 5.8 to 6.5 for PDM, and 6.8 to 7.7 for CSM. The low powdered skimmed milk content and the presence of both probiotic and prebiotics (inulin and FOS) in SDM may have been responsible for its better acceptability compared with PDM. These results demonstrate that the presence of *Lactobacillus acidophilus* La-5, sucralose, inulin and FOS, as well as the time of storage, were responsible for significant changes in SDM instrumental texture profile, but not for its sensory acceptability, and that sucralose could be a good sucrose substitute in mousses.

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Chapter 3

Effect of the consumption of a synbiotic diet mousse containing *Lactobacillus acidophilus* La-5 by individuals with metabolic syndrome: a randomized controlled trial

ABSTRACT

This study aimed to evaluate the impact of a synbiotic diet mousse containing *Lactobacillus acidophilus* La-5 and the prebiotics inulin and fructooligosaccharides on the health of volunteers with metabolic syndrome (MetS). In a randomized, double-blind, placebo-controlled trial, forty-five volunteers with MetS were assigned into two groups, each receiving 40 g/day of: synbiotic diet mousse (SDM) (n=23) and placebo diet mousse (PDM) without pro- and prebiotics (n=22). Anthropometric and blood pressure measurements, biochemical, haematological, inflammatory, and immunologic parameters were measured at the beginning and after 8 weeks of intervention. The daily intake of the SDM and the PDM led to significant reductions of total cholesterol and HDL-cholesterol, as well as of immunoglobulins (A and M), and interleukin-1β in both groups (p<0.05). These results suggest that the presence of the probiotic and prebiotic ingredients in the diet mousse did not exert any additional effects on the health of volunteers with MetS.

Keywords: Probiotic; Prebiotic; Clinical trial; Metabolic Syndrome; Mousse.

1. INTRODUCTION

The metabolic syndrome (MetS) has received a great deal of attention from the scientific community in recent years. This is largely influenced by the increase in the prevalence of MetS in the last two decades especially in countries with increased calorie consumption and decreased physical activities (Mazidi et al., 2016). In general, MetS is a group of risk factors comprising obesity (particularly abdominal obesity), insulin resistance, atherogenic dyslipidaemia, and hypertension, which are associated with increased risk of cardiovascular disease (CVD) and type 2 diabetes mellitus (Grundy et al., 2005; Kakafika et al., 2006). Moreover, subjects with these features usually show prothrombotic and proinflammatory states (Grundy et al., 2005). Clinical and epidemiological studies have indicated that low-grade inflammation may contribute to the development of metabolic disorders associated with obesity (Cani & Hul, 2015). In this sense, MetS is known to be a low grade systemic inflammatory condition (Synetos et al., 2016).

It has been shown that an intestinal dysbiosis which could also be associated to MetS. In this context, a number of studies using animal models and clinical trials have reported a relationship between the composition of the intestinal microbiota and MetS risk factors, including obesity and diabetes (Larsen et al., 2010; Ley et al., 2005; Tremaroli & Bäckhed, 2012). In general, the microbiota associated with obese inviduals has been characterized by an increased *Firmicutes*/*Bacteroidetes* ratio (Ley et al., 2006; Jonkers, 2016; Turnbaugh et al., 2009). Nevertheless, according to Scavuzzi et al. (2015), there is still no consensus as to the mechanisms relating intestinal microbiota modifications and the potential metabolic changes. On the other hand, these researchers reported that mechanisms possibly involve gut barrier alterations and low-grade inflammation.

The MetS may have several origins; however, diet and lifestyle are considered important aspects that may influence the susceptibility of humans to MetS (Kovatcheva-Datchary & Arora, 2013). Since, dietary approaches to manipulate the intestinal microbiota, in particular the use of probiotic microorganisms and/or prebiotic compounds, have demonstrated health-improving effects on the host. These approaches were proposed for MetS management (Bernini et al. 2016; Kovatcheva-Datchary & Arora, 2013; Scavuzzi et al., 2015). Nonetheless, it is noteworthy that studies evaluating the impact of probiotics on obesity-related inflammation are limited and especially based on animal studies (de Moreno de LeBlanc & Perdigon, 2010; Gøbel et al., 2012).

Some researchers reported beneficial effects of the consumption of probiotic, prebiotic, and synbiotic products on parameters related to MetS (Barreto et al., 2014; Bernini et al., 2016; Gøbel et al., 2012). Akkasheh et al. (2016) observed significant decreases in serum insulin concentrations and the homoeostasis model of assessment of insulin resistance (HOMA-IR) after the daily consumption of one probiotic capsule containing *Lactobacillus acidophilus* YAB, *Lactobacillus casei* TD_2, and *Bifidobacterium bifidum* B12 during 8 weeks. Studies have also investigated the possible role of probiotic bacteria and prebiotic fibres on different risk factors of MetS, such as the reduction of cardiovascular disease (CVD) risk (Al-Sheraji et al., 2012; Gøbel et al., 2012). Along these lines, a meta-analysis of randomized controlled trials conducted by Guo et al. (2011) showed that the consumption of probiotics led to a decrease in the total cholesterol and the LDL-C in individuals with high, borderline high, and normal cholesterol levels.

Recently, inflammatory processes have also been considered as biomarkers in clinical trials with MetS patients (Brito-Luna et al., 2016; Karaman et al., 2015; Panahi et al., 2016). Barreto et al. (2014) observed that the consumption of fermented milk containing *Lactobacillus plantarum* Lp 115 led to a significant decrease in IL-6 levels in patients with MetS after 90 days of study.

It is noteworthy that probiotic beneficial effects, as well as mechanisms of action, are considered as strain specific. In addition, it has been suggested that different food formats in which the probiotic bacteria are incorporated may influence their functionality, and, consequently, their potential health effects (Forssten, Sindelar, & Ouwehand, 2011; Sanders & Marco, 2010). Besides, there are indications that synbiotic products may be more effective than either probiotics or prebiotics alone (Sanders & Marco, 2010).

To the best of our knowledge, no study is available in the scientific literature on the impact of a synbiotic diet dessert on subjects with MetS. The aim of this study was therefore to assess the impact of a synbiotic diet dessert (mousse) containing *L. acidophilus* La-5 and the prebiotic ingredients inulin and fructooligosaccharides (FOS) on biochemical (plasmatic glucose, TC, HDL-C, LDL-C, TG, and insulin), inflammatory (TNF-α, CD40, IL-1β, IL-6, IL-8, IL-10, and IL-12), haematological (erythrocytes, leukocytes, lymphocytes, erythrocytes, neutrophils, eosinophils, monocytes, and haemoglobin), and immunological (IgA, IgE, IgG, and IgM) parameters of volunteers with MetS.

2. SUBJECTS AND METHODS

2.1. Production of synbiotic and placebo diet mousses

The diet desserts were produced under suitable hygiene and sanitation criteria at the Laboratory of Food Technology of the Department of Biochemical and Pharmaceutical Technology of the School of Pharmaceutical Sciences of the University of São Paulo (SP, Brazil), according to the method described by Buriti, Castro, and Saad (2010). The amounts of ingredients employed in the production of the diet desserts shown in Table 1, while Table 2 lists their macronutrient composition and energy contribution.

Table 1. Amounts of ingredients employed in the production of synbiotic diet mousse (SDM) and placebo diet mousse (PDM).

Ingredients (g/100 g)	**SDM**	**PDM**
Skimmed milk[1]	61.7	61.7
Skimmed milk powder[2]	4.0	14.0
Sucralose[3]	1.1	1.1
Pasteurized and frozen guava pulp[4]	20.0	20.0
Emulsifier/stabilizer[5]	2.8	2.8
FOS[6]	6.0	0.0
Inulin[7]	4.0	0.0
Lactic acid[8]	0.4	0.4
Lactobacillus acidophilus La-5[9]	0.05	0.0
Total	100.0	100.0

[1]Paulista (Danone, Guaratinguetá, SP, Brazil); [2]Molico (Nestlé, Araçatuba, SP, Brazil); [3]Sucralose (Línea Sucralose, São Paulo, SP, Brazil); [4]Icefruit Comércio de Alimentos (Icefruit Comércio de Alimentos, Tatuí, SP, Brazil); [5]Cremodan Mousse 30 (Danisco, Cotia, SP, Brazil); [6]Beneo P95 (Orafti, Oreye, Belgium); [7]Beneo HP (Orafti); [8]Purac (Purac Sínteses, Rio de Janeiro, RJ, Brazil; 85 g/100 g food-grade solution); [9]Strain La-5 (Christian Hansen, Hoersholm, Denmark).

Table 2. Chemical composition, energy contribution of macronutrients, and total energy values (TEV) of synbiotic diet mousse (SDM) and placebo diet mousse (PDM) in 100 g of whole mousses (dry weight).

	SDM	**PDM**
Composition (g/ 100g)		
Ash	0.90 (0.06) ^{B}	1.42 (0.17) ^{A}
Proteins	6.77 (0.37) ^{B}	8.55 (0.33) ^{A}
Simple carbohydrates	10.24 (0.97) ^{B}	17.53 (1.01) ^{A}
Fructans	9.63 ^{B,*}	0.00 ^{A}
Lipids	0.22 (0.05) ^{A}	0.12 (0.06) ^{A}
Moisture	72.24 (1.59) ^{A}	72.38 (1.84) ^{A}
Total	100.0	100.0
Energetic value (kJ/ 100 g)		
Proteins	113.30 (6.67)	143.09 (5.52)
Lipids	8.28 (2.07)	4.52 (2.26)
Simple carbohydrates	171.38 (31.07)	293.24 (16.90)
Fructans	60.46	0.00
TEV	353.42 (27.91)	440.85 (11.42)

Values are expressed as mean (standard deviation). Different uppercase letters in the same line indicate statistically significant differences (p<0.05) between the two diet formulations. *Estimate based on information given by the supplier (Orafti) for the prebiotic ingredients (Beneo P95 and Beneo HP).

Diet desserts were packaged in polypropylene plastic pots for food products (100 mL of capacity) (Tries Aditivos Plásticos, São Paulo, Brazil) in portions of 40 g. The pots were sealed with metallic covers with varnish in a sealer (Delgo Metalúrgica, Cotia, Brazil). The products were stored frozen (-18 °C) and delivered to each volunteer in plastic vials labelled

with the dates of manufacture and expiration. Microbiological analyses of the synbiotic product showed that the average population of *L. acidophilus* La-5 ranged between 9.2 and 9.5 log CFU (colony-forming units) per daily serving portion (40 g) during the experimental period. Therefore, the probiotic population was above the minimum recommended level (6 log CFU/g) suggested for beneficial health effects (Health Canada, 2009; Champagne et al., 2011; Ministero della Salute, 2013). Coliforms, *Escherichia coli*, and yeasts and moulds were not detected during the products' storage period.

2.2. Participants

Sixty subjects with MetS, aged between 19 and 65, were recruited (August 2014 up to June 2015) from the ambulatory of the University Hospital (São Paulo, SP, Brazil). The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and approved by the Research Ethical Committees involving humans of the School of Pharmaceutical Sciences of the University of São Paulo (CAAE 30539214.6.0000.0067) and of the University Hospital (Protocol Number 663.138). All subjects provided written consent form before participating in the study. According to the National Cholesterol Education Program, Adult Treatment Panel III (NCEP/ATP III) (Grundy et al., 2005), the subjects were eligible for the study if they had at least three of the following five factors: 1) abdominal obesity (waist circumference of ≥ 88 cm for women and ≥ 102 cm for men); 2) high TG levels (≥150 mg/dL); 3) low HDL-C levels (<50 mg/dL for women and <40 mg/dL for men); 4) high blood pressure (systolic ≥ 130 mmHg and diastolic ≥ 85 mmHg); 5) high fasting glucose levels (≥100 mg/dL). The exclusion criteria were thyroid, renal, hepatic, gastrointestinal or oncological disease and use of drugs (including hormone replace therapy) that interfere with the lipids and/or glycaemic profile.

2.3. Study design

The present study was a randomized, double-blind, placebo-controlled trial in which subjects with MetS were randomly divided into two groups: group S (synbiotic group - individuals who consumed 40 g/d of SDM; n=23) and group P (placebo group - individuals who consumed 40 g/d of PDM; n=22). The participants were paired by age, gender, ethnicity, and consumption of antihypertensive drugs (Simão et al., 2013). Throughout the study (8 weeks), the subjects were encouraged to maintain their lives as they normally would, with no change in their usual diets or physical activity. However, the volunteers were instructed to avoid the consumption of probiotic and prebiotic products during the 7 days that preceded the beginning of the intervention (run-in).

2.4. Anthropometric, heart rate, and laboratory blood analysis

Fasting blood samples, anthropometric, heart rate, and blood pressure measurements were collected at baseline (T0) and at the end of week 8 (T8). Body mass index (kg/m^2) was calculated as body weight (kg) divided by squared height (m). Waist circumference was determined using a tape measure. After the subjects had been seated for five minutes, three blood pressure measurements, obtained at one-minute intervals, were recorded. The average of the last two measurements was used. These clinical and anthropometric parameters were measured according to Mill et al. (2013).

After fasting for 12 h, blood samples were drawn from the forearm vein into Vacutainer tubes (Becton Dickinson, Rutherford, USA). Samples were immediately centrifuged at 3000 rpm for 15 min at 4 °C (Eppendoff, Hamburg, Germany), and the serum was collected and stored at -80 °C until the analysis. The plasmatic glucose levels and the serum levels of TC, HDL-C, LDL-C, TG, IgA, IgE, IgG, and IgM were assayed by an

automated biochemical analyser (Labmax 240, Tokyo, Japan), using specific enzyme kits (Labtest Diagnostics, Lagoa Santa, GO, Brazil). Plasma insulin level was determined by chemiluminescence microparticle immunoassay (Architect, Abbott Laboratory, IL, USA). TNF-α, CD40, IL-1β, IL-6, IL-8, IL-10, and IL-12 were evaluated using commercially available immunoassay kit #HCYTOMAG-60K (Millipore, Billerica, MA, USA) with a series of magnetic beads and the MAGPIX system (Luminex, Austin, TX, USA). Haematological parameters were evaluated at the University Hospital Clinical Laboratory of the University of São Paulo through an automated haematology analyser (Sysmex-XT 2000i, Kobe, Japan), using routine analysis based on electrical impedance (erythrocytes), flow cytometry (leukocytes, lymphocytes, erythrocytes, neutrophils, eosinophils, and monocytes) and colorimetric (haemoglobin) methods.

2.5. Statistical analysis

The chi-squared test was used to evaluate the differences between synbiotic and placebo groups with respect to the gender, ethnicity, consumption of antihypertensive drugs, and student-t test to age. The Mann-Whitney test was performed to compare differences among parameters of groups at baseline and differences across treatment groups (intergroup changes). The Wilcoxon matched pairs test was performed to verify changes from baseline (intragroup changes). Data were presented as median (25%-75%), and the significance was declared when the p-value was <0.05. Statistical analyses were carried out using the Statistica version 12.0 (Statsoft Inc, Tulsa, USA) and GraphPad Prism version 3.0 (GraphPad Software Inc, La Jolla, USA) programs.

