Co-fermentation of onion and whey: a promising synbiotic combination

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Federica Tinello[a†], Veronica Vendramin[a†], Vitória Barros Divino[b], Laura Treu[ac], Viviana Corich[a*], Anna Lante[a], Alessio Giacomini[a]

[a]Department of Agronomy Food Natural Resources Animals and Environment (DAFNAE) Agripolis, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy

[b] Universitade Federal de Ouro Preto, MG, Brasile

[c] Department of Environmental Engineering, Technical University of Denmark, Kongens Lyngby, Denmark

[†] These authors contributed equally to this work

[*]Corresponding author. Viviana Corich

E-mail address: viviana.corich@unipd.it

ABSTRACT

Juice from three different onion varieties was mixed with sweet whey and used as growth substrate for four lactic acid bacteria strains, isolated from agri-food by-products, to evaluate the possibility to exploit such substrates, known to be reach in bioactive molecules, as fermented drinks for human consumption. Results show good growth performance for *Lactobacillus fabifermentans*, *L. plantarum* and *Streptococcus macedonicus*. On the contrary *S. thermophilus* did not grow in the mixture while *S. macedonicus* did not develop in pure onion juice. After 48 h the overall sugar content decreased significantly. In particular, glucose was not utilized while inulin was completely preserved. Moreover, MS/MS analysis revealed the presence of the rare trisaccharide lactosucrose.

In the light of these considerations, the formulation obtained may be considered a potential synbiotic product with pleasant taste and beneficial effects for consumers and also an eco-friendly solution to convert an agro-food by-product into value added products.

Keywords: prebiotic, lactosucrose, *Lactobacillus*, *Streptococcus*, functional food

Highlights

- Onion juice and whey were used for growth of *Lactobacillus* and *Streptococcus* strains
- Lactosucrose was produced by bacteria during growth in onion juice and whey
- Inulin present in onion juice remained in the medium after bacterial growth

1. Introduction

Currently, the research of new functional foods is focused on synbiotic obtained by combining probiotics and prebiotics in order to further improve human health benefits (Pandey, Naik, & Vakil, 2015; Krumbeck, Maldonado-Gomez, Ramer-Tait, & Hutkins, 2016).

Lactic acid bacteria (LAB) are the most widely used microorganisms for production of fermented foods (Kumar, Vijayendra, & Reddy, 2015) for their capability to enhance shelf-life, safety, organoleptic and nutritional properties (Lante, Nardi, Zocca, Giacomini, & Corich, 2011). They are widely recognized for their beneficial effects towards gastrointestinal diseases, human immune system, lactose intolerance, allergies and fungal infections (Masood, Qadir, Shirazi, & Khan, 2011) as a consequence of their metabolic activities, immunomodulation, and interaction with the intestinal microbiota (Parvez, Malik, Ah Kang, & Kim, 2006; De Almada, De Almada, Martinez, & De Souza Sant'Ana, 2015). The key role of LAB in vegetable fermentation has been definitely established (Tamang, Watanabe, & Holzapfel, 2016). During growth, microbial metabolism transforms plant material leading to the production of bioactive compounds and/or improve the final pleasantness of the foods (Di Cagno, Coda, De Angelis, & Gobbetti, 2013). Spontaneously fermented foods (Pogačić et al., 2010; Paramithiotis, Doulgeraki, Karahasani, & Drosinos, 2014; Paramithiotis, Kouretas, & Drosinos, 2014; Lucena-Padrós, & Ruiz-Barba, 2016) or even by-products from food production (Bovo et al. 2012; Favaro, Corich, Giacomini, Basaglia, & Casella, 2013) contain autochthonous microbial communities that constitute the source for the isolation of new potentially interesting bacteria and yeasts.

