Springer

Dear Author,

Here are the proofs of your article.

- You can submit your corrections **online**, via **e-mail** or by **fax**.
- For **online** submission please insert your corrections in the online correction form. Always indicate the line number to which the correction refers.
- You can also insert your corrections in the proof PDF and **email** the annotated PDF.
- For fax submission, please ensure that your corrections are clearly legible. Use a fine black pen and write the correction in the margin, not too close to the edge of the page.
- Remember to note the **journal title**, **article number**, and **your name** when sending your response via e-mail or fax.
- **Check** the metadata sheet to make sure that the header information, especially author names and the corresponding affiliations are correctly shown.
- **Check** the questions that may have arisen during copy editing and insert your answers/ corrections.
- **Check** that the text is complete and that all figures, tables and their legends are included. Also check the accuracy of special characters, equations, and electronic supplementary material if applicable. If necessary refer to the *Edited manuscript*.
- The publication of inaccurate data such as dosages and units can have serious consequences. Please take particular care that all such details are correct.
- Please **do not** make changes that involve only matters of style. We have generally introduced forms that follow the journal's style.
  Substantial changes in content, e.g., new results, corrected values, title and authorship are not allowed without the approval of the responsible editor. In such a case, please contact the Editorial Office and return his/her consent together with the proof.
- If we do not receive your corrections **within 48 hours**, we will send you a reminder.
- Your article will be published **Online First** approximately one week after receipt of your corrected proofs. This is the **official first publication** citable with the DOI. **Further changes are, therefore, not possible.**
- The **printed version** will follow in a forthcoming issue.

Please note

After online publication, subscribers (personal/institutional) to this journal will have access to the complete article via the DOI using the URL: http://dx.doi.org/[DOI].
If you would like to know when your article has been published online, take advantage of our free alert service. For registration and further information go to: http://www.link.springer.com.

Due to the electronic nature of the procedure, the manuscript and the original figures will only be returned to you on special request. When you return your corrections, please inform us if you would like to have these documents returned.

Metadata of the article that will be visualized in OnlineFirst

Please note: Images will appear in color online but will be printed in black and white.

ArticleTitle	Malt sprout, an underused beer by-product with promising potential for the growth and dehydration of lactobacilli strains	
Article Sub-Title		
Article CopyRight	Association of Food Scientists & Technologists (India) (This will be the copyright line in the final PDF)	
Journal Name	Journal of Food Science and Technology	
Corresponding Author	Family Name	**Gómez-Zavaglia**
	Particle	
	Given Name	**Andrea**
	Suffix	
	Division	
	Organization	Center for Research and Development in Food Cryotechnology (CIDCA, CCT-CONICET La Plata)
	Address	Calle 47 y 116, 1900, La Plata, Buenos Aires, Argentina
	Phone	+54(221)4890741
	Fax	
	Email	angoza@qui.uc.pt
	URL	
	ORCID	http://orcid.org/0000-0002-8705-0160
Author	Family Name	**Cejas**
	Particle	
	Given Name	**Luján**
	Suffix	
	Division	
	Organization	Center for Research and Development in Food Cryotechnology (CIDCA, CCT-CONICET La Plata)
	Address	Calle 47 y 116, 1900, La Plata, Buenos Aires, Argentina
	Phone	
	Fax	
	Email	
	URL	
	ORCID	
Author	Family Name	**Romano**
	Particle	
	Given Name	**Nelson**
	Suffix	
	Division	
	Organization	Center for Research and Development in Food Cryotechnology (CIDCA, CCT-CONICET La Plata)
	Address	Calle 47 y 116, 1900, La Plata, Buenos Aires, Argentina

	Phone	
	Fax	
	Email	
	URL	
	ORCID	
Author	Family Name	**Moretti**
	Particle	
	Given Name	**Ana**
	Suffix	
	Division	
	Organization	Center for Research and Development in Food Cryotechnology (CIDCA, CCT-CONICET La Plata)
	Address	Calle 47 y 116, 1900, La Plata, Buenos Aires, Argentina
	Phone	
	Fax	
	Email	
	URL	
	ORCID	
Author	Family Name	**Mobili**
	Particle	
	Given Name	**Pablo**
	Suffix	
	Division	
	Organization	Center for Research and Development in Food Cryotechnology (CIDCA, CCT-CONICET La Plata)
	Address	Calle 47 y 116, 1900, La Plata, Buenos Aires, Argentina
	Phone	
	Fax	
	Email	
	URL	
	ORCID	
Author	Family Name	**Golowczyc**
	Particle	
	Given Name	**Marina**
	Suffix	
	Division	
	Organization	Center for Research and Development in Food Cryotechnology (CIDCA, CCT-CONICET La Plata)
	Address	Calle 47 y 116, 1900, La Plata, Buenos Aires, Argentina
	Phone	
	Fax	
	Email	
	URL	
	ORCID	

Schedule	Received	

	Revised	28 September 2017
	Accepted	9 October 2017

Abstract	Malt sprout (MS), a by-product of the malt industry obtained by removing rootlets and sprouts from the seed of germinated barley (*Hordeum vulgare* L.), was used as culture, dehydration and storage medium of three strains of lactobacilli: *Lactobacillus salivarius* CM-CIDCA 1231B and CM-CIDCA 1232Y and *Lactobacillus plantarum* CIDCA 83114. The three strains were grown in MS and MS supplemented with 20% w/v fructo-oligosaccharides (MS FOS). Bacterial growth was determined by registering the decrease of pH and by plate counting. Results comparable with those of microorganisms grown in MRS (controls) were observed in terms of *lag* times, ΔpH and acidification rates. Furthermore, during fermentation, a significant increase of DP6 (FOS with degree of polymerization 6) was observed at expenses of inulin and DP7, probably indicating their hydrolysis. A concomitant decrease of DP3, sucrose and monosaccharides was also observed, as result of their bacterial consumption during growth. The presence of FOS in the fermented media protected microorganisms during freeze-drying and storage, as no decrease of culturability was observed after 60 days at 4 °C (> 10^8 CFU/mL). Using MS appears as an innovative strategy for the production at large scale, supporting their use for the elaboration of functional foods containing prebiotics and probiotics.