3. RESULTS AND DISCUSSION

The present study evaluated the impact of a synbiotic diet mousse containing *L. acidophilus* La-5 and the prebiotic ingredients inulin and FOS on some biochemical, haematological, inflammatory, and immunological parameters of subjects with MetS through a randomized, double-blind, and placebo-controlled trial.

In general, the two experimental groups were similar (Table 3) since there were no significant differences between the synbiotic and the placebo groups related to age, gender, ethnicity, and the consumption of antihypertensive drugs at baseline ($p>0.05$).

Table 3. General characteristics of the participants of the placebo and synbiotic groups at the beginning of the study.

Parameters	Group P (n=22)	Group S (n=23)	*p*
Gender (M/F)	(10/12)	(13/10)	0.4578
Antihypertensive (yes/no)	(6/16)	(5/18)	0.6659
Non-Caucasian/Caucasian	(11/11)	(10/13)	0.6611
Age (years)	49.5 (39.5-59.5)	47.0 (41.0-53.0)	0.4674

Values are expressed as median (25%-75%). Group P: Placebo group; Group S: Synbiotic group. M/F: male/female.

During the period of daily diet mousse consumption (from T0 to T8), there were no significant differences for anthropometric and haematological parameters, systolic and diastolic blood pressure levels, heart rate, glucose, TG, LDL-C, TC/HDL-C and LDL-C/HDL-C ratios, insulin, TNF-α, CD40, IL-8, IL-10, IL-12, and IgG (Tables 4, 5, and 6) for both groups. The exceptions were verified for haemoglobin (Hb) levels (p=0.0356), IgE (p=0.0451), and IL-6 (p=0.0396), since the placebo group showed a decrease in these parameters after 8 weeks of the study (Table 5 and 6). In relation to the other parameters, there were significant reductions in TC, HDL-C, IgA, IgM, and IL-1β for both groups

throughout the experimental period (Table 4 and 6). Comparing the median differences (T8 to T0) between the two groups, the trend for LDL-C decrease was higher in group S (15.0 mg/dL) (p=0.0606) than in group P (2.5 mg/dL) (p=0.1292). Nevertheless, regarding inter-group changes, no significant differences were verified for all parameters at baseline and after 8 weeks of diet dessert consumption (p>0.05).

Table 4. Clinical and biochemical parameters at baseline (T0) and after 8 weeks (T8) of mousse consumption.

Parameters	Groups	T0	T8	*p*
Weight (kg)	P	87.8 (61.9-96.5)	88.1 (78.1-97.3)	0.6158
	S	84.6 (59.9-99.3)	84.0 (72.2-100.5)	0.5392
BMI (kg/m^2)	P	33.9 (29.1-37.1)	33.9 (29.0-37.4)	0.5536
	S	30.7 (28.0-33.4)	30.9 (28.6-33.1)	0.3764
WC (cm)	P	101.9 (94.5-112.8)	103.0 (96.0-113.8)	0.8552
	S	99.0 (91.5-112.2)	97.5 (91.0-109.5)	0.6671
SBP (mm Hg)	P	130.0 (115.5-139.5)	127.0 (115.0-137.5)	0.9759
	S	133.0 (127.0-142.0)	131.0 (127.0-142.0)	0.6089
DBP (mm Hg)	P	81.5 (74.0-89.0)	80.0 (74.0-87.0)	0.3543
	S	82.0 (73.0-91.0)	82.0 (77.0-89.0)	0.7779
Heart rate (bmp)	P	77.0 (65.8-86.2)	76.0 (65.5-85.3)	0.6017
	S	71.0 (66.0-78.0)	70.0 (66.0-76.0)	0.4885
Glucose (mg/dL)	P	94.5 (89.0-99.0)	91.5 (79.0-96.0)	0.1469
	S	95.0 (88.0-99.0)	95.0 (83.0-101.0)	0.2592
TG (mg/dL)	P	127.0 (93.5-163.0)	120.0 (75.5-168.0)	0.5645
	S	123.0 (89.0-159.0)	131.0 (102.0-194.0)	0.2511
TC (mg/dL)	P	183.5 (149.5-211.5)	160.0 (133.0-209.5)	0.0023
	S	203.0 (194.0-229.0)	189.0 (168.0-206.0)	0.0064
LDL-C (mg/dL)	P	109.0 (87.5-133.0)	106.5 (74.0-137.5)	0.1292
	S	123.0 (103.0-137.0)	108.0 (103.0-118.0)	0.0606
HDL-C (mg/dL)	P	46.0 (37.0-51.0)	43.0 (35.5-48.5)	0.0054
	S	48.0 (38.0-60.0)	44.0 (38.0-51.0)	0.0050
TC/HDL-C	P	4.0 (3.5-5.2)	4.1 (13.4-4.6)	0.0785
	S	4.2 (4.0-5.1)	4.3 (3.7-4.9)	0.4702
LDL-C/HDL-C	P	2.4 (2.2-3.0)	2.5 (2.1-2.7)	0.6321
	S	2.5 (2.3-3.1)	2.6 (2.1-2.9)	0.8137
Insulin (µU/mL)	P	12.3 (8.5-17.5)	11.9 (8.4-16.7)	0.7086
	S	13.3 (8.7-18.4)	12.3 (9.1-16.2)	0.0830

Values are expressed as median (25%-75%). Wilcox matched pairs test was performed to verify changes from the baseline (intragroup changes). Mann-Whitney was performed to compare differences at baselines and across treatments groups (intergroup changes). The differences were significant for $p<0.05$ and $p<0.01$. No differences between groups were found. BMI: body mass index; WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; TG: triglycerides; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TC/HDL-C: total cholesterol to HDL-cholesterol ratio; LDL-C/HDL-C: LDL-cholesterol to HDL-cholesterol ratio; IgA: immunoglobulin A; IgM: immunoglobulin M. Group P: individuals who consumed the placebo product (n=22); Group S: individuals who consumed the synbiotic diet mousse (n=23). T0: baseline; T8: 8 weeks of daily consumption of diet desserts.

Table 5. Haematological parameters at baseline (T0) and after 8 weeks (T8) of mousse consumption.

Parameters	**Groups**	**T0**	**T8**	***p***
Haemoglobin (g/dL)	P	14.5 (13.1-15.3)	13.9 (13.0-15.1)	0.0356
	S	14.6 (13.3-15.6)	14.7 (13.2-15.7)	0.8771
Leukocytes (mm^3)	P	7160.0 (6025.0-9275.0)	7060.0 (5910.0-9875.0)	0.6083
	S	6960.0 (5730.0-7570.0)	6830.0 (6270.0-7970.0)	0.3989
Limphocytes (mm^3)	P	2124.0 (1960.0-2511.0)	2148.0 (1775.0-2598.0)	0.9935
	S	2241.0 (1869.0-2760.0)	2351.0 (1871.0-2871.0)	0.0528
Erytrocytes (mm^3)	P	$5.02x10^6$ ($4.59x10^6$-$5.38x10^6$)	$4.98x10^6$ ($4.41x10^6$-$5.31x10^6$)	0.2392
	S	$4.93x10^6$ ($4.70x10^6$-$5.29x10^6$)	$5.08x10^6$ ($4.75x10^6$-$5.34x10^6$)	0.4479
Neutrophils(mm^3)	P	4429 (3087-5652)	4176 (3209-5990)	0.7640
	S	3783 (2854-4413)	3953 (2700-4655)	0.5894
Eosinophils (mm^3)	P	190.0 (120.5-255.5)	194.0 (137.5-294.5)	0.1605
	S	160.0 (100.0-314.0)	162.0 (120.0-277.0)	0.9454
Monocytes (mm^3)	P	549.5 (446.0-679.9)	531.5 (469.0-642.0)	0.7029
	S	592.0 (500.0-709.0)	577.0 (542.0-700.0)	0.7553

Values are expressed as median (25%-75%). Wilcox matched pairs test was performed to verify changes from baseline (intragroup changes). Mann-Whitney was performed to compare differences at baselines and across treatments groups (intergroup changes). The differences were significant for $p < 0.05$ and $p<0.01$. No differences between groups were found. Group P: individuals who consumed the placebo product (n=22); Group S: individuals who consumed the synbiotic diet mousse (n=23). T0: baseline; T8: 8 weeks of daily consumption of diet desserts.

Table 6. Inflammatory parameters and antibodies at baseline (T0) and after 8 weeks (T8) of mousse consumption.

Parameters	Groups	T0	T8	*p*
IL-10 (pg/mL)	P	1.9 (1.9-1.9)	0.1 (0.0-6.5)	0.1680
	S	1.9 (1.9-4.9)	0.7 (0.1-2.1)	0.2783
IL-12 (pg/mL)	P	0.3 (0.30-108.9)	0.0 (0.0-27.2)	0.2035
	S	0.3 (0.30-108.9)	0.0 (0.0-27.2)	0.2035
CD40 (pg/mL)	P	6210 (1890-12100)	2690 (2220-26200)	0.9645
	S	4540 (3525-11450)	2690 (2280-6060)	0.1677
IL-1β (pg/mL)	P	2.8 (2.9-7.6)	1.1 (0.5-2.1)	0.0137
	S	2.8 (2.9-2.9)	1.5 (0.8-2.3)	0.0360
IL-6 (pg/mL)	P	1.9 (1.9-1.9)	0.00 (0.0-0.0)	0.0396
	S	1.9 (1.9-1.9)	0.00 (0.0-1.2)	0.1274
IL-8 (pg/mL)	P	15.3 (7.0-20.9)	15.6 (6.5-18.6)	0.5195
	S	22.1 (16.3-47.2)	18.6 (16.2-24.0)	0.2163
TNF-α (pg/mL)	P	29.2 (22.8-44.8)	29.3 (27.1-37.6)	0.5195
	S	30.6 (23.1-37.4)	34.0 (29.3-45.2)	0.3757
IgA (mg/dL)	P	201.5 (145.0-249.0)	149.5 (126.5-214.0)	0.0001
	S	186.0 (136.0-292.0)	183.0 (116.0-267.0)	0.0410
IgE (UI/mL)	P	71.5 (25.6-169.9)	67.2 (32.6-232.5)	0.0451
	S	65.9 (27.15-354.5)	75.5 (25.0-319.4)	0.4505
IgG (mg/dL)	P	1142.0 (949.5-1329.0)	1012.0 (787.5-1286.0)	0.0841
	S	1128.0 (957.0-1222.0)	1073.0 (985.0-1208.0)	0.2512
IgM (mg/dL)	P	53.0 (42.5-97.5)	36.0 (14.0-80.0)	0.0028
	S	61.9 (38.0-101.5)	41.0 (19.5-79.5)	0.0003

Values are expressed as median (25%-75%). Wilcox matched pairs test was performed to verify changes from baseline (intragroup changes). Mann-Whitney was performed to compare differences at baselines and across treatments groups (intergroup changes). The differences were significant for $p<0.05$ and $p<0.01$. No differences between groups were found. IL-10: interleukin 10; IL-12: interleukin 12; CD40: cluster of differentiation 40; IL-1β: interleukin 1β; IL-6: interleukin 6; IL-8: interleukin 8; TNF-α: tumor necrosis factor alpha; IgA: immunoglobulin A; IgE: immunoglobulin E; IgG: immunoglobulin G; IgM: immunoglobulin M. Group P: individuals who consumed the placebo product (n=22); Group S: individuals who consumed the synbiotic diet mousse (n=23). T0: baseline; T8: 8 weeks of daily consumption of diet desserts.

We did not find any effect of the intervention with SDM on blood pressure, heart rate, anthropometric, and various laboratory blood parameters (TG, LDL-C, TC/HDL, insulin, glucose, TNF-α, CD40, IL-8, IL-10, IL-12, and IgG) assessed in the present study. Similarly, Bernini et al. (2016) verified that the daily ingestion of fermented milk containing

Bifidobacterium animalis ssp. lactis HN019 by patients with MetS did not cause any significant changes in blood pressure, glucose, WC, TG, HDL-C, insulin, and HOMA-IR. Nevertheless, the authors observed a significant reduction in BMI, TC, and LDL-C in the probiotic group compared to the baseline and the control group values.

On the other hand, in our study a significant reduction of TC and HDL-C levels was observed for both groups after 8 weeks of intervention. It is noteworthy that the TC decrease was higher in the placebo group compared to the synbiotic group (reductions of 23.5 and 14.0 mg/dL, respectively). A possible explanation for this result may be related to the differences found in the food matrix of the PDM and the SDM, in that former product presented 14.0% of skimmed milk powder (non-fat solids source), while the SDM only 4.0% (Table 1).

In this sense, during the intervention period of 8 weeks, the placebo and the synbiotic groups ingested, respectively, 0.14 and 0.04 g/day of skimmed milk powder. Some evidence suggests a possible interaction of the dietary calcium with fatty acids through the formation of chelates during the process of lipids digestion, resulting in a reduction of the absorption of some fatty acids by the precipitation and excretion of the salts formed in faeces (Cominetti, Marreiro, & Cozzolino, 2012; Jolma et al., 2003; Vaskonen et al., 2002). Thus, the highest content of calcium in PDM compared to SDM might have exerted a greater influence on the lipid profile of volunteers. Indeed, Barreto et al. (2014) attributed the reduction of cholesterol levels in the placebo group after 90 days of intervention with unfermented milk (not containing probiotic and prebiotic) to calcium and magnesium.

Although the LDL-C reduction was not statistically significant, there was a higher tendency ($p=0.0606$) of reduction of this parameter in the group S (around 12%) when compared to group P (around 2%). Several hypotheses have been proposed to explain the potential cholesterol-lowering effects of probiotics and/or prebiotics including the production of short chain fatty acids resulting from fermentation of prebiotics, incorporation of

cholesterol into the cell membrane, dissociation of bile salts by specific hydrolases, and conversion of cholesterol into coprostanol by desconjugated bile (Ishimwe et al., 2015).

It is important to point out that the findings of different studies on probiotic and prebiotic hypocholesterolemic effect are still controverse. For instance, studies developed by Ahn et al. (2015) and Jung et al. (2015) did not show any effect of the probiotic strains *Lactobacillus curvatus* HY7601 and *L. plantarum* KY1032 on TC, HDL-C, and LDL-C levels of subjects with triglyceridemia (without diabetes) and overweight, respectively. However, a meta-analysis of randomized controlled trials revealed that the consumption of probiotics has positive health effects on TC and LDL-C in volunteers with high, borderline high and normal cholesterol levels (Guo et al., 2011).