Onion (*Allium cepa* L.), which is taxonomically included in the *Liliaceae* family, is a vegetable whose cultivation is widespread worldwide and accounts for more than 88 million t produced in 2014 (FAO, 2017). Compounds from onion have been reported to have a range of health benefits including anticarcinogenic, antiplatelet, antithrombotic, antiasthmatic and antibiotic activities (Griffiths, Trueman, Crowther, Thomas, & Smith, 2002). Onions contain also prebiotic substances such as fructooligosaccharides (FOS) and inulin (Sangeetha, Ramesh, & Prapulla, 2005) which are indigestible to humans enzymes but promote growth and activity of bacterial strains and simultaneously enhance health benefits in the large intestine (Al-Sheraji et al., 2013). Moreover, lactic acid fermentation can reduce the typical pungency flavor of raw onions leading to a more favourable product for the consumers (Roberts & Kidd, 2005).

Sweet whey is the main by-product of cheese production, a highly polluting material produced in high amounts, since only about 10% of the milk is transformed in cheese (Ryan & Walsh, 2016). It is rich in fermentable sugars (ca. 5% lactose) and contains numerous bioactive compounds (Madadlou & Abbaspourrad, 2016).

The present work is aimed at developing a new functional food enriched in prebiotics and lactic acid bacteria by co-fermenting onion juices and sweet milk whey with *Lactobacillus* and *Streptococcus* strains. After evaluating microbial growth performance in juices of different onion varieties, the characteristics of the fermented products have been investigated in order to formulate a potential synbiotic product that, besides being beneficial for consumers, can convert an agro-food by-product into value added products.

2. Materials and methods

2.1 Strain selection and culture conditions

The lactic acid bacteria (LAB) used in this study were from our laboratory collection, namely *Lactobacillus fabifermentans* T30PCM01 (DSM 28391) (Treu, Vendramin, Bovo, Giacomini, & Corich, 2014) and *L. plantarum* T30PCM38 (DSM 28393) (Campanaro et al., 2014) isolated from grape marcs (Maragkoudakis et al., 2013), *Streptococcus macedonicus* 33MO (Vendramin et al., 2014) and *S. thermophilus* TH1436 (Treu et al., 2014) isolated from dairy products.

Lactobacilli were grown in de Man, Rogosa and Sharpe (MRS) broth (Oxoid) at 30°C while streptococci were cultivated in M17 medium (Oxoid) supplemented with 0.5% lactose at 37°C. Growth in liquid media was measured by optical density at 600 nm (OD_{600}).

In order to use the same amount of bacteria for each experiment, Cryobeads, porous beads intended as carriers to support the viability of microorganisms during storage (Thermo Fisher Scientific, Rodano, IT) were prepared according to manufacturer's instruction using stationary phase cultures and stored at -80° C. The number of bacterial cells adherent to each Cryobead was verified cells by plate count to be 10^7. One bead was used as inoculum for each experiment by transferring it into the growth substrate.

2.2 Preparation of onion juices

Onions from red, white and yellow varieties (RO, WO and YO, respectively) were

purchased in a local market and stored at 4 °C in the dark until required. Onions were peeled of their dry outer skin, de-stemmed and finally processed in a centrifugal juicer (Moulinex JU655, Groupe SEB, Ecully, France) to obtain juice (Tinello & Lante, 2017). After pH measurement, juices were centrifuged at 3,864 *g* for 15 min at 4 °C, filtered through a Millipore 0.45 μm filter membrane (Merck Millipore, Billerica, MA, USA) and stored at -20 °C in the dark.

2.3 Fermentation of onion juice

Microbial cultures were grown in 15-ml sterile falcon tubes without agitation. Each strain was inoculated into 10 mL of raw onion juice at the final concentration of 10^6 cells/mL by adding one Cryobead to each tube. Samples of raw onion juice without inoculum were used as control.

Tubes were incubated at 30 °C for lactobacilli and 37 °C for streptococci. Each experiment was repeated three times. After 96 h samples were collected for microbiological and chemical analyses.

2.3 Co-fermentation of onion and whey

Milk whey powder (Lactalis, Laval, France) was dissolved into deionized water to 20% w/v in 250-mL flasks and pasteurized for 15 min at 72 °C (Maragkoudakis et al., 2016).

Fermentation media were prepared by mixing sweet whey with onion juice at 1:1 ratio. Each strain was inoculated into the mixture at a concentration of 10^6 cells/mL and incubated

for 48 hours at 30 °C or 37 °C, depending on the species. One sample of medium without inoculum was used as negative control. Each experiment was repeated three times.