Keywords (separated by '-')	Malt sprout - Culture medium - Fructo-oligosaccharides - Lactobacilli - Dehydration
Footnote Information	

J Food Sci Technol
DOI 10.1007/s13197-017-2927-7

ORIGINAL ARTICLE

Malt sprout, an underused beer by-product with promising potential for the growth and dehydration of lactobacilli strains

Luján Cejas[1] · Nelson Romano[1] · Ana Moretti[1] · Pablo Mobili[1] · Marina Golowczyc[1] · Andrea Gómez-Zavaglia[1]

Revised: 28 September 2017 / Accepted: 9 October 2017

Abstract Malt sprout (MS), a by-product of the malt industry obtained by removing rootlets and sprouts from the seed of germinated barley (*Hordeum vulgare* L.), was used as culture, dehydration and storage medium of three strains of lactobacilli: *Lactobacillus salivarius* CM-CIDCA 1231B and CM-CIDCA 1232Y and *Lactobacillus plantarum* CIDCA 83114. The three strains were grown in MS and MS supplemented with 20% w/v fructo-oligosaccharides (MS FOS). Bacterial growth was determined by registering the decrease of pH and by plate counting. Results comparable with those of microorganisms grown in MRS (controls) were observed in terms of *lag* times, ΔpH and acidification rates. Furthermore, during fermentation, a significant increase of DP6 (FOS with degree of polymerization 6) was observed at expenses of inulin and DP7, probably indicating their hydrolysis. A concomitant decrease of DP3, sucrose and monosaccharides was also observed, as result of their bacterial consumption during growth. The presence of FOS in the fermented media protected microorganisms during freeze-drying and storage, as no decrease of culturability was observed after 60 days at 4 °C (> 10^8 CFU/mL). Using MS appears as an innovative strategy for the production at large scale, supporting their use for the elaboration of functional foods containing prebiotics and probiotics.

Keywords Malt sprout · Culture medium · Fructo-oligosaccharides · Lactobacilli · Dehydration

✉ Andrea Gómez-Zavaglia
angoza@qui.uc.pt

[1] Center for Research and Development in Food Cryotechnology (CIDCA, CCT-CONICET La Plata), Calle 47 y 116, 1900 La Plata, Buenos Aires, Argentina

Introduction

Malt sprout is a by-product of the malt industry, obtained after removing rootlets and sprouts from the seed of germinated barley (*Hordeum vulgare* L.). Because of its bulkiness, malt sprout is usually pelleted to increase density for shipment, the pellets having ca. 95% dry matter. The organic matter accounts ca. 89%, including high contents of proteins (21–25%) and carbohydrates (46%), majorly composed of fructo-oligosaccharides (FOS) (Aborus et al. 2017). Due to this nutritional richness, malt sprout has attracted strong interest as feedstuff (Nurfeta and Abdu 2014). In fact, it has been used as an economical protein and energy source in mixed dairy or beef cattle and horse feeds, and also incorporated in swine and poultry rations (Šidagis et al. 2014). In addition, extracts from malt sprouts have been studied as glutathione sources for bread making as well as amino nitrogen sources for beer yeast fermentation, and its proteolytic activity has also been reported (Waters et al. 2013; Brestenský et al. 2013; Kondo et al. 2016). Moreover, malt sprout has also been reported as bacterial nutrient in culture media for the production of antibiotics, pectinases, amylase, L-lysine, citric acid, butanol, acetone and lactic acid, as well as for yeasts and mold cultivation (Hujanen et al. 2001).

Lactic acid bacteria have an important role in food and biotechnology industries, as they are widely used as starters for the manufacturing of food and probiotic products. Although MRS is a well-established culture medium at a laboratory scale, its high cost is not compatible with large-scale commercial applications. Therefore, the production of lactic acid starters at an industrial level requires cost effective culture media allowing an adequate production of bacterial biomass. For this reason, industries are continuously seeking for cost effective media, nutritionally

Journal : Large 13197 | Dispatch : 10-10-2017 | Pages : 9
Article No. : 2927 | LE | TYPESET
MS Code : JFST-D-17-01377 | CP | DISK

valuable by-products being of special interest. In this regard, whey, whey permeate, okara and several other agro-wastes have been proposed as alternative culture media for lactic acid bacteria production (Golowczyc et al. 2013; Londero et al. 2012, 2014, 2015; Quintana et al. 2017). Malt sprout also fulfills these desired requirements, and thus, it has been used in the past to grow *Bacillus* and *Rhizobium* species (Bioardi and Ertola 1985) or more recently added as nitrogen source in other culture media (Liu et al. 2010; Yegin et al. 2017). Regarding lactic acid bacteria, malt sprout itself has been proposed as an efficient culture medium for large-scale production of lactobacilli, with results similar to those obtained in MRS medium (Laitila et al. 2004).

The nutritional value of malt sprout could also go beyond its efficiency as culture medium. In this sense, it is worth to mention that the presence of FOS of different degrees of polymerization (DP) in malt sprout, could be considered as an added value to develop novel applications. In fact, prebiotics present in other fermented by-products (i.e., okara or whey permeate), have been reported as protective compounds when such media are subsequently used for bacterial dehydration (Golowczyc et al. 2013; Quintana et al. 2017). The carbohydrate nature of FOS has been reported as responsible for their protective effect during dehydration and other technological processes, as well as during storage (Quintana et al. 2017; Romano et al. 2015, 2016; Santos et al. 2014). Therefore, the FOS present in malt sprout could provide additional technological benefits when incorporated in the formulation of functional foods or feeds.

For all these reasons, the aim of this work was to use malt sprout as culture, dehydration and storage medium for three strains of lactobacilli (*Lactobacillus salivarius* CM-CIDCA 1231B *L. salivarius* CM-CIDCA 1232Y and *Lactobacillus plantarum* CIDCA 83114), performing a comprehensive study of the role of malt sprout oligosaccharides during bacterial stabilization.

Materials and methods

Preparation of malt sprout medium (MS) and determination of its composition

AQ2 Malt sprouts were obtained from a local brewery industry (Malteria PAMPA S.A.). After reception, they were soaked in distilled water (1 L water for 70 g of dry material), placed in a microwave oven for 3 min and then, sieved to eliminate nitrogen-rich roots and grains. Then, they were sterilized in an autoclave at 110 °C for 30 min, cooled to room temperature, and filtered through a 0.22 mm filter to remove the non-dissolved material. The filtered medium was supplemented with 20% w/v FOS.

The composition of the freshly filtered medium (MS) was determined as recommended by the Association of Official Analytical Chemists (AOAC 1995). Lipids were assessed by extraction with diethyl ether/petroleum ether solvent (1:1 ratio) in a Soxhlet system (AOAC 1995). Ash content was determined by carbonization of the dried samples followed by incineration in a muffle furnace at 550 °C. Total nitrogen was determined using the micro-Kjeldahl method (conversion factor to transform nitrogen into protein: 6.25). The composition was expressed in g/100 g dry basis (d.b.), and total carbohydrates, estimated by difference (100 − total grams of humidity, protein, lipids, and ash).

Growth conditions

The filtered medium obtained in the previous section was then used to grow *L. salivarius* CM-CIDCA 1231B, *L. salivarius* CM-CIDCA 1232Y and *L. plantarum* CIDCA 83114 isolated from kefir grains (Garrote et al. 2001). The strains were maintained frozen at − 80 °C in 120 g/L non-fat milk solids (Difco, MI, USA), and activated for 24 h in de Man, Rogosa, Sharpe (MRS) broth at 37 °C in aerobic conditions. The resulting culture was inoculated (inoculum 1%) in fresh MRS and incubated in the same conditions. Cultures in the stationary phase were used to inoculate 100 mL of MS and MS supplement with 20% w/v FOS (MS FOS) (inoculum 2%). They were then incubated at 37 °C. Microorganisms grown in MRS broth were used as controls.