Regarding the haematological parameters, we observed a significant reduction ($p<0.05$) in the Hb level only in the placebo group after 8 weeks of study. However, no significant difference was observed between the groups at the end of the intervention period. Studies suggest a relationship between Hb levels and the risk of developing MetS (Chuang et al., 2016; Hu, Kuo, & Wu, 2016). It is important to emphasize that hypertrophy and hyperplasia are characteristic features of obesity and lead to a reduction in the blood supply to adipocytes, due to the adipocytes enlargement. This reduction causes a tissue hypoxia, leading to metabolic changes, besides stimulating erythropoietin production and Hb synthesis (Chuang et al., 2016). In this sense, the Hb levels may be correlated to MetS and used to predict this syndrome in subjects (Chuang et al., 2016). Past studies showed that among various CVD risk factors associated with Hb levels are white blood cell counts, cigarette smoking, diastolic blood pressure, and serum albumin (Shimakawa & Bild, 1993).

Regarding proinflammatory cytokines, the present study showed that PDM as well as SDM consumption led to a reduction in IL-1β after 8 weeks. On the other hand, a significant decrease in the IL-6 levels was found only in the placebo group. Nevertheless,

there were no significant differences between the experimental groups after 8 weeks of intervention. Further studies are necessary to investigate the role of each mousse ingredient on the inflammatory parameters of MetS subjects. However, it is noteworthy that lactic acid bacteria, including probiotic strains, differ in their immunomodulatory properties regarding their differences in their cytokine profile and regulatory T cell responses (Ashraf et al., 2014).

Studies have suggested a great potential for immune and inflammatory response associated with daily supplementation with lactobacilli strains (Akoğlu et al., 2015; Matsusaki et al., 2016; Štofilová et al., 2016). Nevertheless, a double-blind, randomized, placebo-controlled trial conducted by Tonucci et al. (2017) showed that the intake of fermented goat milk containing *L. acidophilus* La-5 and *Bifidobacterium animalis* Bb-12 did not influence the IL-6 levels in subjects with type 2 diabetes mellitus after 6 weeks of intervention.

Additionally, an immunostimulatory effect of the bioactive peptides resulting from the digestive process of milk proteins was suggested by Solieri, Rutella, and Tagliazucchi (2015), since milk proteins, particularly casein, are precursors of biologically active peptides. Besides, these researchers reported that bioactive peptides can be released from milk proteins by gastrointestinal digestion or by enzymatic hydrolysis during food processing and fermentation. Moreover, they may exert several beneficial properties, including the fact that immunomodulation, and immunomodulatory peptides can increase immune cell functions, such as lymphocyte proliferation, natural killer cell activity, antibody synthesis, and cytokine regulation (Singh, Vij, & Hati, 2014). In this context, the effect of the diet mousses studied, in particular the placebo product, on the reduction of the IL-1β and IL-6 levels might be related to an increased formation of bioactive peptides in the products. As mentioned before, the amount of milk proteins in the placebo product was higher compared to the synbiotic product. Bioactive peptides may inhibit inflammatory biomarkers such as IL-1β, cyclooxygenase-2, and TNF-α mRNA expression (Ma et al., 2016).

Regarding the immunoglobulins, we observed a significant reduction in IgM and IgA levels for both experimental groups studied. According to Gonzalez-Quintela et al. (2007), high levels of IgA may represent an important immunological marker for the prevalence of obesity and MetS. In addition, these researchers reported an association between serum levels of IL-6 and IgA between people with this profile of metabolic abnormalities. In the present study, IL-6 and IgA levels showed a significant reduction ($p<0.05$) in the placebo group.

According to Song et al. (2014), IgM is reactive to several autoantigens and is implied to be important for autoimmunity, suggesting that this immunoglobulin may be a potential risk factor for MetS. In this context, these researchers designed a cross-sectional study with around one thousand subjects to evaluate the relationship between IgM and MetS. The results showed that IgM may be a useful predictive factor for MetS in an adult population. Although further studies are required to explain the exact mechanisms of IgM in MetS, we could observe a significant reduction in IgM levels in both experimental groups after 8 weeks of study.

In the present study, a significant decrease in IgE levels was only verified in the group that received the placebo product. Evidence suggests that high levels of IgE in plasma or tissues contribute to the activation of mast cells in the extracellular environment involved in the inflammation process and immunity (Madjene et al, 2015; Wang et al., 2013). Studies suggest the association between high levels of IgE and mast cells, which are important biomarkers for the development of type 2 diabetes mellitus in humans (Wang et al., 2011; Wang et al., 2017). Besides, Zhang and Shi (2012) related the mast cell presence to other diseases associated with MetS, including obesity, insulin resistance, hypertension, and dyslipidemia.

Some limitations of the present study, including the duration of the intervention and sample size, need to be considered to analyse the results obtained. Long-term interventions using a larger number of volunteers would be required to confirm the effects of the synbiotic product on anthropometric, biochemical, haematological, inflammatory, and immunological parameters. Moreover, we believe that the aggressive conditions of the gastrointestinal tract (GIT) (digestive agents present in the gastric and pancreatic secretions, as well as other physiological factors) may have affected the *L. acidophilus* La-5 survival and functionality, which could explain why the results of the synbiotic group were not so expressive in the present study. Buriti, Castro, and Saad (2010) demonstrated the low tolerance of *L. acidophilus* La-5, incorporated in a mousse similar to the one applied in the present study, to artificial gastrointestinal juice in an assay that simulated the GIT conditions. The survival of this probiotic strain was drastically reduced after 6 h of the in vitro assay. On the other hand, in a study conducted by our research group, we tested a synbiotic mousse containing *L. acidophilus* La-5 microencapsulated with inulin, where the probiotic strain showed a high survival rate (around 82%) when submitted to simulated gastrointestinal conditions, suggesting that the microencapsulation may be an alternative to increase the strain survival and potential health effects (unpublished data). This technology enables the development of more stable probiotic products, preserving the viability of microorganisms throughout processing, distribution, storage, and especially during the passage through the gastrointestinal tract (Amine et al., 2014). Nevertheless, further studies will be required to verify whether the mousse incorporated with microencapsulated *L. acidophilus* La-5 could have a greater influence on risk factors related to MetS. In brief, our results suggest that the presence of probiotic and prebiotic ingredients in the diet mousse did not significantly affect the parameters evaluated after 8 weeks of intervention in the volunteers with MetS.

4. CONCLUSION

The observations here reported suggest that daily consumption of either the synbiotic mousse or the placebo product contributed to the reduction of TC, HDL-C, IL-1ß, IgA, and IgM in the volunteers with MetS. Therefore, these results suggest that the presence of probiotic and prebiotic ingredients in the diet mousse did not significantly influence the risk factors related to MetS. Further clinical studies are necessary to support the results here reported, such as a long-term experimental protocol and the inclusion of an evaluation of the intestinal microbiota to determine whether the synbiotic dessert might cause specific changes in the composition and/or activity of the intestinal microbiota of subjects with MetS.

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Chapter 4

Microencapsulation of *Lactobacillus acidophilus* La-5 using inulin as coating agent by spray drying and its survival under in vitro simulated gastrointestinal conditions

ABSTRACT

The objective of this study was the optimization of the spray drying process for the microencapsulation of *Lactobacillus acidophilus* La-5, using inulin as coating agent to increase its gastrointestinal survival. Microencapsulation process conditions were optimized at 80 mL/min, 82% and 10%, for feed flow, aspiration rate and inulin concentration, respectively. Subsequently, a synbiotic diet mousse (SDM) was produced with the addition both of the free and of the microencapsulated probiotic strain, and its *in vitro* gastrointestinal resistance was evaluated. The microencapsulated probiotic strain incorporated in mousse survived significantly better during the gastric phase: 5.68 log CFU/g (83.3%), the enteral phase I: 5.61 log CFU/g (82.3%), the enteral phase II: 5.56 log CFU/g (81.4%), in relation the other samples evaluated ($p<0.05$). Overall, these results confirm the appropriateness of the spray drying process to encapsulate the probiotic strain evaluated using inulin as coating agent and providing resistance to the microencapsulated microorganism.

Keywords: Probiotic; Prebiotic; Atomization; Gastrointestinal tolerance; Mousse.

1. INTRODUCTION

Microencapsulation of probiotic bacteria leads to cell protection against unfavorable conditions in the food matrix, as well as along the gastrointestinal tract (Würth et al., 2015). Microencapsulation technologies enable development of more stable probiotic products with the preservation of the viability of the microorganisms during processing, distribution, storage, and especially during the digestive process (Amine et al., 2014).

The microencapsulation technique through spray drying consists of forming a suspension containing microorganism and coating agents. Next, this suspension is nebulized with hot air or nitrogen (Encina et al., 2016; Venil et al., 2016). This process is convenient in terms of energy requirements, operating costs, and leads to high process yield and is often used for probiotic encapsulation (Pinto et al., 2015). In general, the prolonged viability of dehydrated probiotic culture after this process makes it a good investment for food industries (Peighambardoust et al., 2011).

Moreover, it is essential that the capsule is able to provide good protection against hydrochloric acid, which leads to damage of the probiotic cells (Cook et al., 2012; Pinto et al., 2015). The coating agent should not present cytotoxicity and anti-microbial activities, which would otherwise compromise the viability of the probiotic culture (Cook et al., 2012). Studies indicate that some encapsulating polymers such as gum arabic (Rajabi et al., 2015), gelatin (Gomez-Mascaraque et al., 2016b), pectin (Tamm et al., 2016), and inulin (Fritzen-Freire et al., 2012; Silva et al., 2016; Zamora-Vega et al., 2013) present a great potential as coating material. In fact, these materials promote conditions suitable for the microorganisms' survival, increasing their stability during the storage period after the spray drying process (Salar-Behzadi et al., 2013).

Interaction between microorganisms and the polymer is an important factor during the choice of the coating agent (Anekella & Orsat, 2013). In addition, the microencapsulated probiotic ought to maintain viability in the product and its release in the gut has to occur in a controlled manner (Corona-Hernandez et al., 2013). Therefore, the objective of this study was the optimization of the spray drying conditions for the microencapsulation of *L. acidophilus* La-5, using inulin as coating agent. Survival of the microencapsulated microorganism incorporated in a synbiotic mousse to *in vitro* simulated gastrointestinal conditions was evaluated.

2. MATERIAL AND METHODS

2.1. Microencapsulation of *Lactobacillus acidophilus* La-5 through *spray drying*

2.1.1. Preparation of the encapsulanting solution

Inulin HP (High Performance) (Beneo-Orafti, Oreye, Belgium) was used as coating agent. Inulin is a polysaccharide with a degree of polymerization (DP) above 23, with purity levels of 99.5%. The commercial freeze-dried probiotic culture of *L. acidophilus* La-5 (Christian Hansen, Hoersholm, Denmark), DVS type (direct vat set - for direct addition in milk), was added to a sterilized pre-mixture containing 6% (w/v) of fructooligosaccharides (FOS) (Beneo-Orafti, Oreye, Belgium) dissolved in UHT (ultra-high temperature) skimmed milk (COOP, Casalecchio di Reno, Italy). The suspension was then mixed for 120 minutes at 37 °C (Komatsu et al., 2013). The activated probiotic strain was inoculated into the suspension containing the coating agent in a ratio of 1:9 (v/v).

2.1.2. The spray drying process

The spray drying process was performed according to Fritzen-Freire et al. (2013) with some modifications in the process parameters. For the experiments, a Buchi B-290 Mini spray dryer (Buchi, Flawil, Switzerland) was used with air inlet temperature of 120 °C. Encapsulating agent solution containing *L. acidophilus* La-5 was maintained under magnetic stirring at room temperature before being used. A drying air flow rate of 55 m^3/h and the compressor air pressure of 4 bar were used.

2.2. Optimization of spray drying process parameters through Box-Behnken experimental design

The response surface methodology and the Box-Behnken experimental design with three factors and three levels were chosen to optimize and investigate the influence of the process variables feed flow (X_1), aspiration rate (X_2), and inulin concentration (X_3) in terms of the survival rate after the spray drying process (Y_1), probiotic cell counts (Y_2), and survival rate in acidic conditions (Y_3). The complete design consisted of 15 experiments with three replicates (used to estimate experimental error) of the central point (Table 1).

Table 1. Box–Behnken experimental design matrix employed.

Factors		**Levels**		
		-1	**0**	**+1**
x_1	Feed Flow (mL/min)	4	7	10
x_2	Aspiratition rate (%)	70	80	90
x_3	Inulin concentration (%)	10	15	20

Response surface methodology was applied to further optimize probiotic microencapsulation. A quadratic polynomial model was fitted to each response following the equation given below:

$$Y_i = \beta_0 + \beta_i \sum X_i + \beta_{ii} \sum X_i^2 + \beta_{ij} \sum_i \sum_j X_i X_j \quad (1)$$

where Y is the response, β_0 the constant, β_i the linear coefficient, β_{ii} the quadratic coefficient, and β_{ij} the interaction coefficient, whereas X_i and X_j are the independent variables (Ismail & Nampoothiri, 2010).

2.3. Microcapsules powder analysis

2.3.1. Count of microencapsulated and free cells of *Lactobacillus acidophilus* La-5

Counts of probiotic cells were determined by the standard plate count method, according to the methodology described by Gomez-Mascaraque et al. (2016a) with some modifications for the microencapsulated probiotic cells. The microcapsules obtained by spray drying (1.0 g) were re-suspended in a ratio of 1:9 with sterile peptone water (0.1% w/v) and kept under mixing for 3 minutes to release the cells. For the count of viable probiotic cells, samples were serially diluted in peptone water solution and seeded in MRS agar modified by the addition of maltose solution (50% w/v) using the pour plate method and with incubation at 37 °C for 48 h, as proposed by International Dairy Federation (IDF, 1995). The experiments were performed in triplicate. The survival rate (%) compared to the initial count before the spray drying was calculated according to Equation (2) (Guo et al., 2009; Pinto et al., 2015):

$$\text{Survival rate } (\%) = \frac{\log \text{CFU/g N}}{\log \text{CFU/g N}_0} \quad (2)$$

Where, N is the total viable count of probiotic strains after the spray drying process and N_0 is the total viable count of probiotic strains before the start of the process.

2.3.2. Survival of encapsulated probiotic strain in acid conditions

The survival rate in acidic conditions was evaluated according to Costa et al. (2017). Other adjustments were necessary and proceeded as follows: microencapsulated probiotic cells were added to a saline solution (0.5% w/v) with pH adjusted to 2.0-2.5 through the addition of a solution containing 1 N HCl (Merck, Darmstadt, Germany), without pepsin and lipase. The samples were incubated at 37 °C for 2 h. After the incubation period, the microencapsulated probiotic cells were released, and their viability was measured in triplicate using the method described in section 2.3.1.

2.3.3. Moisture content

The mean moisture content (% wet basis) resulting from the spray drying process was measured gravimetrically. A sample of known mass (0.5 g) was placed in an aluminum pan and dried in a hot air oven at 105 ± 2 °C for a period of 24 h. The sample was then removed and immediately weighed. Initial and final weights were used to calculate the moisture content on a wet basis. The experiments were performed in triplicate.