2.4 Microbiological analyses

Viable cell counts were determined by the spread plate method. Samples were diluted in sterile saline solution (0.9% NaCl w/v) and spread on plates. Plate Count Agar (Oxoid) was used for total bacterial counts, incubated at 30 °C for 24 h.

2.5 High performance liquid chromatography (HPLC) analysis of sugars

Sugars (fructose, galactose, glucose, inulin, and sucrose) contents were determined by HPLC analysis using a SpectraSYSTEM^{TM} UV6000LP HPLC system (Thermo Finnigan, San Jose, CA, USA) with diode-array detection and an Aminex HPX-87C anion exchange column (Bio-Rad, Hercules, CA) in accordance with the method proposed by Kagkli, Corich, Bovo, Lante, & Giacomini (2016). Before injection into the column, samples were filtered through Millipore 0.22 μm filter membranes (Merck Millipore, Billerica, MA, USA). The mobile phase was deionized water, the temperature of analysis was 85°C and the flow rate was 0.6 mL/min. Sugars were identified by comparing their retention times with those of commercial standards.

2.6 Tandem mass spectrometry (MS/MS) analysis

Further analysis of the peaks collected from the HPLC elution of the fermented mixtures of sweet whey and yellow onion juice (YOW) with *L. fabifermentans, L. plantarum* and *S. macedonicus* (retention time of 9.02 min) was made by MS/MS analysis using a Thermo Scientific™ LTQ Orbitrap XL™ Hybrid Ion Trap-Orbitrap Mass Spectrometer system (Thermo Fisher Scientific, Waltham, MA, USA) with Fourier Transform (FT) detector. Prior to analysis the sample was dissolved in methanol/0.1% v/v formic acid (1:1 v/v). The flow rate of the constant infusion was 3 μL/min. The capillary voltage and temperature were 4 kV and 200 °C, respectively. Identification of the compounds composing the peak was performed according to the mass-to-charge ratio (m/z) of its corresponding fragment ions.

2.7 Statistical analysis

Data, which were presented as means ± standard deviation (SD) of three replicates, were subjected to one-way analysis of variance (ANOVA), after verifying the normal distribution and homogeneity of variance, using the PROC GLM of SAS (Statistical Analysis System, 2013). The model included the onion cultivars and LAB strains as fixed effects. Differences among means with $P \leq 0.05$ were accepted as representing statistically significant differences in accordance with the Tukey's multiple range test.

3. Results and discussion

3.1 Evaluation of onion juices

Firstly, WO, RO and YO juices were assessed for their suitability as substrate for LAB growth, since it is well known that these microbes have complex nutritional requirements (Ozcelik et al. 2016).

Four strains were chosen: *L. fabifermentans* T30PCM01 and *L. plantarum* T30PCM38 isolated from grape marcs; *S. macedonicus* 33MO and *S. thermophilus* TH1436 coming from dairy products. LAB belonging to the genera *Lactobacillus* and *Streptococcus* are widely recognized for their health benefits as probiotics (Parvez, Malik, Ah Kang, & Kim, 2006; Masood, Qadir, Shirazi, & Khan, 2011. At species level *L. plantarum* (Seddik et al., 2017, Fijan, 2014) and *S. thermophilus* (Uriot et al., 2017, De Vries, Vaughan, Kleerebezem & de Vos, 2006) are known to include strains with probiotic properties. *L. fabifermentans* is a recently introduced species, but it is closely related to *L. plantarum* (De Bruyne et al., 2009). Several data are also available on probiotic characteristics of *S. macedonicus* strains (Zoumpopoulou et al., 2008). We wanted to check primarily if these strains could act as starters in the mixture and, in case of positive response, further work will be devoted to the study of their probiotic potential.

Regarding pH (Table 1), all onion juices showed similar, slightly acidic values. The juices were also rich in sugars but with significantly different ($P \leq 0.001$) contents (Table 1). In details, the juices from RO showed about twice the sugar content of WO, while YO had intermediate values. Moreover, RO juices showed the highest content of fermentable monosaccharides, especially fructose and glucose, which represented 39% and 47% respectively of total sugars determined by HPLC analysis, while YO juice had the highest

content of sucrose. Interestingly, inulin, which is a prebiotic used in human diets for its known health benefits (Peshev, & Van den Ende, 2014, Al-Sheraji et al., 2013) was present in YO sample at double concentration with respect to RO and WO. Galactose content was negligible in all juices.

