LWT - Food Science and Technology 79 (2017) 205–212

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Inulin increases *Bifidobacterium animalis* Bb-12 *in vitro* gastrointestinal resistance in margarine

Cínthia Hoch Batista de Souza [1, *], Luiz Antonio Gioielli, Susana Marta Isay Saad

Departamento de Tecnologia Bioquímico-Farmacêutica, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Av. Prof. Lineu Prestes, 580, 05508-000 São Paulo, SP, Brazil

ARTICLE INFO

Article history:
Received 20 July 2016
Received in revised form
6 January 2017
Accepted 12 January 2017
Available online 13 January 2017

Keywords:
Synbiotic
Probiotic
Prebiotic
Spread
Gastrointestinal resistance

ABSTRACT

This study aimed to investigate the effect of inulin, whey protein concentrate (WPC), and/or casein-omacropeptide (CMP), in different proportions up to 3%, on the viability and resistance to simulated gastric and enteric conditions of *Bifidobacterium animalis* Bb-12 added in probiotic and synbiotic margarine, during 35 days of storage at 5 °C. Supplementation was important, since the control margarine presented very low Bb-12 populations. Inulin at 3% resulted in higher Bb-12 counts (8 log cfu g^{-1}). CMP contributed for higher Bb-12 counts, compared to WPC. Inulin increased Bb-12 *in vitro* survival significantly after 6 h ($P<0.05$). In margarines with WPC at 3%, Bb-12 populations decreased drastically during the *in vitro* assays for all storage periods. For the other formulations, Bb-12 populations decreased 2 log cfu g^{-1} after 2 h. Taken together, results on viability of *B. animalis* Bb-12 and on *in vitro* GI resistance of the strain incorporated in margarine produced with inulin were unique, but the addition of inulin was necessary.

1. Introduction

Probiotics are “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (Hill et al., 2014). One of the well-investigated probiotics is *Bifidobacterium animalis* subsp. *lactis* Bb-12, with demonstrated efficacy through clinical trials (Haschke et al., 1998). In order to exert their functional properties, probiotics need to be delivered to human intestine in an active and viable form (Vandenplas, Huys, & Daube, 2015). Therefore, it is important to develop probiotic food with suitable probiotic populations throughout shelf life and that are ingested as part of a normal diet for maintaining a regular probiotic intake. Nevertheless, ingested microorganisms are exposed to several stress factors that influence their viability through the human gastrointestinal tract (GIT). Among these factors, probiotics must tolerate the stomach acidic environment and also the reduced water activity and the presence of bile in the upper small intestine (Vandenplas et al., 2015).

Once probiotic bacteria must preserve viability and arrive at high populations in the colon after surviving the food processing steps and the digestive process, a careful selection of the food matrix is an important factor that should be considered in developing a probiotic product. The food matrix is considered as one of the major factors in regulating colonization of microorganisms in the GIT. Food helps to buffer the bacteria through the stomach and may contain other functional ingredients that could interact with probiotics to alter their functionality. Fat content, concentration and type of proteins, sugars, and pH of foods are some factors that could affect probiotic growth and survival in food products (Ranadheera, Baines, & Adams, 2010). These features might help probiotic survival during passage through the GIT, where survival is dependent on both the strain and the food matrix involved. Likewise, the supplementation of food with some ingredients like the prebiotic fibre inulin and milk proteins may stimulate probiotic bifidobacteria growth and also protect them during GIT passage (Janer, Peláez, & Requena, 2004; Kos, Šušković, Goreta, & Matošić, 2000).

Limitations of dairy products such as the presence of allergens and the requirement for cold storage facilities, as well as an increasing demand for new foods and tastes have initiated a trend in non-dairy probiotic product development (Martins et al., 2013; Rößle, Auty, Brunton, Gormley, & Butler, 2010; Tripathi & Giri,

* Corresponding author.
E-mail addresses: cinthia@kroton.com.br (C.H.B. Souza), susaad@usp.br (S.M.I. Saad).
[1] Present address: Mestrado em Ciência e Tecnologia de Leite e Derivados, Universidade Norte do Paraná. Rua Marselha, 591, 86041-140 - Londrina, PR, Brazil.

http://dx.doi.org/10.1016/j.lwt.2017.01.032
0023-6438/

2014). Among these new food matrices, margarine has excellent perspectives as a matrix for probiotic incorporation. Margarine has several advantages over yoghurt-type products in terms of delivery of viable probiotics, including its higher pH. Moreover, desirable characteristics for probiotic foods as higher fat content and more solid consistency (Ong, Henriksson, & Shah, 2006) are observed in a margarine food matrix and may offer protection to probiotics through the GIT transit. Also, spreads, like margarine, are a particularly interesting vehicles for functional ingredients, because they are eaten daily. Therefore, the aim of this study was to investigate the effect of inulin, WPC, and/or CMP, in different proportions up to 3%, on the viability and resistance to simulated gastric and enteric conditions of a commercial probiotic strain of *Bifidobacterium animalis* Bb-12 added in probiotic and synbiotic margarine.