2.3.4. Microencapsulation yield

The microencapsulation yield was determined according to Gomez-Mascaraque et al. (2016a), using the Equation (3):

$$\text{Microencapsulation yield (\%)} = \frac{\text{Mass of spray drying products recovered from collector}}{\text{Mass of solids in the processed suspension}} \times 100 \quad (3)$$

2.3.5. Water solubility index, water absorption index and swelling capacity

The water solubility index (WSI) and the water absorption index (WAI) were determined according to Ahmed et al. (2010), by dissolving 1 g of product in 12 mL of water. The WSI and WAI were calculated according to Equations (4) and (5), respectively.

$$WSI = (DW_{sup}/DW_{part}) \times 100 \quad (4)$$

$$WAI = PW/DW_{part} \quad (5)$$

where DW_{sup} is the dry weight of the supernatant, DW_{part} is the initial weight of the microparticles (dry basis), and PW is the weight of the pellet after centrifugation.

The Equation (6) was applied according to Paini et al. (2015) using Equation (4) to determine the swelling capacity (SC):

$$SC = DW_{sup}/[DW_{part} \times (100–WSI)] \quad (6)$$

2.4. Production of the Synbiotic Diet Mousse (SDM)

The aerated synbiotic diet mousse (SDM) was prepared according to the formulation developed by Buriti et al. (2010) and characterized by Komatsu et al. (2013). Table 2 shows the proportions of the ingredients used in the production of the SDM. For the preparation, a commercial freeze-dried DVS probiotic culture of *L. acidophilus* La-5 was used. Skimmed milk powder and FOS were dissolved in ultra-high temperature skimmed milk on the day before the product preparation in order to make the dissolution of these ingredients easier. The resulting pre-mixture was stored under refrigeration at 4 °C until the addition of the remaining ingredients. One portion (40 mL) of this pre-mixture was sterilized and employed for the

fermentation at 37 °C for 120 min, using the probiotic culture (Komatsu et al., 2013). The activated culture in milk was then, depending on the condition tested, either added directly to the further ingredients of the mixture (free culture) or after being added to a suspension containing the coating agent in a ratio 1:9 and microencapsulated as described above (section 2.1 - microencapsulated culture, obtained through the optimized spray drying process, as described in section 2.2).

Table 2. Proportions of the ingredients used in the production of the synbiotic diet mousse.

Ingredient (g/100 g)	**Synbiotic diet mousse**
Skimmed milk[1]	61.7
Powdered skimmed milk[2]	4.0
Sucralose[3]	1,1
FOS[4]	6.0
Inulin[5]	4.0
Pasteurized and frozen guava pulp[6]	20.0
Stabilizer/emulsifier[7]	2.8
Lactic acid[8]	0.4
Lactobacillus acidophilus La-5[9]	0.05
Total	100.0

[1]Latte UHT scremato (COOP, Gmunden, Austrian); [2]Latte scremato in polvere (Ristora, Montichiari, Italy); [3]Sucralose (COOP, Casalecchio di Reno, Italy); [4]Beneo P95 (Orafti, Oreye, Belgium); [5]Beneo HP (Orafti); [6]Fruteiro do Brasil (Nectarvis Processamento de Frutas, Ceará-Mirim, RN, Brazil); [7]Cremodan Mousse 30 (Danisco, Cotia, SP, Brazil); [8]Lactic acid (Sigma-Aldrich, Steinheim, Germany); [9]Strain La-5 (Christian Hansen, Hoersholm, Denmark).

The other ingredients listed in Table 2 were added and mixed until becoming homogenous. The mixture obtained was pasteurized in the same mixer at 85 °C for 5 min, allowed to cool to 40 °C and supplemented with fermented milk containing the activated probiotic culture. The mixture was then kept refrigerated (4 °C) for subsequent aeration at a

temperature between 10 and 15 °C, during which its volume increased by 80-85%. Figure 1 schematically illustrates the main steps of the production of the SDM.

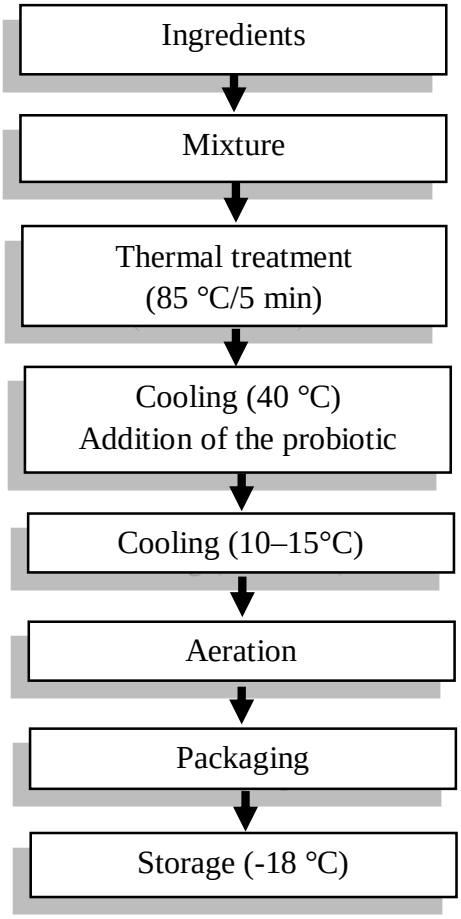

Figure 1. The main steps of synbiotic diet mousse production.

2.5. Survival of microencapsulated *Lactobacillus acidophilus* La-5 to *in vitro* simulated gastrointestinal conditions after storage

Free cells were prepared according to Yonekura et al. (2014). The freeze-dried probiotic culture was transferred to MRS broth (Oxoid Ltd., Basingstoke, UK), and, under aerobic conditions, incubated for 48 h at 37 °C. The MRS broth, modified by the addition of a maltose solution (described in section 2.3.1), was centrifuged at 3000 ×g for 5 min in aseptic conditions. Supernatants were discarded, and cell pellets were washed with phosphate

buffered saline (Sigma-Aldrich, Steinheim, Germany), and re-centrifuged. After discarding the supernatants, the collected bacterial cell pellets were suspended in the drying carrier media. The preparation of free cells was performed in triplicate.

Microencapsulated and free cells were compared for their ability to survive *in vitro* digestion simulating the human gastric and enteric conditions, according to Liserre et al. (2007), with the modifications suggested by Buriti et al. (2010). Other adjustments were necessary and proceeded as follows.

The microencapsulated and the free probiotic cells (1 g) were placed in 9 mL (ratio of 1:9) of a saline solution (0.5% w/v). The first 2 h represented the gastric phase (pH 2.0–2.5), with a solution containing 1 N HCl (Merck, Darmstadt, Germany), pepsin (3 g/L) (from porcine stomach mucosa, Sigma-Aldrich, St Louis, USA), and lipase (0.9 mg/L) (Amano lipase G, from *Penicillium camemberti*, Sigma-Aldrich). Bile (10 g/L) (bovine bile, Sigma-Aldrich) and pancreatin (1 g/L) (pancreatin from porcine pancreas, Sigma-Aldrich) were added to the enteric I (4 h of total assay) and the enteric II (6 h of total assay) phases with the pH adjusted, respectively, to 4.5–5.5 and 6.5–7.5.

After the respective incubation periods, microencapsulated and free cells were removed, and the viability of the entrapped cells was measured in triplicates using the method described in section 2.3.1. The results were presented as log CFU/g of fresh probiotic culture. Each assay was performed in triplicate. The pH values of the samples at each step of the assay were monitored using a pH meter (Hanna Instruments, Woonsocket, USA).

2.6. Statistical analysis

The response surface modelling was conducted using the STATISTICA v.10.0 software (Statsoft Inc., Tulsa, OK, USA). The statistical analysis of the Box-Behnken model

and adjusted coefficient of determination (R^2) was performed through the analysis of variance (ANOVA), followed by the Tukey post-hoc test ($p<0.05$). The optimization was obtained using the methodology described by Balasubramani et al. (2015). The optimization techniques of the Design-Expert software (Version 7.0 Trial, Stat-Ease, Minneapolis, MN, USA) were used for the simultaneous optimization of the multiple responses. The desired goals for each variable and response were chosen. All of the independent variables were kept within the range, while the responses were either maximized or minimized.

3. RESULTS AND DISCUSSION

3.1. Box–Behnken experimental design of the spray drying conditions

The experimental design matrix along with the measured responses for all the 15 experiments is given in Table 3. The probiotic survival rate after spray drying ranged between 62.6% and 86.5% and its survival rate to acidic conditions ranged between 41.8% and 79.3%. Probiotic counts varied from log 6.3 CFU/g up to log 9.3 CFU/g. The moisture and microencapsulation yield ranged between 4.2% and 10.6% and from 81.5% to 98.2%, respectively. The water solubility index ranged between 36.9% and 74.0%, the water absorption index from 0.9 g/g_{DP} to 2.0 g/g_{DP}, and the swelling capacity from 0.059 g/g_{DP} to 0.0310 g/g_{DP}.

Khem et al. (2016) reported an average survival rate for *L. plantarum* A17 after spray drying from 34.7% to 82.8%, using whey protein isolate powder as coating agent and an inlet temperature between 90 °C and 130 °C. In another study, Fritzen-Freire et al. (2013) using different coating agents to encapsulate *Bifidobacterium animalis* subsp. *lactis* BB-12, by spray drying observed a survival rate of 70.6% and 75.7% in reconstituted skimmed milk

with FOS and FOS-enriched inulin at 150 ℃, respectively. Huang et al. (2016) verified approximated survival of 40.0% for *L. casei* microencapsulated with sweet protein at 140 ℃.

Table 3. Box–Behnken experimental design matrix and responses.

Run	Feed Flow (mL/min) X_1	Aspiration rate (%) X_2	Inulin concentration (%) X_3	Survival rate after spray drying (%) Y_1	Survival rate to acid conditions (%) Y_2	Probiotic count (log UFC/g) Y_3	Moisture (%) Y_4	Microencapsulation yield (%) Y_5	Water solubility index (%) Y_6	Water absorption index (g/g_{DP}) Y_7	Swelling capacity (g/g_{DP}) Y_8
1	4 (-1)	70 (-1)	15 (0)	64.86	56.59	6.68	8.00	95.73	51.50	1.64	0.0107
2	10 (+1)	70 (-1)	15 (0)	73.51	46.39	7.68	8.00	93.53	38.76	1.78	0.0063
3	4 (-1)	90 (+1)	15 (0)	62.57	54.70	6.33	9.20	94.73	51.80	1.73	0.0105
4	10 (+1)	90 (+1)	15 (0)	75.57	73.15	7.82	4.40	89.27	44.89	1.71	0.0083
5	4 (-1)	80 (0)	10 (-1)	77.26	44.30	8.41	6.93	98.10	57.73	1.04	0.0138
6	10 (+1)	80 (0)	10 (-1)	86.49	79.33	9.30	6.86	93.50	44.41	1.64	0.0080
7	4 (-1)	80 (0)	20 (+1)	64.50	52.90	6.57	4.26	95.25	67.37	0.89	0.0206
8	10 (+1)	80 (0)	20 (+1)	76.22	63.08	7.95	6.45	81.45	73.95	0.98	0.0310
9	7 (0)	70 (-1)	10 (-1)	73.77	63.58	7.89	9.24	92.40	44.54	1.58	0.0080
10	7 (0)	90 (+1)	10 (-1)	77.59	56.57	7.85	10.64	98.20	40.80	1.87	0.0069
11	7 (0)	70 (-1)	20 (+1)	74.03	42.43	7.52	4.34	82.85	41.90	1.69	0.0072
12	7 (0)	90 (+1)	20 (+1)	77.45	44.57	7.87	4.23	94.45	38.93	1.86	0.0064
13	7 (0)	80 (0)	15 (0)	78.01	51.14	7.70	7.64	88.73	61.90	1.03	0.0163
14	7 (0)	80 (0)	15 (0)	78.36	54.41	7.76	9.17	91.33	49.92	1.30	0.0100
15	7 (0)	80 (0)	15 (0)	77.08	41.84	8.00	7.40	91.27	36.87	1.99	0.0059

Regarding the survival rate in acidic conditions, the results obtained can be attributed to the nature of the coating agent and the porosity of the microparticles (Gomez-Mascaraque et al., 2016). The highest survival rate (79.3%) was obtained when the microorganism was encapsulated using a feed flow of 10 mL/min, an aspiration rate of 80%, and the lowest concentration of inulin (10%). Darjani et al. (2016) reported that *L. casei* 431 microencapsulated with a mixture of alginate and inulin in a saline solution with pH adjusted to 1.5 demonstrated a 69.8% decrease after 120 min. The authors noticed an increased survival up to 85.1% under the same conditions when a mixture of alginate/inulin/chitosan was used as coating agent.

The moisture content is an important factor that can affect the microorganisms' viability and storage. This parameter might be influenced by several process variables, including the inlet temperature, the coating agent properties, and the feed flow rate (Khem et al., 2016). According to our experimental results, when our feed was injected at the lowest flow rate of 7 mL/min, and in the presence of the highest quantity of inulin (20%), the resulting product had the lowest moisture content (4.2%), which is an acceptable value in food products (Yonekura et al., 2014). Fernandes et al. (2016), who investigated the effect of inulin as coating agent in relation to the moisture content, obtained a moisture value from 3.3% to 3.5% using a mixture of inulin with arabic gum or modified starch as coating agents at the high temperature inlet (170 °C).

The microencapsulation yields were mostly above 90% and were much higher than the results obtained by Anekella and Orsat (2013). In that study, the microencapsulation process of lactobacilli (*Lactobacillus rhamnosus* NRRL B-4495 and *L. acidophilus* NRRL B-442) was performed using a combination of raspberry juice with maltodextrin as wall material, and the microencapsulated yield was 48.8% at 100 °C inlet temperature, with a coating agent ratio of 1:1, and an inlet feed rate of 40 mL/min.

The swelling capacity (SC) is another parameter to investigate the resistance of capsules before their bulk dissolution. Therefore, a higher SC is associated to the higher physical integrity of capsules before dissolution (Cheow et al., 2014). From data shown in Table 3, it can be observed that the highest SC (0.031 g/g_{DP}) corresponded to the highest survival rate after spray drying and also to the highest survival rate under acidic condition, confirming the hypothesis of stronger integrity of particles. The lowest survival rate under acidic condition (41.8%) with the minimum SC once more confirmed the poor physical integrity of the particles resulting in the faster dissolution.

The adequacy of experimental results to fit quadratic polynomial models was analyzed using the statistical software (data not shown). The fitted models were not significant (R^2<65%), and the results of ANOVA for the only significant model related to the survival rate are listed in Table 4.

Table 4. Results of variance analysis.