3.1 Fermentation of onion juices

In order to verify the capability of onion juice to support the growth of the selected strains, they were inoculated in RO, WO e YO juices and incubated for 96 h at 30 °C for lactobacilli and 37 °C for streptococci. Streptococci did not grow on any of the onion juices. This could be ascribed to their inability to use the sugars present, but it has also been reported the possible toxic activity of onion for some species of *Streptococcus* (Kim, 1997). Both lactobacilli grew very well in all the juices increasing their population to more than 10^8 cfu/ml (Table 2). *L. fabifermentans* did not show significant differences among the different onion types while *L. plantarum* grew better in WO and YO.

Both lactobacilli lowered the pH between 3.41 and 3.72 (Table 2), a value that very well hampers the growth of bacterial pathogens.

Regarding total sugars, their content significantly decreased ($P \leq 0.001$) after bacterial growth in all onion juices (Table 2). In details, lactobacilli consumed the sugars in a similar manner by significantly decreasing ($P \leq 0.001$) the content of monosaccharides whose averaged percentage reductions of fructose, galactose and glucose were higher in YO (25%, 39%, 39%, respectively) compared with RO (18%, 19%, 28%, respectively) and WO (26%, 27%, 21%, respectively) (Table 2). This could be the consequence of the more

efficient growth of *L. plantarum* in YO with respect to RO and WO (almost 3X higher cell counts).

Instead, lactobacilli did not use sucrose, since its values remained always unchanged (Table 2). Even if *L. plantarum* is generally able to use this sugar (Gänzle & Follador, 2012), in the tested onion juices lactobacilli preferred to use other sugars. Hence they did not need to activate sucrose usage because of the good availability of its constituting monosaccharides, glucose and fructose.

Interestingly, inulin was completely preserved in all fermented onion juices (Table 2).

3.1 Co-fermentation of onion juice and whey

Sweet whey was mixed with onion juices and used as growth substrate for LAB. Among those tested, YO juice was chosen for this study, since it contained the highest amounts of inulin and allowed the growth of the highest number of bacteria (Table 1, 2).

Whey provides mainly lactose, organic nitrogen from whey proteins and mineral salts. Therefore streptococci, that did not grow in onion juices alone, could have a chance to develop in the mixture, hence they were tested again. *L. fabifermentans, L. plantarum, S. macedonicus* and *S. thermophilus* where added singularly to the YOW mixture and incubated for 48 h at 30 °C for streptococci and 37 °C for lactobacilli.

Among streptococci, *S. macedonicus,* that did not grow in pure onion juice, developed very well in the mixture (Table 3), evidently taking advantage from lactose as energy source and probably also from organic nitrogen. Instead, *S. thermophilus* did not grow in the mixture.

Considered that this bacterium was isolated from dairy environment, the absence of growth should evidently be attributed to some substances present in the onion juice that ~~resulted~~ were toxic for the strain.

Lactobacilli grew on the mixture (Table 3) at levels comparable with those obtained in the sole onion juice (Table 2). All three strains lowered the pH to 3.9 (Table 3), a value on average only slightly higher than that reached in onion juices alone (Table 2), but still effective for pathogens control.

Regarding sugars, their overall content decreased significantly ($P \leq 0.01$), but not dramatically, after fermentation (Table 3). In details, fructose was consumed mainly by *L. plantarum* (36%) and only weakly by *S. macedonicus* (16%). In RO juice alone, lactobacilli consumed the same amount of fructose (Table 2) while in the mixture *L. fabifermentans* did not use it at all (Table 3).

It is well know that the species used here are able to utilize lactose. Galactose content significantly increased ($P \leq 0.001$) after fermentation, evidently as consequence of lactose degradation inside the cells (Table 3) while the presence of galactose in the medium indicates that the strains are not able to metabolize this monosaccharide.