2. Materials and methods

2.1. Experimental design and margarine manufacture

Seven pilot scale margarine-making trials, named as M1-M7, were produced according to Table 1, using a *simplex-centroid* design, changing the inulin, whey protein concentrate (WPC), and caseinomacropeptide (CMP) proportions in the margarines (three repetitions of each trial were produced on different days). For each formulation, 3 kg of margarine were obtained. Combinations of the ingredients inulin (Beneo ST-Gel, Orafti, Oreye, Belgium), whey protein concentrate (Lacprodan 80, Arla Foods Ingredients, Sønderhøj, Denmark), and caseinomacropeptide (Lacprodan CGMP 10, Arla Foods Ingredients) were used. A control trial, without supplementation with inulin, WPC and/or CMP (M8) was also prepared. All trials were produced using the freeze-dried probiotic culture of *Bifidobacterium animalis* subsp. *lactis* Bb-12 (Christian Hansen, Hørsholm, Denmark).

Margarine production was carried out in five steps: 1) fat phase production, 2) water phase production, 3) emulsification, 4) crystallization process, and 5) packaging and storage. Different ingredients were employed for preparing the fat and water margarine phases (Table 2).

The following commercial ingredients were employed for the preparation of the fat phase: palm oil (Agropalma, Tailândia, Brazil), canola oil (Liza, Mairinque, Brazil), emulsifiers monoacylglycerol (Myvatex Smooth 5Z10729 and Myverol 18−92K) and polyglycerol esters of ricinoleic acid (Admul Wol 1408K) (Kerry Bio-Science, Campinas, Brazil), colouring agent β-carotene (DSM, São Paulo, Brazil), butter flavour (Danisco, Cotia, Brazil), and vitamin A (Fortitech, Campinas, Brazil). For the water phase, the following ingredients were employed: water, NaCl (Cisne, Cabo Frio, Brazil), food-grade lactic acid 85% solution (Purac Sínteses, Rio de Janeiro, Brazil), skimmed powdered milk (Nestlé, Araçatuba, Brazil). Additionally, according to experimental design (Table 1), the following ingredients were also used in the water phase: inulin (Beneo ST-Gel), whey protein concentrate (Lacprodan 80), and caseinomacropeptide (Lacprodan CGMP 10). Water phase ingredients were manually mixed, after which the probiotic inoculum was added.

The probiotic inoculum of Bb-12 was grown in 40 ml of reconstituted skimmed powdered milk for 2 h at 37 °C, prior to its addition to the water phase, in order to obtain Bb-12 populations of a minimum between 8 and 9 log cfu g^{-1} after margarine production (day 1). For the preparation of the fat phase, palm oil was heated at 45 °C and manually mixed with the additional fat phase ingredients mentioned above.

During the emulsification step (step 3), the water phase was gently added to the fat phase under agitation using a mixer (Stand

Table 2
Ingredients used for the production of the margarine trials studied, according to the experimental design described in Table 1.

Ingredients (%)	Margarine formulations	
	M1-M7	M8*
Water phase		
Water	30.999	33.999
Salt[a]	0.760	0.760
Lactic acid[b]	0.010	0.010
Skimmed powdered milk[c]	0.400	0.400
Probiotic culture[d]	0.500	0.500
Fat phase		
Fat**	60.000	60.000
Emulsifiers[e]	3.400	3.400
Colouring agent[f]	0.001	0.001
Butter flavour[g]	0.050	0.050
Vitamin A[h]	0.880	0.880
Mixture***	3.000	–

– = without supplementation with inulin, WPC, and/or CMP.
* Control trial.
** 60% of palm oil (Agropalma, Tailândia, Brazil) + 40% of canola oil (Liza, Mairinque, Brazil).
*** Margarines M1 - M7 were supplemented with a mixture containing inulin, WPC, and/or CMP, according to Table 1.
[a] (Cisne, Cabo Frio, Brazil).
[b] (food-grade lactic acid 85% solution, Purac Sínteses, Rio de Janeiro, Brazil).
[c] (Nestlé, Araçatuba, Brazil).
[d] (Christian Hansen, Hørsholm, Denmark).
[e] (Kerry Bio-Science, Campinas, Brazil).
[f] (β-carotene, DSM, São Paulo, Brazil).
[g] (Danisco, Cotia, Brazil).
[h] (Fortitech, Campinas, Brazil).

Table 1
Simplex-centroid experimental design employed in the present study.

Margarine Trials	Proportion of ingredients in the mixture (x_1, x_2, x_3)	Quantities of each ingredient (%)		
		Inulin[a] (x_1)	WPC[b] (x_2)	CMP[c] (x_3)
M1	(1, 0, 0)	3.0	0	0
M2	(0, 1, 0)	0	3.0	0
M3	(0, 0, 1)	0	0	3.0
M4	(½, ½, 0)	1.5	1.5	0
M5	(½, 0, ½)	1.5	0	1.5
M6	(0, ½, ½)	0	1.5	1.5
M7	($^1/_3$, $^1/_3$, $^1/_3$)	1.0	1.0	1.0
M8[d]	(0, 0, 0)	–	–	–

– = without inulin, WPC and/or CMP addition.
[a] Inulin (Orafti, 92% inulin +8% fructose, glucose, and sucrose).
[b] Whey protein concentrate (Arla Foods Ingredients, Sønderhøj, Denmark; 82% protein).
[c] Caseinomacropeptide (Arla Foods Ingredients, 85% protein).
[d] M8: control trial.