The three growth kinetics were followed by determining the decrease of pH and also by plate counting in MRS agar every 2 h. Results were expressed as log colony forming units per mL (log CFU/mL).

Carbohydrate composition of MS and MS FOS before and after fermentation

The sugar composition of MS and MS FOS before and after fermentation was analyzed by high performance liquid chromatography (HPLC) in a Perkin-Elmer Series 200 equipment (Milford, MA, USA) with refractive index detector and autosampler. A Waters Sugar Pak I chromatographic column for carbohydrate analysis was used to resolve glucose, sucrose, DP3-DP7 (10 µm, 6.5 mm × 300 mm) (Milford, MA, USA). The pump flow rate was 0.5 mL/min; column temperature: 80 °C; injection volume: 20 µL. Column and detector temperatures were maintained at 50 and 40 °C, respectively. To resolve inulin from high DP FOS (i.e., DP7), a Waters Ultrahydrogel Column Linear (10 µm, 7.8 mm × 300 mm) with

Journal : Large 13197 Dispatch : 10-10-2017 Pages : 9
Article No. : 2927 LE TYPESET
MS Code : JFST-D-17-01377 CP DISK

Ultrahydrogel Guard Column (6 µm, 6 mm × 40 mm) (Milford, MA, USA) was used. The pump flow rate was 0.6 mL/min, the column temperature: 20 °C and the injection volume: 20 µL.

Samples were prepared by filtering both fermented and non-fermented MS and MS FOS through 0.22 µm Millipore Durapore membranes (Billerica, MA, USA) and eluted with milli-Q water (mobile phase) at a flow-rate of 0.4 mL/min. Chromatograms were integrated using Total Chrom software (version 6.3.1, Perkin Elmer, USA).

The composition of samples was determined by assuming that the area of each peak was proportional to the weight percentage of the respective sugar on the total sugar mass. The accuracy of such assumption was checked by making a material balance. External standards of fructose, glucose, sucrose, 1-kestose (DP3), nystose (DP4) and 1^F-fructofuranosylnystose (DP5), oligofructose and inulin (Sigma, MO, USA) were used to determine their retention times and check the linear range of the measurements.

Freeze-drying

Aliquots of 1 mL of MS and MS FOS containing microorganisms in the stationary phase were transferred into 5 mL glass vials under aseptic conditions, frozen at − 80 °C for 48 h and freeze-dried − 50 °C for 48 h using a Heto FD4 freeze drier (Heto Lab Equipment, Denmark). Results were expressed as log (N/N_0), where N and N_0 were the CFU/mL after and before freeze-drying, respectively.

Storage

The obtained samples were stored for 60 days at 4°C. Culturability was determined immediately after freeze-drying, and then, at regular intervals. For each determination, samples were re-hydrated in 1 mL 0.85% w/v NaCl. Bacterial suspensions were serially diluted, plated on MS agar [MS to whom 1.5% w/v agar (Difco, MI, USA) were added], and incubated at 37°C for 48 h in aerobic conditions.

Statistical analysis

All experiments were performed on duplicate samples using three independent cultures of bacteria. The relative differences were reproducible irrespective of the cultures used. Analysis of variance (ANOVA) was carried out using the statistical program Infostat v2009 software (Córdoba, Argentina). Differences were tested with paired sample Tukey comparison tests, and if $p \leq 0.05$ the difference was considered statistically significant.

Results

The filtered MS used as culture medium had a humidity of 7.49 ± 1.39 and was composed of 30.92 ± 0.69 g/100 d.b. of proteins, 2.03 ± 0.96 g/100 d.b. of lipids, 9.09 ± 0.32 of ashes and 50.47 g/100 d.b. of carbohydrates. Figure 1a–c show the kinetics of growth corresponding to *L. salivarius* CM-CIDCA 1231B, *L. salivarius* CM-CIDCA 1232Y, and *L. plantarum* CIDCA 83114, respectively, grown in MS, MS FOS and MRS (control medium), as determined by the decrease of pH. Results were adjusted according to Eq. 1 (Romano et al. 2016):

$$pH(t) = \frac{pH_0 - pH_f}{1 + \frac{t^p}{c}} + pH_f \quad (1)$$

where t is the time in hours, pH_0 is the pH of the culture medium at time equal to 0, pH_f is the pH once attained the stationary phase, c is the time at the inflection point and p is an exponential fitting factor. The *lag* time was calculated as the intersection between the tangent line at pH_0 and $t = c$. The acidification rate during the exponential phase was calculated as the module of the slope of the tangent line. The values of the *lag* time, acidification rate, final pH and ΔpH for all the growth kinetics are shown in Table 1. Both MS and MS FOS were intrinsically more acid than the control MRS medium. That is why the curves corresponding to the pH kinetics were shifted along the y-axis (pH). In spite of that, no strong differences in the ΔpH were observed when compared with the corresponding values for microorganisms grown in MS and MS FOS.

The *lag* times for *L. salivarius* CM-CIDCA 1231B and *L. salivarius* CM-CIDCA 1232Y grown in MS were significantly shorter than those of the same strains grown in MS FOS, which in turn were shorter than those of the controls grown in MRS ($p \leq 0.05$) (Table 1). In addition, it is remarkable that the acidification rate in MS was about twice that observed in MS FOS and MRS (Table 1). The behavior of *L. plantarum* CIDCA 83114 was different in regard to some parameters. For example, MS FOS was the medium in which microorganisms grew the best in terms of *lag* times and acidification rates, followed by MRS and MS (Table 1).

The performance of microorganisms grown in MS and MS FOS was also analyzed by plate counting (Fig. 1d–f). MS FOS was the best medium for all the three strains, followed by MS FOS and MRS. *L. plantarum* CIDCA 83114 showed similar growth kinetics in the latter two media (Fig. 1f).