Factors	Sum of square	Degrees of freedom	Mean square	*F*-value	*p*-value	Signicant
X_1	678.93	1	678.93	89.80	<0.0001	*
X_2	18.26	1	18.26	2.41	0.1292	
X_3	196.25	1	196.25	25.96	<0.0001	*
X_1X_2	14.27	1	14.27	1.89	0.1782	
X_1X_3	4.55	1	4.55	0.60	0.4429	
X_2X_3	0.11	1	0.11	0.01	0.9038	
X_1^2	189.15	1	189.15	25.02	<0.0001	*
X_2^2	230.05	1	230.05	30.43	<0.0001	*
X_3^2	65.97	1	65.97	8.73	<0.0056	*
Error	264.62	35	7.56			
Total Sum of square	1671.37	44				
R^2=0.842	R^2_{adj}=0.801					

Highly significant (*p<0.005)

In this model (R^2 = 0.842), all three variables had a significant influence on the response (p<0.0001). The F-value of the quadratic model was also significant ($p < 0.0001$). As we can observe from the quadratic model (Equation 1), the two process variables, i.e. feed flow (x_1) and aspiration rate (x_2), had a positive effect, while inulin concentration (x_3) had a negative effect on the survival rate after spray drying. The offset term (β_0 = 73.65), which corresponds to the predicted value of the survival rate after spray drying at the central point (x_1 = 0; x_2 = 0; x_3 = 0), is not significantly different from the experimental result (78.9%). Hence confirming the suitability of this model.

$$\text{Survival rate after spray drying (\%)} = 73.65+10.64x_1+1.74x_2-5.72x_3+4.13x_1^2+4.56x_2^2-2.44x_3^2 \quad (1)$$

The factors which had the greatest impact were the feed flow (β_1 = 10.64; β_1^2 = 4.13) and the aspiration rate (β_2 = 1.74; β_2^2 = 4.56), while inulin concentration (β_3 = -5.72; β_3^2 = -2.44) was the parameter with the least influence throughout the microencapsulation process. Response surfaces of survival rate after spray drying as a function of three variables are illustrated in Figure 2.

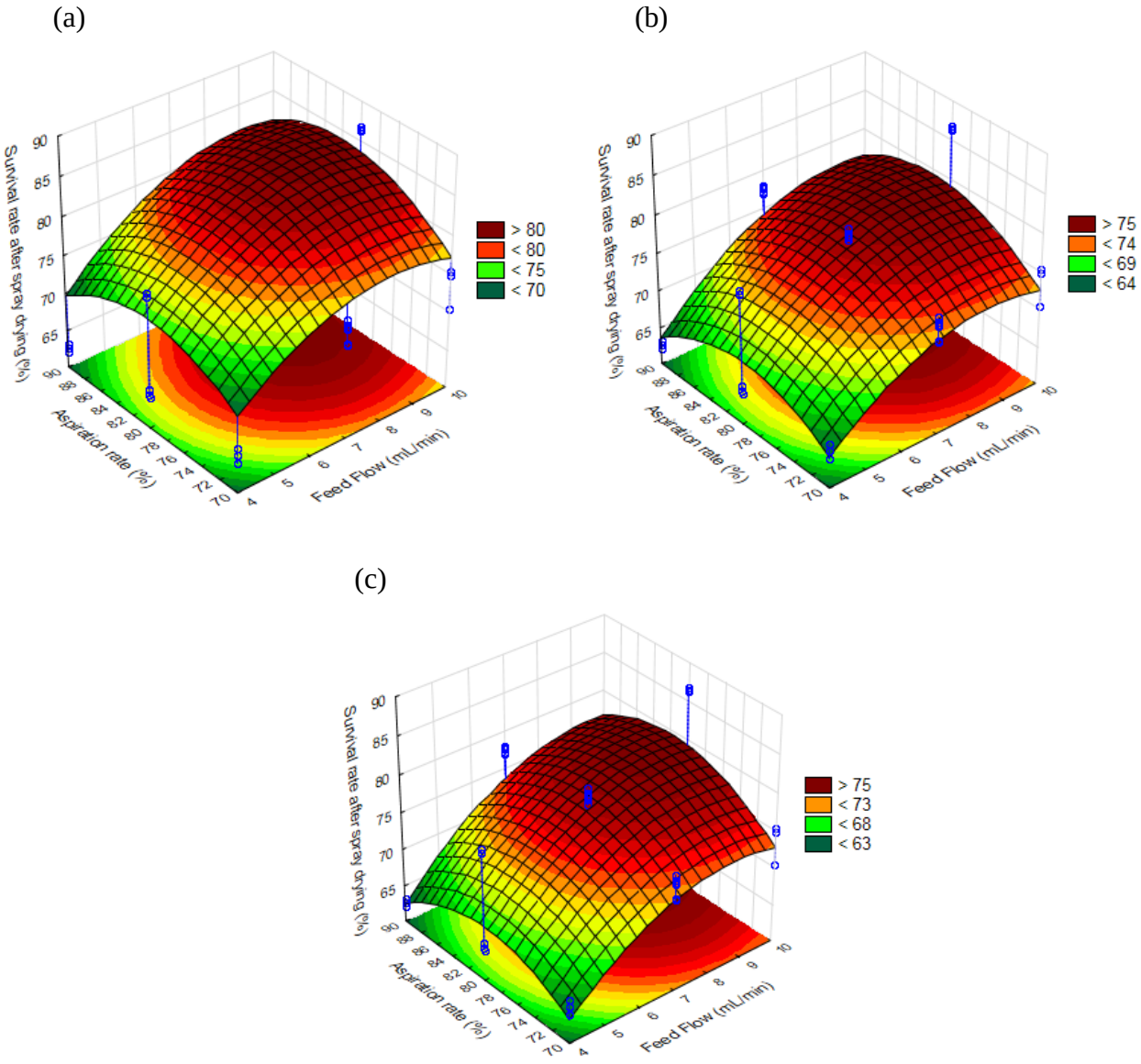

Figure 2. Response surface plot for survival rate after spray drying as a function of aspirated rate and feed flow at inulin concentration of 10% (a), 15% (b), and 20% (c).

That result probably occurred because the high feed flow rate reduces the microencapsulation process time and the cell exposure to the high air inlet temperature. Thus, the process conditions provide the highest microbial viability using spray drying (Alves et al., 2016). Serantoni et al. (2012) also confirmed that high aspiration rate directly positively

influenced the contact time of the granulated material with the cyclone hot air in the drying chamber.

3.2. Optimization of the spray drying conditions

In order to point out the optimal operative parameters for encapsulation of *L. acidophilus* La-5 using spray drying, a numerical optimization was performed. The criteria of optimization (Table 5) were chosen to maximize the survival rate after spray drying, the probiotic cell count, and the survival rate to acidic conditions. The optimum conditions predicted by the model were: inulin concentration of 10%, aspiration rate of 82%, and feed flow of 10 mL/min. In these conditions a high survival rate after spray drying (86.5%), the probiotic cell count (9.0 log CFU/g), and the survival rate to acidic conditions (78.7%) were predicted.

Table 5. Criteria for optimization of process conditions along with responses.

Name	Goal	Lower limit	Upper limit	Importance	Solution	Actual response value
X_1: Feed Flow (mL/min)	Is in range	4	10	3	10	-
X_2: Aspiration (%)	Is in range	70	90	3	82	-
X_3: Inulin concentration (%)	Is in range	10	20	3	10	-
Survival rate after spray drying (%)	Maximize	63.6	89.1	3	86.5	86.5
Probiotic cell count (log CFU/g)	Maximize	6.3	9.3	3	9.0	9.0
Survival rate to acid conditions (%)	Maximize	41.8	79.3	5	79.3	78.7

3.3. *In vitro* simulated gastrointestinal conditions

Free and microencapsulated probiotic cells were added during production of the mousse formulation, and their viability before and after the addition to the mousse over a 6h-assay of *in vitro* simulating gastrointestinal conditions was monitored (Figure 3).

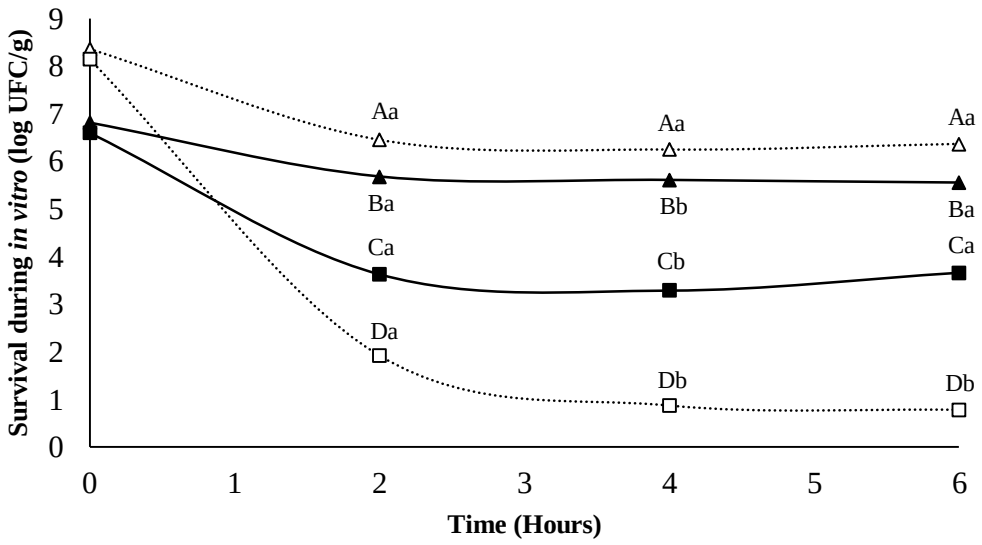

Figure 3. Survival of *Lactobacillus acidophilus* La-5 during exposition to simulated gastrointestinal conditions. (▲) Mousse with microencapsulated cells, (■) Mousse with free cells, (Δ) Microencapsulated cells, and (□) Free cells. Different uppercase letters indicate statistically significant differences (p<0.05) among the four samples for the same time. Different lowercase letters indicate statistically significant differences (p<0.05) among different times for the same parameter.

As can be seen in Figure 3, microencapsulated probiotic cells incorporated in the mousse presented the highest survival rate throughout the *in-vitro* simulated gastrointestinal conditions. As expected, free cells were not stable in this state. The trends of samples were statistically different (p<0.05).

Concerning the viability before all the steps of the simulated gastrointestinal conditions, the mousse with microencapsulated cells showed counts of 6.8 log CFU/g, while

counts of the mousse with free cells, microencapsulated cells, and free cells were of, respectively, 6.6, 8.4, and 8.2 log CFU/g.

A decline in the survival rate of *L. acidophilus* La-5 in the gastric phase was observed in all the samples ($p<0.05$). The greatest reduction of cell counts occurred for the free cells (6.3 log CFU/g), followed by mousse with free cells (3.0 log CFU/g), microencapsulated cells (1.9 log CFU/g), and mousse with microencapsulated cells (1.1 log CFU/g) after 2 h of incubation.

In the enteric phase I (after 4 h of *in-vitro* incubation) the lower variations in relation to the initial population occurred for the mousse containing microencapsulated cells (1.2 log CFU/g) and microencapsulated cells (2.1 log CFU/g), respectively, while mousse with free cells (3.3 log CFU/g) and free cells (7.3 log CFU/g) presented the greatest reductions.

In the enteric phase II, after 6 h, the least variation in survival rate was observed for mousse with microencapsulated cells (1.2 log CFU/g), followed by microencapsulated cells (2.0 log CFU/g), mousse with free cells (2.9 log CFU/g), and free cells (7.4 log CFU/g). After 6 h of *in-vitro* assay, mousse containing microencapsulated cells had the highest survival rate, confiring the efficiency of inulin as protective covering agent.

The resistance of the microencapsulated *L. acidophilus* La-5 along the in-vitro simulated gastrointestinal conditions can be considered suitable when compared to those of other similar studies. According to results obtained by Buriti et al. (2010), a high susceptibility of *L. acidophilus* La-5 during the in vitro assay was observed when the strain was incorporated in a synbiotic light mousse containing sugar and a lower content of guava pulp (12.5%) and inulin (2.0%). The free probiotic cells were drastically reduced after 30 min of the *in-vitro* assay. Gomez-Mascaraque et al. (2016a) observed a viability loss for *L. plantarum* CECT using whey protein concentrate (WPC) powder as coating agent during the gastric phase. Similar results have been previously reported using *L. casei* ATCC 393

microencapsulated with reconstituted skimmed milk that increased cell viability at low pH values (Dimitrellou et al., 2016).

Similarly to what was observed in the present study for the survival of the microencapsulated probiotic strain, Schell and Beermann (2014) observed an increased survival of microencapsulated *L. reuteri* DSM 20016 with sweet whey and shellac during the *in-vitro* gastrointestinal environment. In that study, the results might have occurred due to the cell structure recovery of the injured bacteria to the less stressful conditions of the enteric phase. In this sense, Gandomi et al. (2016) observed that the microencapsulation process of *L. rhamnosus* GG with sodium alginate and inulin using the extrusion technique improved bacterial viability during the gastric and intestinal models. In the present study, microencapsulated *L. acidophilus* La-5 had a high survival rate in the *in vitro* gastric environment. This fact might be attributed to the resistance of inulin to the simulated gastric and intestinal fluids (Karimi et al., 2015). Besides, the low solubility of the long-chain inulin, and consequently slower rehydration of the powder, may have contributed to a delayed release of the bacterial cells in the gastrointestinal tract (Pinto et al., 2015).

Although the digestive enzymes present in the gastric and the pancreatic secretions may be harmful to the cell structure, other factors, such as peristalsis and competitive exclusion in the gut microbiota would be enough to affect the bacterial adhesion ability in the intestinal epithelium (Darilmaz et al., 2011). Moreover, Matias et al. (2016) suggested that a prolonged exposure of a microorganism to distinct environmental conditions present throughout the digestive process, such as the gastric phase and the enteric phases I and II, containing bile and pancreatin, may promote significant changes in its physiology. The authors reported that these changes might affect the surface structure integrity, the cell membrane or the cell wall, due to an adaptation process to the dynamic environment. In the present study, the coating agent was important to protect the probiotic strain throughout the

simulated gastrointestinal conditions, confering a hight survival for the microencapsulated cells, and mainly when incorporated to mousse. Thus, the use of prebiotic ingredients may be an alternative to improve their survival through the gastrointestinal tract (Hernandez-Hernandez et al., 2012).

4. CONCLUSIONS

Spray drying was employed to encapsulate *L. acidophilus* La-5. Optimization of the process parameters demonstrated that an inulin concentration of 10%, an aspiration rate of 82%, and a feed flow of 10 mL/min, ensured a high survival rate after spray drying (86.5%), a high probiotic cell count (9.0 log CFU/g), and a high survival rate to acidic conditions (78.7%). Furthermore, mousse enriched with microencapsulated cells presented the highest survival level among the samples ($p<0.05$) during the *in-vitro* simulated gastrointestinal conditions. The results of this study confirm the appropriateness of the spray drying technique to encapsulate the probiotic strain evaluated using a prebiotic compound such as inulin, especially when the microorganism was incorporated in a synbiotic mousse, leading to an excellent survival rate under *in vitro* simulated gastrointestinal conditions.

