Brazilian Journal of Microbiology (2007) 38:315-317
ISSN 1517-8382

ACTIVITY OF PASSION FRUIT (*PASSIFLORA EDULIS*) AND GUAVA (*PSIDIUM GUAJAVA*) PULPS ON *LACTOBACILLUS ACIDOPHILUS* IN REFRIGERATED MOUSSES

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Submitted: October 02, 2006; Returned to authors for corrections: January 11, 2007; Approved: March 20, 2007.

SHORT COMMUNICATION

ABSTRACT

Five pilot-scale mousse-making trials were produced and supplemented with *Lactobacillus acidophilus* La-5 probiotic culture: M1 – with passion fruit concentrated juice (PJ); M2 – with PJ and prebiotic fibre inulin; M3 – with frozen passion fruit pulp (PP); M4 – with frozen guava pulp (GP); M5 – with GP and lactic acid. The products were stored refrigerated (4ºC) and M2 and M5 were also stored frozen (-18ºC). Viability of *L. acidophilus* decreased up to 4.7 log cfu.g^{-1} in the 21st day for refrigerated mousses containing passion fruit (M1, M2 and M3), whereas the probiotic population remained above 6 log cfu.g^{-1} in the mousses containing guava (M4 and M5). Inhibition due to acidity was discharged. The addition of fruits to probiotic products should be carefully planned because inhibition of probiotic strains might occur.

Key words: *Lactobacillus acidophilus*, *Passiflora edulis*, *Psidium guajava*, mousse, refrigeration, freezing

Aerated dairy desserts have shown a great market potential, as a function of consumer behaviour, interested in lighter and healthier relish products (2). Mousse is an aerated dessert with stabilized foamy structure that, although traditionally home-made, is nowadays produced on an industrial scale and is gaining space in the dessert market (1). Although the industrial production of aerated dairy desserts is delicate, this food product permits addition of ingredients like chocolate, fruit juices or pulps (orange, lemon, strawberry, among others), besides probiotic microorganisms and prebiotic fibres (2). According to FAO/WHO (4), probiotics are presently defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host. In addition, prebiotics are nondigestible food ingredients that selectively stimulate the growth and/or activity of one or more bacterial species in the colon and thereby beneficially affect the host (6). Inulin and oligofructose, non digestible fermentable fructans, are amongst the most studied and well established prebiotics (5). However, there are insufficient data regarding the effect that fruit juices and/or pulps may have on the survival of microbiota of probiotic foods (12), especially when inulin or other prebiotic ingredient is present. Moreover, when developing a dairy probiotic and synbiotic product containing fruit juices and/or pulps like mousse, the possibility of inhibition of the probiotic strains during refrigerated storage of almost 4 weeks or frozen storage of at least 2-3 month ought to be evaluated, as shelf life of this kind of product is rather limited.

In this study, five pilot-scale mousse-making trials were produced and added of freeze dried commercial *Lactobacillus acidophilus* (LA) La-5 probiotic culture (Christian Hansen, Hoersholm, Denmark): M1 – containing passion fruit (*Passiflora edulis*) concentrated juice (PJ) (Maguary, Kraft Foods, Araguari, Brazil, 125g Kg^{-1}) and LA (0.1g Kg^{-1}); M2 – with PJ (125g Kg^{-1}), LA (0.1g Kg^{-1}) and prebiotic fibre inulin (I) (Beneo™ HP Gel, Orafti, Oreye, Belgium, 40g Kg^{-1}); M3 – with pasteurized and frozen passion fruit pulp (PP) (Doce Mel, Frutos da Bahia, Ipiaú, Brazil, 125g Kg^{-1}) and LA (0.2g Kg^{-1}); M4 – with pasteurized and frozen guava (*Psidium guajava*) pulp (GP) Icefruit-Maisa

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(Icefruit Comércio de Alimentos, Tatuí, Brazil, 125g Kg^{-1}) and LA (0.2g Kg^{-1}); M5 – with GP (125g Kg^{-1}), lactic acid (L) (Purac Sínteses, Rio de Janeiro, Brazil, 4g Kg^{-1}) and LA (0.2g Kg^{-1}). All mousse trials were produced with powdered milk (Molico, Nestlé, Araçatuba, Brazil, 40g Kg^{-1}), sucrose (União, Coopersucar-União, Limeira, Brazil, 110g Kg^{-1}), oligofructose (Beneo™ P95, Orafti 60g Kg^{-1}) and emulsifier (Cremodan® Mousse 30-B, Danisco, Cotia, Brazil 28g Kg^{-1}). Except for M2, which contained inulin instead, all mousses were produced with whole milk cream (Nestlé, 160g Kg^{-1}). Commercial skimmed milk (Paulista, Divisão de Beneficiamento da Danone, Guaratinguetá, Brasil, ultra high temperature [UHT]) was added in all trials to achieve 1Kg of mass.