The sugar composition of both MS and MS FOS before and after fermentation is shown in Table 2. Fermentation of MS led to significant differences in the carbohydrate composition, for all the three strains investigated. When compared with MS, fermented MS showed a significant

Journal : Large 13197 Dispatch : 10-10-2017 Pages : 9
Article No. : 2927 LE TYPESET
MS Code : JFST-D-17-01377 CP DISK

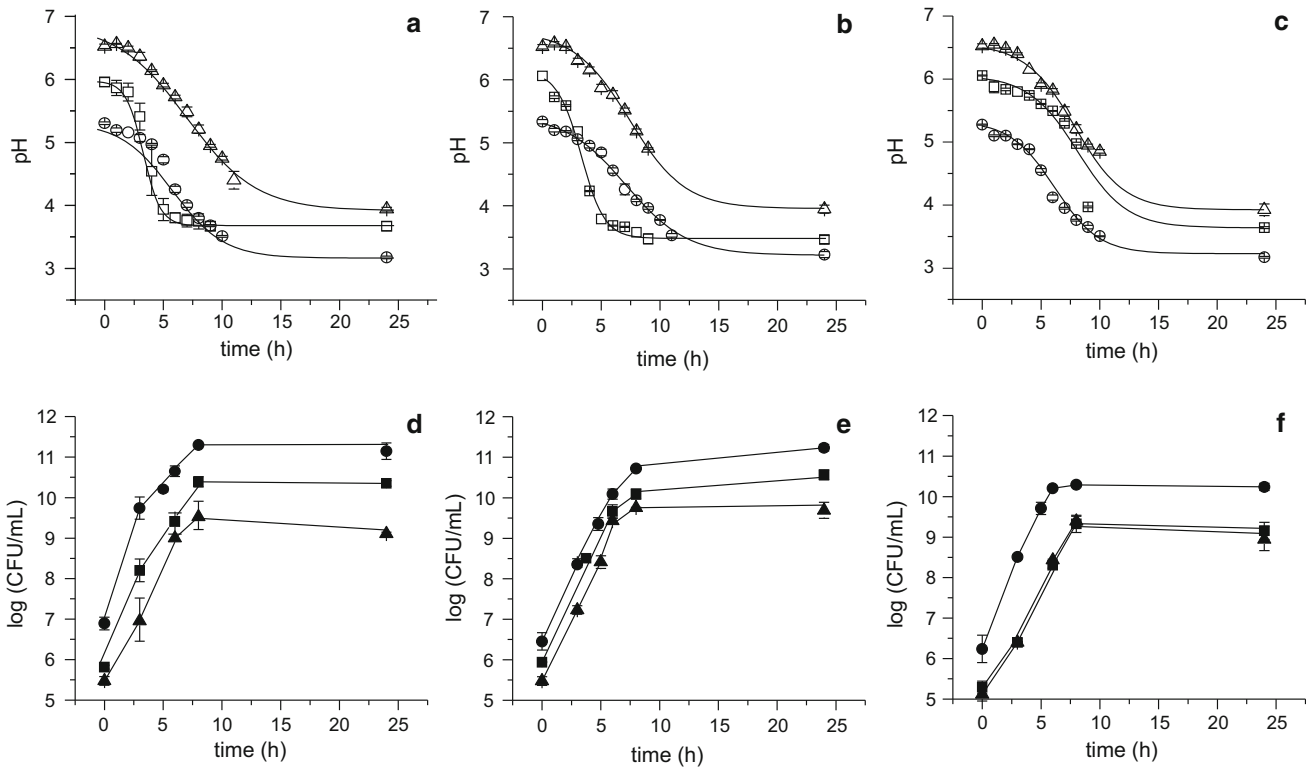

Fig. 1 Growth kinetics of microorganisms in MS (squares), MS FOS (circles) and MRS (triangles) (control), determined by monitoring pH and plate counting: **a**, **d** *L. salivarius* CM-CIDCA 1231B; **b**, **e** *L. salivarius* CM-CIDCA 1232Y; **c**, **f** *L. plantarum* CIDCA 83114

Table 1 Kinetic parameters of microorganisms grown in MS, MS FOS and MRS

	MS	MS FOS	MRS
Lactobacillus salivarius CM-CIDCA 1231B			
ΔpH	2.29	2.13	2.63
Lag time (h)	2.29*	3.66**	3.81***
Medium acidification rate (pH units/h)	0.777*	0.444**	0.321***
pH (final)	3.67	3.18	3.94
R^2	0.998	0.995	0.990
Lactobacillus salivarius CM-CIDCA 1232Y			
ΔpH	2.50	2.23	2.78
Lag time (h)	1.04*	1.92**	2.34***
Medium acidification rate (pH units^{-1}h)	0.520*	0.215***	0.251**
pH (final)	3.47	3.22	3.78
R^2	0.989	0.992	0.995
Lactobacillus plantarum CIDCA 83114			
ΔpH	2.32	2.10	2.63
Lag time (h)	3.37***	2.38*	2.63**
Medium acidification rate (pH units/h)	0.172*	0.278***	0.249**
pH (final)	3.64	3.23	3.93
R^2	0.989	0.994	0.995

Asterisks indicate significant differences

decrease of inulin, DP7, DP3, sucrose and glucose ($p \leq 0.05$), concomitantly with a significant increase of DP6 ($p \leq 0.05$). DP4 and DP5 significantly decreased as well ($p \leq 0.05$), but in a lesser extent than DP3 (Table 2). It is also noteworthy that the absolute concentration of carbohydrates for the fermented MS was lower than that of

Journal : Large 13197 Dispatch : 10-10-2017 Pages : 9
Article No. : 2927 LE TYPESET
MS Code : JFST-D-17-01377 CP DISK

Table 2 Composition of MS and MS FOS before and after fermentation

Carbohydrate	MS (mg/mL)	MS + *L. salivarius* CM-CIDCA 1231B (mg/mL)	MS + *L. salivarius* CM-CIDCA 1232Y (mg/mL)	MS + *L. plantarum* CIDCA 83114 (mg/mL)
Inulin	1.29 ± 0.02 (6.21)*	n.d.**	n.d.**	n.d.**
DP7	11.72 ± 0.32 (56.48)*	4.03 ± 0.13 (40.74)**	4.44 ± 0.35 (40.81)**	5.35 ± 0.71 (41.2)**
DP6	0.63 ± 0.14 (3.01)*	5.39 ± 0.02 (54.56)**	6.20 ± 0.31 (57.05)***	6.53 ± 0.35 (50.3)***
DP5	0.33 ± 0.03 (1.59)*	0.10 ± 0.03 (1.04)**	0.19 ± 0.02 (1.74)**	0.77 ± 0.04 (5.9)***
DP4	0.36 ± 0.14 (1.73)*	0.25 ± 0.02 (2.55)**	0.04 ± 0.01 (0.40)***	0.07 ± 0.01 (0.5)***
DP3	4.58 ± 0.09 (22.08)*	0.06 ± 0.00 (0.59)**	n.d.***	0.21 ± 0.01 (1.6)****
Sucrose	0.72 ± 0.20 (3.46)*	0.05 ± 0.04 (0.52)**	n.d.***	0.06 ± 0.01 (0.5)**
Glucose/ fructose	1.13 ± 0.34 (5.44)*	n.d.**	n.d.**	n.d.**
TOTAL	20.76 ± 0.10 (100)*	9.88 ± 0.32 (100)**	10.87 ± 0.01 (100)***	12.99 ± 1.05 (100)****