5. REFERENCES

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GENERAL CONCLUSION

Based on the results of this study, the synbiotic diet mousse (SDM) stored at -18±2 °C showed populations of the probiotic strain *Lactobacillus acidophilus* La-5 above 7.8 log CFU/g for 112 days of storage, ensuring the minimum recommended amount of 8.0 log CFU/daily portion for this product to be considered a probiotic food. Regarding the instrumental texture of SDM, hardness and gumminess increased and cohesiveness decreased throughout storage, while adhesiveness and springiness kept almost unvaried. Moreover, sensory acceptability was good throughout storage, with average scores from 6.67 to 7.03 for the SDM, 5.77 to 6.50 for the placebo diet mousse (PDM), and 6.83 to 7.67 for the control synbiotic mousse (CSM). The results of the clinical trials demonstrated that SDM and PDM contributed to reduce total cholesterol, HDL-cholesterol, IL-1β, IgA, and IgM in volunteers with metabolic syndrome (MetS). These findings suggest that the presence of probiotic and prebiotic ingredients in the diet mousse did not influence the risk factors related to MetS significantly. The optimization of the process conditions via spray drying for the microencapsulation of *L. acidophilus* La-5, using inulin as coating agent, led to an increased survival rate after spray drying (86.5%), probiotic cell count (9.0 log CFU/g), and survival rate to acidic conditions (78.7%). In this sense, the microencapsulated probiotic strain incorporated in SDM sample showed the highest *in vitro* gastrointestinal survival among samples evaluated ($p<0.05$) in the different phases of the assay (the gastric phase: 5.7 log CFU/g (83.3%), the enteric phase I: 5.6 log CFU/g (82.3%), the enteric phase II: 5.7 log CFU/g (81.4%). Thus, the incorporation of the microencapsulated *L. acidophilus* La-5 with inulin into SDM becomes an interesting alternative to increase the viability of the probiotic cell throughout the digestive process. However, future studies are needed to evaluate the influence of daily supplementation of microencapsulated *L. acidophilus* La-5 incorporated into a diet mousse on the MetS risk factors, as well as to determine whether this dessert might cause specific changes on the composition and/or activity of the intestinal microbiota of subjects with MetS.

SCIENTIFIC RESULTS CONCERNING THE PRESENT PhD THESIS

This PhD thesis also resulted in 5 abstracts presented at international scientific meetings, of which three were abroad. In ascending chronological order, they are listed as follows:

- **XAVIER-SANTOS, D**.; SOLANO, J. H.; PIMENTEL, J. A.; LIMA, E. D.; SIMÃO, A. N. C.; BEDANI, R.; SAAD, S. M. I. Potential Effect of Synbiotic Diet Dessert Containing *Lactobacillus acidophilus* La-5 on Individuals with Metabolic Syndrome: A randomized Controlled Trial. In: Probiota 2016: connecting the global business and science of pre and probiotics, Amsterdam, Holland, 2016.
- **XAVIER-SANTOS, D**.; CASAZZA, A. A.; ALIAKBARIAN, B.; BEDANI, R.; SAMPAIO, F. C.; CONVERTI, A.; SAAD, S. M. I.; PEREGO, P. Microencapsulation of *Lactobacillus acidophilus* La-5 using spray drying. In: 24th Innference ICFMH Conference, Food Micro 2016: One Health Meets Food Microbiology: Food Micro, Dublin, Irland, 2016.
- **XAVIER-SANTOS, D**.; BEDANI, R.; CONVERTI, A.; PEREGO, P.; SAAD, S. M. I. Texture profile and sensory acceptance of a synbiotic diet aerated mousse containing *Lactobacillus acidophilus* La-5, inulin, and fructooligosaccharide. In: X CIGR Section IV International Technical Symposium, Gramado, Brazil, 2016.
- **XAVIER-SANTOS, D**.; SOLANO, J. H.; SIMÃO, A. N. C.; BEDANI, R.; LIMA, E. D.; SAAD, S. M. I. Potential effect of a synbiotic diet dessert containing *Lactobacillus acidophilus* La-5 on hematological parameters in patients with metabolic syndrome. In: Probiota 2017 - Connecting the global business and science of pre and prebiotics, Berlin, Germany, 2017.

- **XAVIER-SANTOS, D**.; PIRES, I. S. O.; PIMENTEL, J. A.; BEDANI, R.; LIMA, E. D.; SIMÃO, A. N. C.; SAAD. S. M. I. Impacto da sobremesa simbiótica *diet* contendo *Lactobacillus acidophilus* La-5 sobre os parâmetros inflamatórios e imunológicos em indivíduos com síndrome metabólica: um ensaio clínico randomizado. In: 19° Fórum Paulista de Pesquisa em Nutrição Clínica e Experimental, 2017, São Paulo.

The following scientific papers related to this PhD thesis were submitted for publication in peer-reviewed journals:

- **XAVIER-SANTOS, D**.; LIMA, E. D.; SIMÃO, A. N. C.; BEDANI, R.; SAAD. S. M. I. Effect of the consumption of a synbiotic diet mousse containing *Lactobacillus acidophilus* La-5 by individuals with metabolic syndrome: a randomized controlled trial. *Journal of Functional Foods,* Elsevier.

- **XAVIER-SANTOS, D**.; CASAZZA, A. A.; ALIAKBARIAN, B.; BEDANI, R.; SAAD, S. M. I.; PEREGO, P. Microencapsulation of *Lactobacillus acidophilus* La-5 using inulin as coating agent by spray drying and its survival under *in vitro* simulated gastrointestinal conditions. Prepared for submission in the next days to *International Journal of Biological Macromolecules,* Elsevier.

The following scientific articles related to this PhD thesis are being prepared for publication in indexed journals:

- **XAVIER-SANTOS, D**.; B.; BEDANI, R.; CONVERTI, A.; SAAD, S. M. I.; PEREGO, P. Texture profile and sensory acceptance of a synbiotic diet aerated mousse containing

Lactobacillus acidophilus La-5, inulin, and fructooligosaccharides. *Science Food and Agriculture*, Elsevier.

- **XAVIER-SANTOS, D.**; BEDANI, R.; LIMA, E. D.; SAAD, S. M. I. Probiotics, Prebiotics and Metabolic Syndrome. *Nutrition*, Elsevier.

PARTICIPATION IN EVENTS, CONGRESSES, AND EXHIBITIONS

The double PhD degree also resulted in 14 participations in scientific events and exhibitions, of which four were abroad. In ascending chronological order, they are listed as follows:

- XVIII Encontro Nacional e IV Congresso Latino Americano de Analistas de Alimentos - ENAAL 2013, São Paulo, Brazil, 2013 (Congress).
- Simpósio Renali - Ensaios de Proficiência e Materiais de Referência - Ferramentas de Garantia da Qualidade Analítica, São Paulo, Brazil, 2013 (Symposium).
- Danish-Brazilian research seminars on bioactive compounds, gut microbiota, inflammation, and metabolic disorders?, São Paulo, Brazil, 2014 (Seminars).
- Aprendizagem Baseada Em Grupos, Aplicada ao Ensino de Fisiopatologia, São Paulo, Brazil, 2014 (Seminars).
- Feira de Ciências e Tecnologia 2014: ciências e empreendedorismo, 2014. Colégio Bandeirantes, São Paulo, Brazil, 2014 (Appraiser).
- 10° Simpósio de Síndrome Metabólica do Hospital das Clínicas da FMUSP, São Paulo, Brazil, 2015 (Symposium).
- Probiota 2016: connecting the global business and science of pre and probiotics, Amsterdam, Holland, 2016 (Congress).
- UniverCity. Ingegneria alimentare e industria 4.0: dalla ricerca ai nuovi prodotti, Genoa, Italy, 2016 (Exhibition).

- 25th International ICFMH Conference – Food Micro 2016, One Health Meets Food Microbiology, Dublin, Irland, 2016 (Congress).
- XXV Congresso Brasileiro de Ciência e Tecnologia de Alimentos, Gramado, Brazil, 2016 (Congress).
- X CIGR Section IV International Technical Symposium, Gramado, Brazil, 2016 (Congress).
- 15° Evento da Série de Workshops Internacionais sobre Alimentos com Alegações de Propriedades Funcionais e/ou de Saúde: Microbioma, Probióticos e Saúde, São Paulo, Brazil, 2016 (Seminars).
- Probiota 2017 - Connecting the global business and science of pre and prebiotics, Berlin, Germany, 2017 (Congress).
- 19° Fórum Paulista de Pesquisa em Nutrição Clínica e Experimental, June 14th to 17th, São Paulo, Brazil, 2017 (Congress).

COLLABORATION IN SCIENTIFIC INITIATION PROJECT

- Collaboration as co-tutor in the scientific initiation study "Efeito do armazenamento refrigerado de musse de goiaba simbiótico diet sobre a viabilidade de *Lactobacillus acidophilus* La-5 e a sua sobrevivência em ensaios de sobrevivência *in vitro* das condições gastrintestinais", which was conducted by the undergraduate student, Juanita Hernández Solano, in Biotechnology (Federal University of São Carlos) at the University of São Paulo, Brazil.
- Collaboration as co-tutor in the graduation thesis "Microencapsulation of *Lactobacillus acidophilus* La-5 using spray drying", which is being conducted by the undergraduate student, Chiara Mucetti, in Chemistry and Pharmaceutical Technologies at the University of Genoa, Italy.

UNDERGRADUATE CLASSES TAUGHT IN HIGHER EDUCATION

- Academic support in the laboratory training activities of the Course “FBT0201 Food Technology” for undergraduate students in “Nutrition” (6 hours/week) at the University of São Paulo - Brazil.
- Academic support in the laboratory training activity of the Disciplines “FBT0530 Industrial Physics” to the students of the students of the Graduation Program in “Pharmacy-Biochemistry” (6 hours/week) at University of São Paulo - Brazil.
- Academic support in the laboratory training activity of the Disciplines “Industrial Microbiology & Chemistry and Biotechnology of Fermentations” to the students of the Graduation Program in “Biotechnology” (8 hours) and “Industrial and Environmental Biotechnologies” to the students of the Post-graduation Program in “Chemical Engineering” (8 hours) at University of Genoa - Italy.
- Academic support in the laboratory training activity of the course of “Processi e Impianti dell'Industria Alimentare” to the students of the Graduation Program in “Chemical Engineering” (8 hours) at University of Genoa - Italy.

ANNEXES

ANNEX 1 - Approval protocol of the Research Ethics Committee of the Faculty of Pharmaceutical Sciences

FACULDADE DE CIÊNCIAS FARMACÊUTICAS DA UNIVERSIDADE DE SÃO

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Sobremesa aerada simbiótica: desenvolvimento de um produto diet e avaliação dos efeitos da ingestão sobre indivíduos com síndrome metabólica

Pesquisador: Susana Marta Isay Saad

Área Temática:

Versão: 2

CAAE: 30539214.6.0000.0067

Instituição Proponente: Faculdade de Ciências Farmacêuticas da Universidade de São Paulo

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 663.138

Data da Relatoria: 26/05/2014

Apresentação do Projeto:

Alguns autores enfatizam que um alimento funcional deve promover efeitos benéficos de forma satisfatória a uma ou mais funções-alvo no organismo, melhoria da saúde e prevenir o risco do desenvolvimento de doenças, além da nutrição básica. No Brasil, os alimentos com alegação de propriedade funcional ou de saúde são regulamentados pela Agência Nacional de Vigilância Sanitária através das Resoluções 16, 18 e 19 de abril de 1999, devendo ser obrigatoriamente registrados junto ao órgão competente e serem seguros para o consumo sem a supervisão médica. Atualmente, os consumidores estão se interessando cada vez mais na incorporação de compostos bioativos como ingredientes funcionais nas dietas, em virtude dos seus diversos efeitos benéficos promovidos à saúde. Dentre os alimentos funcionais, destacam-se os probióticos, prebióticos e simbióticos. A definição aceita internacionalmente é que os probióticos são micro-organismos vivos, administrados em quantidades adequadas, que conferem benefícios à saúde do hospedeiro. A influência benéfica dos probióticos sobre a microbiota intestinal humana inclui fatores como efeitos antagônicos, competição e efeitos imunológicos, resultando em um aumento da resistência contra patógenos. O grupo de pesquisa proponente tem realizado vários trabalhos no desenvolvimento de produtos lácteos simbióticos, como manjar branco e queijos. Paralelamente, foram

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desenvolvidos trabalhos relacionados com o desenvolvimento de sobremesa aerada simbiótica do tipo musse, com baixo teor de gordura, processada com a
adição de uma cultura probiótica de L. acidophilus La-5, dos ingredientes prebióticos oligofrutose e inulina. A sobremesa desenvolvida deu origem ao depósito de uma patente e foi desenvolvida uma musse de goiaba potencialmente simbiótica, suplementada com a bactéria comprovadamente probiótica L. acidophilus La-5 e com o prebiótico oligofrutose, onde o creme de leite foi parcialmente ou totalmente substituído pelo prebiótico inulina e/ou por concentrato proteico de soro. Escassos são os estudos avaliando o efeito dos micro-organismos probióticos sobre a saúde, quando incorporados em produtos alimentícios não convencionais, como sobremesas aeradas tipo musse, particularmente quando esses produtos também contém ingredientes prebióticos. Sendo assim, a ideia de adaptar um produto simbiótico desenvolvido e bem caracterizado e com teor reduzido de gordura para um equivalente diet, sem a adição de açúcar, e testar o efeito desse produto light e diet em pacientes com parâmetros sanguíneos indicativos de síndrome metabólica torna-se um alvo de investigação bastante atraente. Desse modo, torna-se relevante à realização de uma pesquisa sobre os possíveis efeitos benéficos de uma musse aerada simbiótica diet sobre a saúde dos voluntários com síndrome metabólica, o que pode contribuir de maneira significativa ao entendimento do desempenho desde micro-organismos no organismo humano.

Objetivo da Pesquisa:

O presente projeto de pesquisa tem por objetivos:- Desenvolver uma sobremesa aerada simbiótica diet tipo musse, processada com a adição da cultura probiótica de Lactobacillus acidophilus La-5 e dos ingredientes prebióticos oligofrutose (FOS) e inulina, a partir da formulação com teor reduzido de gordura, mas contendo sacarose desenvolvida anteriormente;- Comparar o efeito do congelamento sobre as características tecnológicas, sensoriais e sobre viabilidade do Lactobacillus acidophilus La-5 no produto diet desenvolvido ao longo de seu armazenamento a -18oC e quando comparado à musse diet não simbiótica (controle) e ao similar com sacarose anteriormente desenvolvido e que será produzido para esse fim;- Verificar a influência da administração da musse aerada simbiótica diet sobre os parâmetros antropométricos, bioquímicos, hematológicos,
inflamatórios e relacionados com a síndrome metabólica durante o tempo 0, 4 e 8 semanas de intervenção com as sobremesas lácteas congeladas e após 2 semanas (tempo de 10 semanas) sem administração dos produtos diet.