For mousse manufacture, about 20 ml of skimmed milk was previously separated for fermentation at 37ºC, during 150 minutes, at the proportion of *Lactobacillus acidophilus* (LA) described above for each trial. Meanwhile, the further ingredients were mixed until complete homogenization of the mass. The mass obtained, for each trial, was pasteurized at 85ºC, during 25 seconds. Next, the temperature was reduced to 40ºC for the addition of the skimmed milk previously fermented with the LA culture. The mass was cooled to 15ºC for aeration. In this process, the mass achieved a volume of about 80-85% of its initial volume, according to Andreasen & Nielsen (1). Subsequently, the mousses were packaged in plastic pots and sealed with a metallic cover. The batches of mousses M2 (with passion fruit juice plus inulin) and M5 (with guava pulp plus lactic acid) were divided in two halves: a half was stored under refrigeration at 4ºC and the other one was stored under freezing at -18ºC. The batches of mousses M1 (with passion fruit juice), M3 (with passion fruit pulp) and M4 (with guava pulp) were stored only at 4ºC.

Refrigerated mousse samples were analyzed in the first day and after 21 days of storage. M2 frozen samples were used for analysis after 1, 21 and 69 days of storage. M5 frozen samples were used for analysis after 1, 7 and 56 days of storage. At each sampling day, at least two pots of mousse from the same trial were used for analysis. For *Lactobacillus acidophilus* counts, 25 g were collected aseptically, after a quick homogenisation of the mousse inside the pot with a sterilized spoon, blended with 225 mL of 0.1% peptone water in a Bag Mixer 400 (Interscience, St. Nom, France) and serially diluted using the same diluent. The pH values of mousses were determined in the remaining samples with a pH-meter Analyser Model 300 M (Analyser, São Paulo, Brazil) equipped with a penetration electrode model 2AO4 GF (Analyser). *Lactobacillus acidophilus* was counted by pour-plating 1 mL of each dilution in modified DeMan-Rogosa-Sharpe (MRS) agar, prepared as a basal medium containing maltose, as described by the International Dairy Federation (7), after 2 days of aerobic incubation at 37ºC. Descriptive analysis was used for the comparison of trials.

To provide health benefits, the suggested concentration for probiotic bacteria is, at least, 6 log CFU ml^{-1} or g^{-1} of a product during its shelf life (8, 10). In the refrigerated mousses M1, M2 and M3, produced with passion fruit, the viability of *Lactobacillus acidophilus* started above 6 log cfu g^{-1} in the first day of storage. Population of probiotic decreased, respectively, 3.2 and 4.2 log cfu.g^{-1} cycles after 21 days of storage for refrigerated mousses M1 (with passion fruit juice) and M3 (with passion fruit pulp) (Table 1). The reduction of viability of *Lactobacillus acidophilus* was higher in refrigerated mousse M2 (with passion fruit juice plus inulin), and decreased 4.7 log cfu.g^{-1} in the 21st day of storage.

On the other hand, in refrigerated mousses M5 and M4, both with guava and, respectively, with and without lactic acid, the viability of *Lactobacillus acidophilus* remained above 6 log cfu.g^{-1} until the end of the storage period of 21 days. Although the pH of refrigerated mousses with passion fruit M1, M2 and M3 was lower than the pH of mousse M4 (with guava), this factor was not responsible for the decreased viability of the probiotic. This statement is based on the fact that mousse M5, produced with guava, as well as M4, presented high viability of the probiotic even when the pH was reduced by the addition of lactic acid. In this case, the decrease in viability was due to the chemical composition of the passion fruit, instead of its pH (Table 1). Vinderola *et al.* (12) verified that one *Lactobacillus acidophilus* strain (CNRZ 1881) had its growth affected by strawberry, pineapple and kiwi juices, but this viability was not reduced when the pH of juices was neutralized. In the case of passion fruit juice and pulp, some possible compounds involved in the inhibition of *Lactobacillus acidophilus* growth could be ascorbic acid, carotenoids, aroma compounds (thiols, terpenes, alcohols) and fatty acid esters, like ethyl butanoate, ethyl hexanoate and hexyl butanoate (9,11).