Carbohydrate	Composition commercial FOS (mg/mL)[a]	MS FOS (mg/mL)	MS FOS + CM-CIDCA 1231B (mg/mL)	MS FOS + CM-CIDCA 1232Y (mg/mL)	MS FOS + CIDCA 83114 (mg/mL)
Inulin	n.d.*	1.29 ± 0.03 (0.60)**	n.d.*	n.d.*	n.d.*
DP7	8.40 ± 1.00 (4.20)*	8.15 ± 0.37 (3.83)**	7.83 ± 0.18 (4.03)***	8.65 ± 1.87 (4.03)*	7.18 ± 0.01 (4.02)****
DP6	40.80 ± 0.80 (20.38)*	44.86 ± 0.86 (21.06)**	47.64 ± 0.18 (22.74)***	50.57 ± 1.98 (23.52)***	42.76 ± 0.88 (23.96)**
DP5	25.40 ± 0.20 (12.69)*	33.04 ± 0.37 (15.51)**	32.94 ± 0.74 (15.37)**	31.74 ± 0.14 (14.77)**	25.87 ± 0.01 (14.50)*
DP4	50.20 ± 0.20 (25.07)*	51.07 ± 1.37 (23.98)*	49.39 ± 0.56 (23.05)*	48.82 ± 0.71 (22.71)*	39.80 ± 0.05 (22.30)**
DP3	58.00 ± 1.20 (28.97)*	51.31 ± 1.65 (24.10)**	48.74 ± 0.55 (22.74)**	49.06 ± 1.41 (22.82)**	40.39 ± 0.01 (22.64)***
Sucrose	8.00 ± 2.00 (4.00)*	10.33 ± 0.08 (4.85)**	11.13 ± 0.76 (5.19)***	10.56 ± 0.07 (4.91)**	8.99 ± 0.00 (5.04)*
Glucose/ fructose	9.40 ± 1.60 (4.70)*	12.94 ± 3.56 (6.08)**	16.63 ± 2.02 (7.76)***	15.58 ± 0.96 (7.25)***	13.44 ± 0.56 (7.53)****
TOTAL	200.20* (100)	213.00 ± 0.98 (100)**	214.30 ± 0.77 (100)***	214.99 ± 1.07 (100)***	178.44 ± 0.27 (100)****

Numbers in parentheses denote the percentage composition

n.d. not detected

[a]Determined on 20% w/v solutions

Asterisks in rows indicate significant differences

the non-fermented medium. For MS FOS, the concentration of total carbohydrates was much higher than that of MS, as result of the supplementation with FOS (Table 2). The main carbohydrates present in MS FOS were DP6, DP5, DP4 and DP3, all of them accounting more than 10% of the total carbohydrates and having a similar concentration than in commercial FOS. After fermentation, a pretty similar behavior than that observed in fermented MS was remarked (Table 2).

In a further step, the efficiency of fermented MS and MS FOS as dehydration media during freeze-drying was investigated. To this aim, bacteria in the stationary phase previously grown in those media were freeze-dried. The microbial logarithm decay after the process is shown in Fig. 2. Dehydrating microorganisms in 0.85% w/v NaCl or in not neutralized MS were the worst situations for all the three strains (Fig. 2, numbers 1 and 2). The suspension of microorganisms in fresh medium (with almost neutral pH) slightly improved the recovery of all the three strains (number 3). Neutralization of the growing media before freeze-drying noticeably enhanced the bacterial recovery (numbers 4 and 5). Although some minor strain dependent differences were observed for bacteria grown in MS or in MS FOS, both neutralized growing media significantly improved the recovery of all the three strains after freeze-drying ($p \leq 005$). The effect of FOS during freeze-drying is shown in numbers 6, 7 and 8 of Fig. 2. The only addition of FOS in the growth medium did not contribute to

Journal : Large 13197 Dispatch : 10-10-2017 Pages : 9
Article No. : 2927 LE TYPESET
MS Code : JFST-D-17-01377 CP DISK

stabilize bacteria during the process (number 6). On the contrary, the addition of FOS just during freeze-drying bacteria grown in MS or in MRS (numbers 7 and 8, respectively) were the best conditions to stabilize microorganisms during freeze-drying (Fig. 2). In summary, it can be concluded that neutralizing the culture medium (MS or MS FOS) and the presence of FOS in the dehydration medium are key factors to improve the bacterial recovery during freeze-drying. On this basis, these three conditions (numbers 4, 5 and 7 in Fig. 2) were selected to investigate bacterial stability during storage at 4 °C (Fig. 3). No significant logarithmic decays were observed in none of the conditions assayed up to 60 days of storage (Fig. 3).

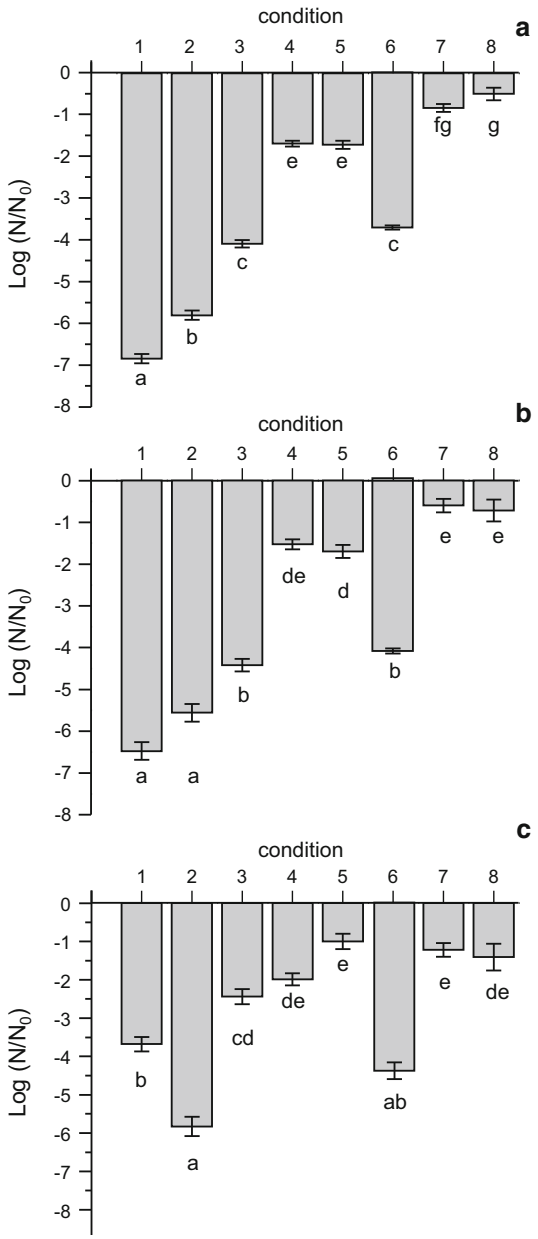

Fig. 2 Log (N/N_0) after freeze-drying. N_0 and N are the culturability of microorganisms before and after freeze-drying, respectively. **a** *L. salivarius* CM-CIDCA 1231B; **b** *L. salivarius* CM-CIDCA 1232Y; c: *L. plantarum* CIDCA 83114. Different letters (a, b) denote statistically significant differences ($p \leq 0.05$). Numbers in the x-axes denote: 1–4: bacteria grown in MS and freeze-dried in: 1: the same culture medium; 2: 0.145 mol/L NaCl; 3: fresh MS medium; 4: the same medium after neutralization; 5: bacteria grown in MS FOS and freeze-dried after neutralization; 6: bacteria grown in MRS FOS and freeze-dried in 0.85% w/v NaCl; 7: bacteria grown in MS and freeze-dried after neutralization and addition of 20% w/v FOS; 8: bacteria grown in MRS and freeze-dried in 20% w/v FOS