Avaliação dos Riscos e Benefícios:

Riscos: Os participantes podem sentir algum desconforto provocado pela picada da agulha no local

da punção, poderá haver alguma dor decorrente da punção da pele e pessoas mais sensíveis poderão ficar com a pele arroxeada (pequeno hematoma) decorrente de uma pequena perda de sangue da veia no local da punção, por até 48 horas.

Benefícios: Os participantes estarão contribuindo de forma voluntária para o desenvolvimento de uma tese de doutorado que contribuirá para o desenvolvimento de um produto diet que poderá promover efeitos benéficos aos consumidores através de ingredientes bioativos (possuem funções específicas) que irá contribuir para melhorar a qualidade de vida e bem-estar da população.

Comentários e Considerações sobre a Pesquisa:

No projeto pretende-se utilizar uma formulação classificada como funcional (musse de goiaba com probióticos e prebióticos, diet) desenvolvidas anteriormente e analisar os efeitos bioquímicos em pessoas com síndrome metabólica atendidas pelo HU/USP. Os pacientes serão indicados pela equipe médica que os atende e a participação é voluntária. O projeto é bastante importante para a área de tecnologia de alimentos e o desenvolvimento de alimentos destinados aos pacientes com síndrome metabólica.

Considerações sobre os Termos de apresentação obrigatória:

Os pesquisadores implementaram alterações no projeto e no TCLE: a correção de 'funcionários' para 'postulantes a participantes da pesquisa' e no TCLE foi incluída informação de que, mesmo havendo a constatação de benefícios com o uso do alimento, não será possível seu fornecimento após o término da pesquisa.

Recomendações:

Recomenda-se a aprovação do projeto na forma em que se encontra.

Conclusões ou Pendências e Lista de Inadequações:

Recomenda-se a aprovação do projeto na forma em que se encontra.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

Tendo em vista as considerações acima, este CEP entende que o adendo ao projeto pode ser aprovado.

Endereço: Av. Prof. Lineu Prestes, 580, Bloco 13A, sala 112
Bairro: Butantã **CEP:** 05.508-000
UF: SP **Município:** SAO PAULO
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FACULDADE DE CIÊNCIAS FARMACÊUTICAS DA UNIVERSIDADE DE SÃO

Continuação do Parecer: 663.138

SAO PAULO, 27 de Maio de 2014

Assinado por:
Mauricio Yonamine
(Coordenador)

Endereço: Av. Prof. Lineu Prestes, 580, Bloco 13A, sala 112
Bairro: Butantã **CEP:** 05.508-000
UF: SP **Município:** SAO PAULO
Telefone: (11)3091-3622 **Fax:** (11)3031-8986 **E-mail:** cepfcf@usp.br

ANNEX 2 - Approval protocol of the Research Ethics Committee of the University Hospital

São Paulo, 07 de julho de 2014.

Il^mo(a). S^r(a).
Profa. Dra. Susana Marta Isay Saad
Departamento de Tecnologia Bioquímico-Farmacêutica
Faculdade de Ciências Farmacêuticas
UNIVERSIDADE DE SÃO PAULO

REFERENTE: **Projeto de Pesquisa** "Sobremesa aerada simbiótica: desenvolvimento de um produto diet e avaliação dos efeitos da ingestão sobre indivíduos com síndrome metabólica"
Pesquisador(a) responsável: Profa. Dra. Susana Marta Isay Saad
Equipe de pesquisa: Douglas Xavier dos Santos, Egidio Lima Dorea, Gelba Almeida Pinto, Maria Cecilia Gusukuma, Marina Baquerizo Martinez
CAAE: 30539214.6.3002.0076
Registro CEP-HU/USP: 1358/14

Prezado(a) Senhor(a)

O Comitê de Ética em Pesquisa do Hospital Universitário da Universidade de São Paulo, em reunião ordinária realizada no dia 27 de junho de 2014, analisou o Projeto de Pesquisa acima citado, considerando-o como **APROVADO**, bem como o seu **Termo de Consentimento Livre e Esclarecido**.

Lembramos que cabe ao pesquisador elaborar e apresentar a este Comitê, relatórios parciais e final, de acordo com a Resolução nº 466/2012 do Conselho Nacional de Saúde, inciso XI.2, letra "d".

O primeiro relatório está previsto para 27 de dezembro de 2014.

Atenciosamente,

Dr. Mauricio Seckler
Coordenador do Comitê de Ética em Pesquisa
Hospital Universitário da USP

COMITÊ DE ÉTICA EM PESQUISA DO HOSPITAL UNIVERSITÁRIO DA USP
Avenida Professor Lineu Prestes, 2565 – Cidade Universitária – 05508-000 São Paulo – SP
Tel.: (11) 3091-9457 – E-mail: cep@hu.usp.br

ANNEX 3 - Term of Free Consent and Enlightened

Universidade de São Paulo
Faculdade de Ciências Farmacêuticas

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO – TCLE

1. Informações do Sujeito da Pesquisa

Nome:			
Documento de Identidade nº:			Sexo: ( ) M ( )F
Data de Nascimento: / /			
Endereço:		Nº	Complemento:
Bairro:	Cidade:		Estado:
CEP:	Telefones:		

Título do Projeto de Pesquisa: "Sobremesa aerada simbiótica: desenvolvimento do produto *diet* e avaliação dos efeitos da ingestão sobre indivíduos com síndrome metabólica"
2. Duração da Pesquisa: 3 anos
3. Nome do pesquisador responsável: Dra. Susana Marta Isay Saad
Cargo/ Função: Professora
Instituição: Faculdade de Ciências Farmacêuticas/USP

Você está sendo convidado(a) a participar da pesquisa: "Sobremesa aerada simbiótica: desenvolvimento do produto *diet* e avaliação dos efeitos da ingestão sobre indivíduos com síndrome metabólica". O projeto é de responsabilidade da Professora Dra. Susana Marta Isay Saad e conta com a colaboração do doutorando Douglas Xavier dos Santos, ambos pertencentes ao Departamento de Tecnologia Bioquímico-Farmacêutica da Faculdade de Ciências Farmacêuticas/USP.

A definição aceita internacionalmente é que os probióticos são micro-organismos vivos, administrados em quantidades adequadas, que conferem benefícios à saúde do hospedeiro (pessoa). Os prebióticos, por sua vez, são atualmente definidos como 'ingredientes seletivamente fermentáveis que permitem modificações específicas na composição e/ou na atividade da microbiota gastrintestinal (micro-organismos que sobrevivem no intestino) que resultam em benefícios ao bem-estar e à saúde do hospedeiro'. O sinergismo (ação combinada) entre os micro-organismos probióticos e os prebióticos, principalmente no caso de produtos lácteos (à base de leite) está sendo cada vez mais usado na formulação de alimentos conhecidos como simbióticos (probiótico+prebiótico). A referida pesquisa tem por objetivo adaptar uma musse aerada simbiótica *diet* (produto sem açúcar e gordura) e avaliar os efeitos de sua ingestão (consumo) em participantes adultos com síndrome metabólica (classificação clínica de pessoas com maior possibilidade de desenvolver doenças cardiovasculares e/ou diabetes mellitus tipo II). Como forma de atingir os objetivos propostos, serão realizadas análises sanguíneas.

O experimento terá a duração de 70 dias e serão selecionados 60 participantes com idades entre 18 e 65 anos (preferencialmente da comunidade USP), de ambos os sexos, que queiram participar da pesquisa e consumir a musse simbiótica *diet*.

_____ _____

Rubrica do sujeito de pesquisa Rubrica Dra. Susana Marta Isay Saad

Universidade de São Paulo
Faculdade de Ciências Farmacêuticas

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO – TCLE

Participarão deste experimento, os pacientes com síndrome metabólica indicados pelo Dr. Egidio Lima Dorea (CRM: 65920-SP), um dos responsáveis pelo ambulatório de doenças metabólicas do Hospital Universitário/USP, que possuirão pelo menos três das cinco seguintes características: 1) Obesidade abdominal: circunferência da cintura ≥ 88cm para a mulher e ≥ 102cm para o homem; 2) hipertrigliceridemia (níveis elevados de triglicerídeos) ≥150mg/dL; 3) Baixos níveis de colesterol-HDL (conhecido popularmente como colesterol "bom") ≤50mg/dL para a mulher e ≤40mg/dL para o homem; 4) Pressão arterial elevada: ≥130/85mmHg e 5) Glicose de jejum elevada ≥100 mg/dL. Além disso, não serão selecionados os participantes portadores de doenças da tireoide (níveis de hormônio tiroidiano alterados), renais (doenças nos rins), hepáticas (doenças no fígado), gastrointestinais (doenças nos intestinos), oncológicas (doenças cancerígenas), medicados com hipolipemiantes (reduzem os níveis de colesterol e triglicerídeos), hiperglicemiantes (reduzem os níveis de glicose sanguínea) e em terapia de reposição de estrogênio (reposição hormonal).

A principal motivação dos participantes da pesquisa está relacionada com a ingestão de uma sobremesa láctea agradável sensorialmente (visão, paladar, olfato e tato) e que não possuirá efeitos colaterais. Os indivíduos medicados com anti-hipertensivos não serão excluídos da seleção, podendo seguir normalmente com o seu medicamento. Nenhum dos participantes seguirá uma dieta específica antes do início e durante a ingestão das musses *diet*.

Os participantes serão orientados a manter suas dietas habituais, prevendo a possível ingestão de bebidas com teor alcoólico, mantendo os níveis de atividades físicas ou outros estilos de vida durante todo o período de intervenção.

As musses serão acondicionadas em um *freezer* no Departamento de Tecnologia Bioquímico-Farmacêutica (endereço na última página). Os participantes poderão passar no departamento (se preferirem) para retirarem 14 embalagens contendo 40g do produto a cada 2 semanas ou poderão receber suas amostras em locais de conveniência. Estes participantes serão orientados a estocar os produtos no *freezer* (temperatura de -18°C) em sua residência.

Durante a coleta da primeira amostra sanguínea (antes do período de ingestão das sobremesas lácteas), será entregue uma informativo para cada participante sobre a forma como o produto deverá ser consumido e armazenado até a entrega das próximas amostras, além de informações adicionais.

Os indivíduos farão jejum de 12 horas após a última refeição do dia. No dia seguinte, estes participantes serão submetidos à punção venosa (picada de agulha) para coleta de amostras sanguíneas nos tempos 0, 4 e 8 semanas de intervenção com as musses *diet* e após 2 semanas (tempo de 10 semanas) sem administração das musses *diet*. O sangue coletado será de aproximadamente 30mL, devido a necessidade de diferentes tubos de coleta com conservantes específicos para cada tipo de análises (hematológicas, inflamatórios e metabólicas). As coletas serão realizadas no Centro de Pesquisa do Hospital Universitário da USP (HU).

_____ Rubrica do sujeito de pesquisa

_____ Rubrica Dra. Susana Marta Isay Saad

Universidade de São Paulo
Faculdade de Ciências Farmacêuticas

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO – TCLE

Para coleta saguínea será realizada apenas uma picada com a utilização de intima (equipamento de coleta sanguínea) que possibilita a coleta de sangue sem a necessidade de mais de uma picada. Neste procedimento, o participante pode sentir algum desconforto provocado pela picada da agulha no local da punção, poderá haver alguma dor decorrente da punção da pele e pessoas mais sensíveis poderão ficar com a pele arroxeada (pequeno hematoma) decorrente de uma pequena perda de sangue da veia no local da punção, por até 48 horas. A coleta de sangue será realizada em local limpo e confortável, por técnicos treinados. Todo material utilizado será descartável e estéril. Posteriormente, as análises do material coletado serão realizadas nos laboratórios credenciados do Serviço de Laboratório Clínico do HU da USP, por métodos quantitativos apropriados.

Todo o procedimento será supervisionado pelo doutorando Douglas Xavier dos Santos. A coleta de sangue será realizada por equipe de profissionais treinados do HU/USP. Se houver algum benefício detectado durante o período de intervenção com as musses diet aos voluntários, não nos comprometeremos a fornecer o alimento após o término do experimento em nenhuma hipótese.

Direito dos participantes:

- ✓ Quaisquer danos resultantes da pesquisa serão indenizados;
- ✓ Você poderá recusar ou desistir do experimento a qualquer momento, sem prejudicar o acompanhamento médico realizado pela equipe do HU/USP;
- ✓ A qualquer momento você poderá solicitar que os seus dados sejam excluídos da pesquisa;
- ✓ Você receberá os resultados das análises laboratoriais (sanguíneas) individualmente e qualquer dúvida a respeito dos exames será sanada através de um profissional qualificado que possa interpretá-los;
- ✓ Você poderá solicitar explicações todas as vezes que achar necessário sobre o experimento que estará participando;
- ✓ Todas as amostras sanguíneas coletadas serão descartadas após as análises laboratoriais;
- ✓ Todos os participantes serão identificados por um código para evitar que o seu nome seja relacionado aos resultados obtidos e quando os resultados destes experimentos forem publicados em eventos e revistas científicas especializadas, os nomes não serão divulgados;
- ✓ Os participantes terão ressarcimento com os custos de transporte, caso eles tenham que se deslocar até o Departamento de Tecnologia Bioquímico-Farmacêutica para retirarem suas amostras de musse *diet*.
- ✓ Todos os participantes deverão permanecer no local da coleta sanguínea durante 30 minutos para recuperação após a coleta, com o direito a alimentação para a quebra do jejum.

_____ _____

Assinatura do sujeito de pesquisa Assinatura do pesquisador responsável

Universidade de São Paulo
Faculdade de Ciências Farmacêuticas

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO – TCLE

Benefícios:

- ✓ Os participantes estarão contribuindo de forma voluntária para o desenvolvimento de uma tese de doutorado que contribuirá para o desenvolvimento de um produto *diet* que poderá promover efeitos benéficos aos consumidores através de ingredientes bioativos (possuem funções específicas) que irá contribuir para melhorar a qualidade de vida e bem-estar da população.

Em caso de dúvidas, intercorrências clínicas ou reações adversas, o participante da pesquisa será encaminhado pela equipe do projeto de pesquisa ao HU/USP.

Endereço: Depto de Tecnologia Bioquímico-Farmacêutica da FCF/USP
Av. Prof. Lineu Prestes, 580 Bloco 16 05508-900 São Paulo - SP
Telefone: (011) 3091-2378 e-mail: susaad@usp.br.
Ou com o Pesquisador colaborador: Me Douglas Xavier dos Santos, cel: (11) 99210-1834 e e-mail: douglas_xavier@usp.br.