Interestingly, glucose, that was used during growth in onion juices (Table 2), was not utilized by any of the bacteria in the mixture (Table 3), since it was found completely in the liquid after fermentation.

Also inulin was completely preserved (Table 3). This in an important result since, in view of a functional food preparation, it is very important that this prebiotic material could remain intact.

Sucrose was not used by bacteria in sole juice fermentation (Table 2). In the mixture

this molecule has been eluted at the same retention time (9.02 min) of lactose, as confirmed by HPLC and MS/MS analysis (data not shown), therefore it was not possible to establish the level of utilization of these two sugars. In this regard, the percentage reduction of the chromatographic peak containing lactose and sucrose was around 16% for all the strains (Table 3). Moreover, the MS/MS analysis of the HPLC peak corresponding to YOW with *L. fabifermentans, L. plantarum* and *S. macedonicus* at the retention time of 9.02 min revealed in the m/z range of 400-600 the presence of lactosucrose (O-β-D-galactopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,2)-β-D-fructofuranoside) (Fig.1S). This rare trisaccharide is a kind of indigestible carbohydrate with a high quality taste similar to sucrose, it has good prebiotic effects, promotes intestinal mineral absorption and it is selectively utilized by intestinal *Bifidobacterium* (Al-Sheraji et al. 2013). This molecule is formed inside the cell from lactose and sucrose by enzymatic transglycosylation (Kawase, Pilgrim, Araki, & Hashimoto, 2001). A number of bacteria have been reported to produce this molecule, but to date no lactic acid bacteria, although the presence of levansucrase has been reported in several lactobacilli (Teixeira, McNeill, & Gänzle, 2012).

The enzymatic reaction that produces lactosucrose generates also a glucose molecule from the cleavage of sucrose. Our data show that glucose levels did not decrease at all in the mixtures after fermentation (Table 3), while in the sole juice glucose was used by the bacteria (Table 2). This different behaviour could be determined by the generation of internal glucose from production of lactosucrose that blocks that need to import external glucose.

The new synbiotic combination, which could be designed for human consumption as soft drink, could not only have beneficial effects on human health by combining potential

293 probiotics and prebiotics, but could also meet consumer acceptability. In this regard, no
294 off-flavors and off-odors have been detected (data not shown) in the co-fermented product
295 which was also tasted in our laboratory selecting odor and flavor as the most important
296 quality characteristics.
297
298 **4. Conclusions**
299
300 In this study we demonstrate that onion juice and whey together allow abundant growth of
301 L. *fabifermentans*, L. *plantarum* and S. *macedonicus* with interesting effects on the mixture
302 composition. Further studies will be necessary to study the probiotic potential of the strains
303 used but, even in case of negative results, they could anyway be used as starters and the
304 addition of a probiotic strain could be done separately.
305 ~~First of all,~~ The prebiotic inulin was not used by bacteria and this is an important result in
306 view of a functional food preparation. In addition, lactosucrose, another indigestible
307 carbohydrate with good prebiotic performance was produced by these bacteria. In brief,
308 there are all the ingredients for a cheap and eco-friendly functional food based on agro-food
309 by-products. However, a deeper sensory evaluation will be performed to correlate the
310 organoleptic attributes with technological analysis, and consumer acceptability of this novel
311 synbiotic product.
312
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Table 1 – Chemical parameters of onion juices from red, white, and yellow cultivars (RO, WO, and YO, respectively).

Parameters	RO	WO	YO	Probability
pH	5.54 ± 0.02	5.60 ± 0.02	5.70 ± 0.01	NS
Sugars (g/L)				
Fructose	38.66 ± 2.64[a]	24.58 ± 0.88[b]	22.47 ± 0.00[b]	***
Galactose	0.55 ± 0.26[a]	0.19 ± 0.00[b]	0.44 ± 0.00[c]	***
Glucose	46.56 ± 2.24[a]	25.44 ± 0.71[b]	29.90 ± 0.00[c]	***
Inulin	2.89 ± 0.57[a]	2.97 ± 0.49[a]	6.33 ± 0.00[b]	***
Saccarose	10.32 ± 0.41[a]	2.68 ± 0.07[b]	11.28 ± 0.00[c]	***
Total sugars	98.99 ± 5.28[a]	55.86 ± 1.17[c]	70.40 ± 0.00[b]	***

'NS' $P > 0.05$, '***' $P \leq 0.001$.
[a, b, c] Each value is expressed as mean ± SD (n = 3). Means within each row and with different lowercase letters are statistically different ($P \leqslant 0.05$).