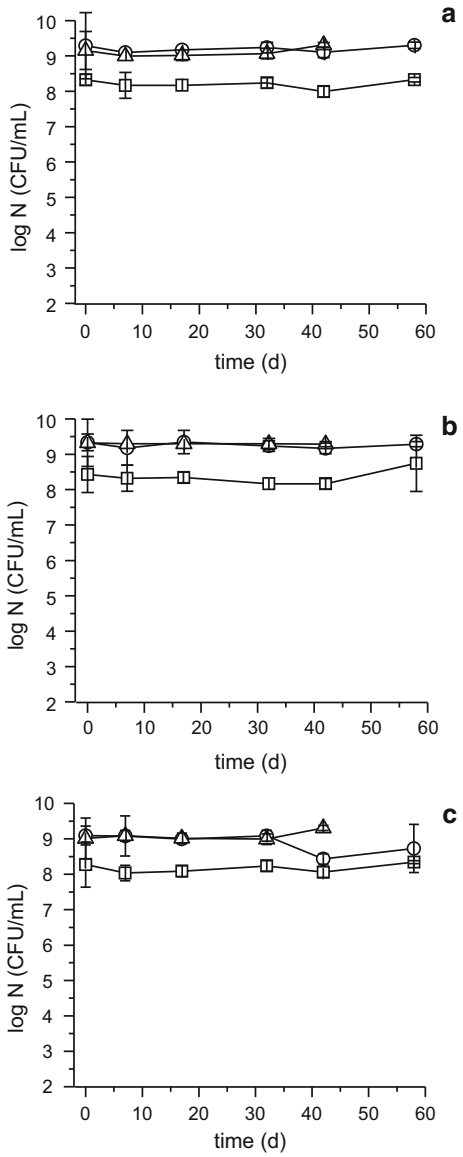

Fig. 3 Log N during storage at 4 °C. N: viability after each time of storage. **a** *L. salivarius* CM-CIDCA 1231B; **b** *L. salivarius* CM-CIDCA 1232Y; **c** *L. plantarum* CIDCA 83114. Squares: bacteria grown in MS and freeze-dried in the same medium after neutralization; Circles: bacteria grown in MS FOS and freeze-dried in the same medium after neutralization; triangles: bacteria grown in MS and freeze-dried after addition of 20% w/v FOS

Journal : Large 13197 Dispatch : 10-10-2017 Pages : 9
Article No. : 2927 LE TYPESET
MS Code : JFST-D-17-01377 CP DISK

Discussion

Using by-products for the production of lactic acid bacteria is a current trend at an industrial level. The industrial production of lactic acid bacteria requires the use of cost-effective media and milk derivatives are mostly used to this aim. The worldwide increasing environmental concerns have stimulated the investigation of different applications for agro-industrial by-products, also contributing to add them value. This led to the development of products of high added value, with great possibilities of being incorporated in the formulation of novel foods and feeds. In this context, the composition of MS, rich in proteins and carbohydrates (sources of nitrogen and energy, respectively), supports its use as culture medium for lactic acid bacteria. In the past, MS has showed to be an efficient medium for the production of rhizobia biomass (Bioardi and Ertola 1985), and a source of nitrogen when used to supplement commercial culture media (Liu et al. 2010). However, it has been very scarcely used to produce lactobacilli biomass (Laitila et al. 2004). Therefore, using such a nutritionally rich by-product as culture medium appears as an interesting strategy to both add value to a by-product and employ a cost-effective medium for the production of lactic acid bacteria starters at a large scale.

In this work, MS was used to grow three strains of lactobacilli, *L. salivarius* CM-CIDCA 1231B, *L. salivarius* CM-CIDCA 1232Y and *L. plantarum* CIDCA 83114. It is noticeable that the first two strains grown in MS were those with the shortest *lag* time and the highest acidification rate (Table 1 and Fig. 1). This can be explained considering the nutritional richness and availability of nutrients in MS, which enabled a quick adaptation to the medium, even faster than in the widely recognized MRS medium (control) (Fig. 1). On the contrary, even when MS was also an adequate medium for *L. plantarum* CIDCA 83114, it was only comparable with the traditional MRS when supplemented with FOS (Table 1). Note that the performance of this latter strain was similar for MRS and MS FOS.

Analyzing the evolution of the different carbohydrates after fermentation enables a comprehensive interpretation of the obtained results. Whereas the decrease of inulin and DP7 in fermented MS is the result of hydrolysis, that of shorter FOS and simple sugars indicates their use as energy source for lactobacilli growth. Note also that mono, disaccharides and DP3 decreased to undetectable levels (Table 2), which supports their preferable use before that of larger FOS. This behavior also explains why the absolute concentration of carbohydrates decreased from 20.76 ± 0.10 to 10–13 mg/mL after fermentation (Table 2), as the shortest FOS were presumably used to produce organic acids.

Supplementing MS with FOS enhanced its capacity as culture medium, when growing *L. salivarius* CM-CIDCA 1231B and *L. salivarius* CM-CIDCA 1232Y, converting it in a medium even better than the traditional MRS (control) (Fig. 1). Although the addition of FOS to MS led to a decrease of pH, microorganisms were able to grow properly in MS FOS, attaining the highest CFU/mL in the stationary phase (circles in Fig. 1d–f). The lower pH, together with the increase in osmolarity (resulting from the addition of 20% w/v FOS) could probably be responsible for the larger *lag* time and lower acidification rates of microorganisms grown in this medium with regard to those grown in MS and MRS. In spite of that, once adapted to the acid medium, microorganisms grew properly, reaching about up to 10^{10}–10^{11} CFU/mL in the stationary phase.

The osmotic stress adaptation has been previously reported (Panoff et al. 2000; Tymczyszyn et al. 2007). In fact, the presence of high concentrations of sugars (i.e., lactose, sucrose or trehalose) or other polyols (i.e., glycerol or polyethylenglycol) promotes bacterial adaptation and leads to a better resistance to dehydration processes. In the context of this work, this explains not only the larger *lag* times of microorganisms grown in MS FOS, but also their better resistance to subsequent stresses (Fig. 2, number 6) (Panoff et al. 2000; Tymczyszyn et al. 2007; Ferreira et al. 2005). As a matter of fact, prior growing in low water activity media not only improves the bacterial yield during growth, but also the efficiency of sugars such as sucrose as protective compounds when drying sensitive microorganisms (Tymczyszyn et al. 2007).