Uma via deverá será entregue a você e outra via ficará com o pesquisador responsável, pelo tempo de 5 (cinco) anos.

Consentimento Pós-Esclarecido:

Declaro que, após convenientemente esclarecido pelo pesquisador e ter entendido o que me foi explicado, consinto em participar do presente Protocolo de Pesquisa.

São Paulo, de de .

_____ _____

Assinatura do sujeito de pesquisa Assinatura do pesquisador responsável

Em caso de dúvida, esclarecimento ou reclamação sobre aspectos éticos dessa pesquisa, favor entrar em contato:

- Comitê de Ética em Pesquisas da Faculdade de Ciências Farmacêuticas da USP – Av. Prof. Lineu Prestes, 580 - Bloco 13A – Cidade Universitária – CEP: 05508-900 – São Paulo/SP, Fone: (11) 3091-3622, Fone-Fax: 3091-3677 – E-mail: cepfcf@usp.br

- Comitê de Ética em Pesquisa do Hospital Universitário da USP - Av. Prof. Lineu Prestes, 2565 – Cidade Universitária - CEP: 05508-000 – São Paulo/SP - Fone: (11) 3091-9457, Fax: (11) 3091-9479 E-mail: cep@hu.usp.br

Universidade de São Paulo
Faculdade de Ciências Farmacêuticas

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO – TCLE

Informações do Sujeito da Pesquisa

Nome:			
Documento de Identidade nº:			Sexo: ( ) M ( )F
Data de Nascimento:__/__/_____			
Endereço:		Nº	Complemento:
Bairro:	Cidade:		Estado:
CEP:	Telefones:		

Título do Projeto de Pesquisa: **Sobremesa aerada simbiótica: desenvolvimento do produto *diet* e avaliação dos efeitos da ingestão sobre indivíduos com síndrome metabólica.**	
Duração da Pesquisa: 3 anos (análise sensorial será realizada a partir de abril de 2014).	
Nome do pesquisador responsável: **Profª. Drª. Susana Marta Isay Saad**	
Cargo/ Função: Professora Associada	Nº do Registro do Conselho Regional: CRF-8: 9541
Instituição: Faculdade de Ciências Farmacêuticas – USP	

REGISTRO DAS EXPLICAÇÕES DO PESQUISADOR AO PROVADOR SOBRE A PESQUISA

Justificativa, objetivos e procedimentos

Você foi convidado para participar da análise sensorial de um musse de goiaba adicionado de micro-organismo que contribui para função intestinal (*Lactobacillus acidophilus* La-5) e fibra alimentar solúvel (inulina e/ou fruto-oligossacarídeos). Sobremesas lácteas são alimentos que surgem com um grande potencial para uso como veículo de micro-organismos que contribuem para função intestinal, com a vantagem de serem alimentos consumidos por todas as faixas etárias. A goiaba tem recebido destaque nas pesquisas voltadas para alimentos que contribuem para a saúde, devido a presença de carotenoides e a função antioxidante. Esta fruta incorporada a musse é um modo de levar ao consumidor seus benefícios de forma prazerosa. Além disso, a combinação de micro-organismos que contribuem para função intestinal e fibra alimentar solúvel agrega ainda mais valor ao alimento. O desenvolvimento deste produto é feito pelo doutorando Douglas Xavier dos Santos, sob orientação da Profª. Drª. Susana Marta Isay Saad. O produto foi elaborado e acondicionado, de acordo com as Boas Práticas de Fabricação de Alimentos, nos laboratórios do Departamento de Tecnologia Bioquímico-Farmacêutica FCF/USP. Caso você tenha interesse em participar, acomode-se junto a uma das cabines do Laboratório de Análise Sensorial (B16).

O produto a ser avaliado nesta análise pode possuir: leite desnatado, leite em pó desnatado, sucralose, polpa de goiaba pasteurizada, estabilizante, ácido lático alimentício, inulina, fruto-oligossacarídeos e micro-organismo que contribui com a função intestinal (*Lactobacillus acidophilus* La-5). Todos os ingredientes da formulação são utilizados em produtos disponíveis para consumo humano, ou seja, são de grau alimentício.

Para participar desta análise, você: deve ter entre 18 e 60 anos; não possuir histórico de manifestação de alergia, intolerância ou outro tipo de restrição (como doença crônica ou tratamento médico com uso de medicamentos que podem interagir) com os ingredientes; não deve estar gripado, resfriado ou indisposto ou ter entrado em contato com materiais, alimentos ou cosméticos de cheiro forte. Atendendo a essas condições você poderá participar da análise sensorial da musse de goiaba. Caso você tenha menos de 18 anos ou mais de 60 anos, histórico de manifestação de alergia, intolerância ou outro tipo de restrição com os ingredientes da musse de goiaba, esteja gripado, resfriado ou indisposto ou tenha entrado em contato com materiais alimentos ou cosméticos com cheiro forte, você não poderá participar desta análise.

Você receberá três amostras, uma de cada vez, cada uma com uma ficha de avaliação. A amostra contém aproximadamente 20g de musse de goiaba. Prove a amostra e registre na ficha sua opinião com relação ao produto de uma

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maneira geral fazendo um "x" em um lugar na escala de 1 a 9, onde, 1 = desgostei muitíssimo e 9 = gostei muitíssimo. Em seguida, escreva o que você mais gostou e o que menos gostou na amostra.

Sua participação é livre e você poderá comparecer no máximo uma vez a cada dia para de análise sensorial deste produto.

Os riscos de sua participação neste estudo são mínimos. Não foram encontradas evidências de risco ou desconforto relacionado à análise sensorial em estudos deste tipo, com alimentos contendo micro-organismos que contribuem para função intestinal e fibras alimentares solúveis nas proporções adicionadas à este produto. Os demais ingredientes são reconhecidamente seguros, os possíveis desconfortos são mínimos.

Não há nenhum benefício direto. Porém, você contribuirá para o desenvolvimento de alimentos com características sensoriais adequadas às expectativas de futuros consumidores.

Caso você não queira continuar participando da pesquisa, a qualquer momento você pode desistir, sem que haja qualquer prejuízo. Havendo qualquer dúvida com relação aos procedimentos, riscos e benefícios relacionados à pesquisa você deve comunicar ao grupo de pesquisa a qualquer momento. É assegurado que todas as informações pessoais serão confidenciais, o sigilo e privacidade também são garantidos, mesmo que os resultados sejam publicados em periódicos científicos.

Serão adotados cuidados especiais para evitar que indivíduos subordinados ou diretamente ligados ao pesquisador se sintam obrigados a participarem do estudo.

Não haverá remuneração financeira aos participantes das análises sensoriais.

Em caso de intercorrências clínicas e reações adversas durante o estudo, o voluntário deverá procurar o Hospital Universitário da Universidade de São Paulo (USP) - Av. Prof. Lineu Prestes, 2565 . Cid. Universitária. (11) 3091-9200.

Os responsáveis pelo acompanhamento da pesquisa estarão à disposição para contato:

Douglas Xavier dos Santos e Susana Marta Isay Saad.
Faculdade de Ciências Farmacêuticas – Departamento de Tecnologia Bioquímico-Farmacêutica. Av. Prof. Lineu Prestes, 580 CEP 05508-000 São Paulo-SP.
Telefone: (11) 3091-2379 ou (11) 3091-2691

CONSENTIMENTO PÓS-ESCLARECIDO

Declaro que, após convenientemente esclarecido pelo pesquisador e ter entendido o que me foi explicado, consinto em participar do presente Protocolo de Pesquisa.

São Paulo, de _____ de _____.

_____ Assinatura do sujeito de pesquisa

_____ Assinatura do pesquisador responsável
Profª. Drª. Susana Marta Isay Saad

Para qualquer questão, dúvida, esclarecimento ou reclamação sobre aspectos éticos dessa pesquisa, favor entrar em contato com o Comitê de Ética em Pesquisas da Faculdade de Ciências Farmacêuticas da Universidade de São Paulo – Av. Prof. Lineu Prestes, 580 - Bloco 13A – Butantã – São Paulo – CEP 05508-900. Fone: 3091-3622, fone-fax: 3091-3677 – e-mail: cepfcf@usp.br

Universidade de São Paulo
Faculdade de Ciências Farmacêuticas

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO – TCLE

Ficha para teste de aceitação – Musse de goiaba

Nome: _____ Data: ___/___/_____
Amostra: _____

Você recebeu uma amostra de musse de goiaba. Por favor, prove a amostra e avalie de maneira geral (aparência, sabor e textura) usando a escala abaixo o seu grau de aceitação relacionado ao produto.

1 - Desgostei muitíssimo
2 - Desgostei muito
3 - Desgostei regularmente
4 - Desgostei ligeiramente
5 - Não gostei nem desgostei
6 - Gostei ligeiramente
7 - Gostei regularmente
8 - Gostei muito
9 - Gostei muitíssimo

Cite a característica que você **mais** gostou na amostra: ☺ _____

Cite a característica que você **menos** gostou na amostra: ☹ _____

ANNEX 4 - School Records

2017-6-5

Janus - Sistema Administrativo da Pós-Graduação

Universidade de São Paulo
Faculdade de Ciências Farmacêuticas
Documento sem validade oficial
FICHA DO ALUNO

9133 - 8402211/1 - Douglas Xavier dos Santos

Email:	douglas_xavier@usp.br
Data de Nascimento:	28/10/1985
Cédula de Identidade:	RG - 42.552.182-5 - SP
Local de Nascimento:	Estado de São Paulo
Nacionalidade:	Brasileira
Graduação:	Tecnólogo - Centro Paula Souza - Faculdade de Tecnologia "Estudante Rafael Almeida Camarinha" - Marília - São Paulo - Brasil - 2008
Mestrado:	Mestre em Ciência de Alimentos (1) - Universidade Estadual de Londrina - Paraná - Brasil - 2012

Curso:	Doutorado
Programa:	Tecnologia Bioquímico-Farmacêutica
Área:	Tecnologia de Alimentos
Data de Matrícula:	10/06/2013
Início da Contagem de Prazo:	10/06/2013
Data Limite para o Depósito:	12/06/2017
Orientador na USP:	Prof(a). Dr(a). Susana Marta Isay Saad - 10/06/2013 até o presente. Email: susaad@usp.br

Aluno USP em convênio de dupla titulação com instituição estrangeira

Instituição Conveniada:	Università Degli Studi di Genova, Itália
Orientador na Instituição Conveniada:	Patrizia Perego
Proficiência em Línguas:	Inglês, Aprovado em 10/06/2013
Data de Aprovação no Exame de Qualificação:	Aprovado em 29/07/2015
Data do Depósito do Trabalho:	
Título do Trabalho:	
Data Máxima para Aprovação da Banca:	
Data de Aprovação da Banca:	
Data Máxima para Defesa:	
Data da Defesa:	
Resultado da Defesa:	
Histórico de Ocorrências:	Primeira Matrícula em 10/06/2013

Aluno matriculado no Regimento da Pós-Graduação USP (Resolução nº 5473 em vigor de 18/09/2008 até 19/04/2013).
Última ocorrência: Matrícula de Acompanhamento em 06/02/2017
Impresso em: 05/06/2017 00:54:54

Janus - Sistema Administrativo da Pós-Graduação

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2017-6-5

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FICHA DO ALUNO

9133 - 8402211/1 - Douglas Xavier dos Santos

Sigla	Nome da Disciplina	Início	Término	Carga Horária	Cred.	Freq.	Conc.	Exc.	Situação
FBC5719-3/1	Trato Gastrointestinal: Imunomodulação da Colonização e Infecção Bacteriana	02/08/2013	14/11/2013	90	6	100	A	N	Concluída
GEN5711-4/5	Preparação à Docência de Graduação (Instituto de Matemática e Estatística - Universidade de São Paulo)	12/08/2013	29/11/2013	60	0	-	-	N	Matrícula cancelada
AGG5900-12/1	Preparação Pedagógica (Instituto de Astronomia, Geofísica e Ciências Atmosféricas - Universidade de São Paulo)	13/08/2013	25/11/2013	15	1	75	B	N	Concluída
EDM5102-3/5	Preparação Pedagógica PAE (Faculdade de Educação - Universidade de São Paulo)	20/08/2013	30/09/2013	60	0	-	-	N	Pré-matrícula indeferida
FBA5752-1/1	Probióticos em Alimentos e Suas Implicações na Saúde Humana	05/11/2013	16/12/2013	60	4	100	A	N	Concluída
Atividade do Programa	Participou da Etapa de Estágio Supervisionado em Docência do Programa de Aperfeiçoamento de Ensino junto à Disciplina FBT0201 Tecnologia de Alimentos, ministrada aos alunos de graduação do curso de Farmácia e Bioquímica da Faculdade de Ciências Farmacêuticas da Universidade de São Paulo (2)	19/02/2014	25/06/2014	-	1	-	-	-	-
FBT5787-1/3	Aplicação Biotecnológica de Bactérias Láticas	04/03/2014	07/04/2014	45	0	-	-	N	Matrícula cancelada
EDM5100-1/3	A Formação do Professor Universitário (Faculdade de Educação - Universidade de São Paulo)	11/03/2014	02/06/2014	120	0	-	-	N	Matrícula cancelada
EDM5791-6/1	Metodologia do Ensino Superior (Faculdade de Educação - Universidade de São Paulo)	11/03/2014	03/06/2014	120	8	100	A	N	Concluída
FBT5700-3/1	Preparo de Artigos Científicos na Área de Tecnologia Bioquímico-Farmacêutica	03/04/2014	04/06/2014	90	6	100	A	N	Concluída
FBT5738-1/1	Tópicos Especiais em Tecnologia Bioquímico-Farmacêutica III	02/03/2015	10/05/2015	30	2	100	A	N	Concluída

	Créditos mínimos exigidos		Créditos obtidos
	Para exame de qualificação	Para depósito de tese	
Disciplinas:	0	25	28
Estágios:			
Total:	0	25	28

Créditos Atribuídos à Tese: 167

Observações:

1) Curso com validade nacional, de acordo com o disposto na Portaria nº 524, de 29.04.2008..

2) Créditos atribuídos de acordo com o disposto na Portaria GR-3588 e GR-4391 - PAE, de 31.08.09 e aprovados pela Comissão de Pós-Graduação, em Sessão de 25/06/2014.

Conceito a partir de 02/01/1997:

A - Excelente, com direito a crédito; B - Bom, com direito a crédito; C - Regular, com direito a crédito; R - Reprovado; T - Transferência.

Um(1) crédito equivale a 15 horas de atividade programada.

Última ocorrência: Matrícula de Acompanhamento em 06/02/2017

2017-6-5

Impresso em: 05/06/2017 00:54:54

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Comissão julgadora da tese de doutorado:

NUSP	Nome	Vínculo	Função
1828231	Susana Marta Isay Saad	FCF - USP	Presidente

Última ocorrência: Matrícula de Acompanhamento em 06/02/2017
Impresso em: 05/06/2017 00:54:54