Contrary to what was observed in refrigerated mousses with passion fruit, frozen mousse M2 maintained the viability of *Lactobacillus acidophilus* above 6 log cfu.g^{-1} after 69 days of storage at -18ºC. This also occurred in frozen mousse M5, supplemented with guava, after 56 days of storage, in which the viability of the probiotic was higher than in the same refrigerated product after the 21st day of storage (Table 1). In fact, freezing is a more stable state for preservation of biological molecules and microorganisms, like *Lactobacillus acidophilus*, when compared to refrigeration (3).

Therefore, the addition of passion fruit juice or pulp was not appropriated for the production of refrigerated mousses supplemented with *L. acidophilus* and stored at 4ºC. The addition of passion fruit or guava did not affect the viability of *Lactobacillus acidophilus* in frozen mousses, which presented high populations of probiotic until the end of storage. Consequently, freezing of samples was suitable to preserve the viability of *Lactobacillus acidophilus* and also to enlarge shelf-life of mousses. Thus, the addition of fruits to probiotic products should be carefully planed because inhibition of probiotic strains might occur. In counterpart, the inhibitory effect of passion

Table 1. Values of pH and population of *Lactobacillus acidophilus* obtained for the different mousse trials studied.

Mousse trial	Storage temperature	Storage days	pH	*L. acidophilus* (log cfu.g^{-1})
M1 $^{(PJ)}$	4ºC	1	5.00	7.28
		21	4.94 ± 0.02	4.09 ± 0.36
M2 $^{(PJ+I)}$	4ºC	1	4.98 ± 0.02	6.51
		21	4.98	1.78
	-18ºC	1	5.00	6.74
		21	5.03	6.57
		69	5.03	6.35
M3 $^{(PP)}$	4ºC	1	4.99	6.64
		21	5.00	2.45
M4 $^{(GP)}$	4ºC	1	6.05	7.09
		21	6.05	6.94
M5 $^{(GP+L)}$	4ºC	1	4.93 ± 0.01	7.36 ± 0.07
		21	4.93 ± 0.02	6.34 ± 0.15
	-18ºC	1	4.91 ± 0.01	7.25 ± 0.02
		7	5.01 ± 0.01	7.43 ± 0.03
		56	5.06 ± 0.01	7.29 ± 0.06

M1 $^{(PJ)}$ = concentrated passion fruit juice; M2 $^{(PJ+I)}$ = concentrated passion fruit juice + inulin; M3 $^{(PP)}$ = pasteurized and frozen passion fruit pulp; M4 $^{(GP)}$ = pasteurized and frozen guava pulp; M5 $^{(GP+L)}$ = pasteurized and frozen guava pulp + lactic acid. Except for M2, containing inulin instead, mousses were produced with whole milk cream.

fruit observed in the present study against a desirable microorganism ought to be better explored and tested in other food systems, aiming the natural preservation against contaminant and pathogenic microorganisms.

AKNOWLEDGMENTS

The authors are grateful to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (Projects 05/51317-8, 04/13597-6 and 06/51297-0) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support.

RESUMO

Atividade das polpas de maracujá (*Passiflora edulis*) e goiaba (*Psidium guajava*) sobre *Lactobacillus acidophilus* em musses refrigeradas

Cinco musses foram produzidas em escala piloto e suplementadas com a cultura probiótica de *Lactobacillus acidophilus* La-5: M1 - com suco concentrado de maracujá (SM); M2 - com SM e fibra prebiótica inulina; M3 - com polpa congelada de maracujá (PM); M4 - com polpa congelada de goiaba (PG); M5 - com PG e ácido lático. Os produtos foram armazenados refrigerados (4ºC) e M2 e M5 também congelados (-18ºC). A viabilidade de *Lactobacillus acidophilus* diminuiu em até 4,7 log ufc.g^{-1} ao 21º dia nas musses contendo maracujá (M1, M3 e M2), enquanto a população do probiótico permaneceu acima de 6 log ufc.g^{-1} naquelas contendo goiaba (M4 e M5). A inibição devido à acidez foi descartada. A adição de frutas aos produtos probióticos deve ser cuidadosamente planejada, uma vez que pode haver inibição das cepas probióticas.

Palavras-chave: *Lactobacillus acidophilus*, *Passiflora edulis*, *Psidium guajava*, musse, refrigeração, congelamento.

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