A thorough analysis of the composition of fermented MS and MS FOS also allowed the understanding of the role of sugars during freeze-drying and storage (Table 2; Figs. 2, 3). AQ3 The chemical analysis of MS and MS FOS after fermentation showed an increase of FOS at expenses of inulin and DP7. This indicates that the dehydration media were composed of FOS arising from the hydrolysis of inulin and DP7, even in the case that MS had not been previously supplemented with FOS. The role of these FOS as lyoprotectants has been recently investigated (Romano et al. 2016). Fermented MS at low pH was the worst dehydration medium (Fig. 2, number 1), because although FOS are stable at low pH (Vega and Zuñiga-Hansen 2015), microorganisms did not, as previously reported (Golowczyc et al. 2013). Suspending microorganisms grown in MS, in fresh medium (pH 6) significantly improved their recovery after freeze-drying (Fig. 2 number 3), but neutralization of the fermented MS appeared a much better strategy (Fig. 2 number 4). The addition of FOS in the growth medium was not an additional protecting factor, as bacteria grown in MS FOS and freeze-dried in the same medium after neutralization showed no significant improvement with regard to those grown in MS (Fig. 2, numbers 4 and 5). Furthermore, microorganisms grown in MRS FOS and freeze-dried in

Journal : Large 13197 Dispatch : 10-10-2017 Pages : 9
Article No. : 2927 LE TYPESET
MS Code : JFST-D-17-01377 CP DISK

0.85% w/v NaCl showed an important decrease of culturability (Fig. 2, number 6), also supporting this hypothesis. Adding FOS in the dehydration medium did improve the bacterial recovery during freeze-drying (Fig. 2, numbers 7 and 8). This results are consistent with those reported before (Tymczyszyn et al. 2007), for sucrose and trehalose. In fact, these sugars potentiate the recovery of *L. delbrueckii* subsp. *bulgaricus* when added in the dehydration media, and not when added just in the growing media. This indicates that these sugars shall be present in the fermented media during dehydration. This behavior also explains the protective capacity of the fermented MS media (with or without the addition of FOS during growth). As FOS, sucrose and monosaccharides were still present after fermentation (Table 2), and their protective capacity is well-known (Romano et al. 2016; Tymczyszyn et al. 2007, 2008), they acted as protective compounds during freeze-drying. However, the addition of FOS to the dehydration media improved even more the bacterial recovery, especially for *L. salivarius* strains, because of the well-known protective properties of FOS (Romano et al. 2016). These results underline two main issues to be considered: the importance of neutralizing the dehydration medium to improve bacterial recovery, and the presence of FOS in the dehydration media to potentiate bacterial protection. Furthermore, it must not be forgotten that the freeze-dried fermented MS and MS FOS also contained bacterial metabolites, which in turn have been reported to be efficient in promoting body weight and feed conversion in farm animals (Denli et al. 2003). Hence, it could be considered as an additional added value if feedstuff are to be developed.

The potentiality of the studied strains in the formulation of probiotic containing food and feed encouraged the investigation of their stability during storage (Fig. 3), showing no significant decrease of culturability after 60 days at 4 °C. Vitrification has an essential role on bacterial stability during storage (Romano et al. 2016). The presence of FOS with higher DP plays a protective role during storage because they have higher vitreous transition temperatures (Romano et al. 2016; Blanch et al. 2012). The presence of high concentrations of FOS DP6 in both fermented MS and MS FOS supports their protective capacity during storage. In turn, the addition of commercial FOS, with reported protective capacity (Romano et al. 2016), explained the stability of bacteria grown in MS and freeze-dried in externally added FOS during storage (Fig. 3).

Conclusions

Considering that cost-effective culture and dehydration media are mandatory for the production of commercial probiotics, using MS in both functions appears as an innovative strategy that fulfills this aim. Besides containing probiotic bacteria whose culturability did not decrease after 60 days of storage at 4 °C, the dehydrated products included ingredients with great potential in the formulation of functional foods and feeds, namely prebiotic carbohydrates (FOS) and other bacterial metabolites (i.e.: short chain organic acids).

Acknowledgements This work was supported by the Argentinean Agency for the Scientific and Technological Promotion (ANPCyT) and Nitrap S.R.L. (Projects PID/2014/0049 and PICT/2014/0912). M.G., P.M. and A.G.-Z. are members of the research career CONICET. N.R. is postdoctoral fellow from CONICET. L.C. and A.M. are doctoral fellows from ANPCyT and CIC, respectively. The authors acknowledge Advance Biotechnology Company S.A. for the fruitful discussions and for financial support.

References

Aborus NE, Čanadanović-Brunet J, Ćetković G, Šaponjac VT, Vulić J, Ilić N (2017) Powdered barley sprouts: composition, functionality and polyphenol digestibility. Int J Food Sci Technol 52:231–238. doi:10.1111/ijfs.13274

Association of Official Analytical Chemists (AOAC) (1995) Official methods of analysis, 16th edn. Association of Official Analytical Chemists (AOAC), Washington

Bioardi JL, Ertola RJ (1985) Rhizobium biomass production in batch and continuous culture with a malt-sprouts medium. World J Microbiol Biotechnol 1:163–172. doi:10.1007/BF01742581

Blanch M, Goñi O, Sanchez-Ballesta MT, Escribano MI, Merodio C (2012) Characterisation and functionality of fructo-oligosaccharides affecting water status of strawberry fruit (*Fragraria vesca* cv. *Mara de Bois*) during postharvest storage. Food Chem 134:912–919. doi:10.1016/j.foodchem.2012.02.203

Brestenský M, Nitrayová S, Patráš P, Heger J (2013) Standardized ileal digestibilities of amino acids and nitrogen in rye, barley, soybean meal, malt sprouts, sorghum, wheat germ and broken rice fed to growing pigs. Anim Feed Sci Technol 186:120–124. doi:10.1016/j.anifeedsci.2013.09.006

Denli M, Çelik K, Okan F (2003) Comparative effects of feeding diets containing flavomycin, bioteksin-L and dry yeast (*Saccharomyces cerevisiae*) on broiler performance. J Appl Anim Res 23:139–144. doi:10.1080/09712119.2003.9706415

Ferreira V, Soares V, Santos C, Silva J, Gibbs P, Texeira PM (2005) Survival of *Lactobacillus sakei* during heating, drying and storage in the dried state when growth has occurred in the presence of sucrose or monosodium glutamate. Biotechnol Lett 27:249–252. doi:10.1007/s10529-004-8351-x

Garrote GL, Abraham AG, De Antoni GL (2001) Chemical and microbiological characterisation of kefir grains. J Dairy Res 68:639–652. doi:10.1017/S0022029901005210

Golowczyc M, Vera C, Santos M, Guerrero C, Carasi P, Illanes A, Gomez-Zavaglia A, Tymczyszyn E (2013) Use of whey permeate containing in situ synthesied galacto-oligosaccharides for the growth and preservation of *Lactobacillus plantarum*. J Dairy Res 80:374–381. doi:10.1017/S0022029913000356

Hujanen M, Linko S, Linko YY, Leisola M (2001) Optimisation of media and cultivation conditions for L(+)(S)-lactic acid production by *Lactobacillus casei* NRRL B-441. Appl Microbiol Biotechnol 56:126–130. doi:10.1007/s002530000501

Kondo K, Nagao K, Yokoo Y (2016) Process for producing food and beverage products from malt sprouts. US patent 9326542 B2

Journal : Large 13197 | Dispatch : 10-10-2017 | Pages : 9
Article No. : 2927 | LE | TYPESET
MS Code : JFST-D-17-01377 | CP | DISK

Laitila A, Saarela M, Kirk L, Siika-Aho M, Haikara A, Mattila-Sandholm T, Virkajärvi I (2004) Malt sprout extract medium for cultivation of *Lactobacillus plantarum* protective cultures. Lett Appl Microbiol 39:336–340. doi:10.1111/j.1472-765X.2004.01579.x

Liu B, Yang M, Qi B, Chen X, Su Z, Wan Y (2010) Optimizing L-(+)-lactic acid production by thermophile *Lactobacillus plantarum* As. 1.3 using alternative nitrogen sources with response surface method. Biochem Eng J 52:212–219. doi:10.1016/j.bej.2010.08.013

Londero A, Hamet MF, De Antoni GL, Garrote GL, Abraham AG (2012) Kefir grains as a starter for whey fermentation at different temperatures: chemical and microbiological characterisation. J Dairy Res 79:262–271. doi:10.1017/S0022029912000179

Londero A, León Peláez MA, Diosma G, De Antoni GL, Abraham AG, Garrote GL (2014) Fermented whey as poultry feed additive to prevent fungal contamination. J Sci Food Agric 94:3189–3194. doi:10.1002/jsfa.6669

Londero A, Iraporda C, Garrote GL, Abraham AG (2015) Cheese whey fermented with kefir micro-organisms: antagonism against Salmonella and immunomodulatory capacity. Int J Dairy Technol 68:118–126. doi:10.1111/1471-0307.12161

Nurfeta A, Abdu Y (2014) Feeding value of different levels of malt sprout and katikala atella on nutrient utilization and growth performance of sheep fed basal diet of Rhodes grass hay. Trop Anim Health Prod 46:541–547. doi:10.1007/s11250-013-0527-8

Panoff J-M, Thammavongs B, Guéguen M (2000) Cryoprotectants lead to phenotypic adaptation to freeze–thaw stress in *Lactobacillus delbrueckii* ssp. *bulgaricus* CIP 101027T. Cryobiology 40:264–269. doi:10.1006/cryo.2000.2240

Quintana G, Gerbino E, Gómez-Zavaglia A (2017) Okara: a nutritionally valuable by-product able to stabilize *Lactobacillus plantarum* during freeze-drying, spray-drying, and storage. Front Microbiol 8:641–650. doi:10.3389/fmicb.2017.00641

Romano N, Tymczyszyn E, Mobili A, Gómez-Zavaglia A (2015) Prebiotics as protectants of lactic acid bacteria. In: Watson RR, Preedy VR (eds) Bioactive foods in promoting health: probiotics, prebiotics, and synbiotics. Part 1: prebiotics in health promotion, 2nd edn. Academic Press, San Diego, pp 155–164. doi:10.1016/B978-0-12-802189-7.00010-1

Romano N, Schebor C, Mobili P, Gómez-Zavaglia A (2016) Role of mono- and oligosaccharides from FOS as stabilizing agents during freeze-drying and storage of *Lactobacillus delbrueckii* subsp. *bulgaricus*. Food Res Int 90:251–258. doi:10.1016/j.foodres.2016.11.003

Santos MI, Araujo-Andrade C, Esparza-Ibarra Tymczyszyn EE, Gómez-Zavaglia A (2014) Galacto-oligosaccharides and lactulose as protectants against desiccation of *Lactobacillus delbrueckii* subsp. *bulcaricus*. Biotechnol Prog 30:1231–1238. doi:10.1002/btpr.1969

Šidagis D, Uchockis V, Bliznikas S (2014) Effect of malt sprouts on nutrient fermentation in the rumen of cows and their productivity. Vet Med Zoot 65:97–101

Tymczyszyn EE, Gómez-Zavaglia A, Disalvo EA (2007) Effect of sugars and growth media on the dehydration of *Lactobacillus delbrueckii* ssp. *bulgaricus*. J Appl Microbiol 102:845–851. doi:10.1111/j.1365-2672.2006.03108.x

Tymczyszyn EE, Díaz MR, Pataro A, Sandonato N, Gómez-Zavaglia A, Disalvo EA (2008) Critical water activity for the preservation of *Lactobacillus bulgaricus* by vacuum drying. Int J Food Microbiol 128:342–347. doi:10.1016/j.ijfoodmicro.2008.09.009

Vega R, Zuñiga-Hansen ME (2015) The effect of processing conditions on the stability of fructooligosaccharides in acidic food products. Food Chem 15:784–789. doi:10.1016/j.foodchem.2014.10.119

Waters DM, Kingston W, Jacob F, Titze J, Arendt EK, Zannini E (2013) Wheat bread biofortification with rootlets, a malting by-product. J Sci Food Agric 93:2372–2383. doi:10.1002/jsfa.6059

Yegin S, Buyukkileci AO, Sargin S, Goksungur Y (2017) Exploitation of agricultural wastes and by-products for production of *Aureobasidium pullulans* Y-2311-1 xylanase: screening, bioprocess optimization and scale up. Waste Biomass Valoriz 8:999–1010. doi:10.1007/s12649-016-9646-6

Journal : Large 13197 Dispatch : 10-10-2017 Pages : 9
Article No. : 2927 LE TYPESET
MS Code : JFST-D-17-01377 CP DISK

Journal : **13197**
Article : **2927**

Springer
the language of science

Author Query Form

Please ensure you fill out your response to the queries raised below and return this form along with your corrections

Dear Author

During the process of typesetting your article, the following queries have arisen. Please check your typeset proof carefully against the queries listed below and mark the necessary changes either directly on the proof/online grid or in the 'Author's response' area provided below

Query	Details Required	Author's Response
AQ1	Kindly check and confirm the authors given name and family name are correctly identified. Also, kindly confirm the details in the metadata are correct.	
AQ2	Please confirm the section headings are correctly identified.	
AQ3	Kindly check and confirm that Figs. 3 and 4 citation has been changed as Figs. 2 and 3 in near by the text „A thorough analysis of the …". Please check if action taken is appropriate and amend if necessary.	
AQ4	Kindly check and confirm the updated reference Association of Official Analytical Chemists (AOAC) (1995) is correct.	