Table 2 – Microbiological and chemical parameters of unfermented (control) and fermented onion juices from red, white and yellow cultivars (RO, WO, YO, respectively) after 96 hours at 37 °C.

Parameters	Control	*L. fabifermentans*	*L. plantarum*	Probability
Red onion (RO)				
Viable cells (CFU/ml)	–	$3.11 \cdot 10^8 \pm 1.41 \cdot 10^8$	$3.56 \cdot 10^8 \pm 2.45 \cdot 10^7$	NS
pH	5.54 ± 0.02^a	3.49 ± 0.01^b	3.49 ± 0.03^b	***
Sugars (g/L)				
Fructose	40.73 ± 0.44^a	33.51 ± 2.26^b	33.03 ± 0.68^b	***
Galactose	1.77 ± 0.26^a	1.37 ± 0.03^b	1.50 ± 0.07^{ab}	*
Glucose	48.35 ± 1.00^a	33.23 ± 2.68^b	36.10 ± 0.54^b	***
Inulin	2.65 ± 0.40	2.60 ± 0.47	2.75 ± 0.27	NS
Sucrose	9.82 ± 0.14	9.32 ± 0.30	9.62 ± 0.15	NS
Total sugars	103.32 ± 1.07^a	80.03 ± 4.81^b	82.99 ± 0.94^b	***
White onion (WO)				
Viable cells (CFU/ml)	–	$3.71 \cdot 10^8 \pm 2.10 \cdot 10^{7b}$	$7.06 \cdot 10^8 \pm 1.81 \cdot 10^{8a}$	*
pH	5.57 ± 0.03^a	3.41 ± 0.02^b	3.50 ± 0.06^b	***
Sugars (g/L)				
Fructose	33.14 ± 1.16^a	23.51 ± 0.08^b	25.01 ± 0.05^b	***
Galactose	1.67 ± 0.10^c	1.17 ± 0.01^b	1.27 ± 0.10^b	***
Glucose	32.33 ± 1.94^a	24.43 ± 0.14^b	26.43 ± 0.10^b	***
Inulin	2.40 ± 0.14	2.17 ± 0.40	2.27 ± 0.33	NS
Sucrose	2.85 ± 0.12	2.75 ± 0.50	2.80 ± 0.47	NS
Total sugars	72.40 ± 3.15^a	54.04 ± 1.13^b	57.78 ± 0.24^b	***
Yellow onion (YO)				
Viable cells (CFU/ml)	–	$3.85 \cdot 10^8 \pm 2.10 \cdot 10^{7b}$	$9.23 \cdot 10^8 \pm 8.20 \cdot 107^a$	***
pH	5.52 ± 0.00^a	3.72 ± 0.02^b	3.63 ± 0.04^b	***
Sugars (g/L)				
Fructose	33.55 ± 2.41^a	24.55 ± 0.08^b	25.55 ± 1.32^b	***
Galactose	2.30 ± 0.27^a	1.33 ± 0.01^b	1.44 ± 0.03^b	***
Glucose	35.58 ± 1.33^a	20.58 ± 0.14^b	22.58 ± 1.40^b	***
Inulin	5.77 ± 0.99	5.53 ± 0.40	5.53 ± 0.27	NS
Sucrose	12.13 ± 0.78	11.03 ± 0.47	11.08 ± 0.38	NS
Total sugars	89.33 ± 5.19^a	63.02 ± 1.65^b	66.18 ± 2.47^b	***

'NS' $P > 0.05$, '*' $P \leq 0.05$, '**' $P \leq 0.01$,'***' $P \leq 0.001$.

[a, b, c] Each value is expressed as mean ± SD (n = 3). Means within each row and with different lowercase letters are statistically different ($P \leqslant 0.05$).

Table 3 - Microbiological and chemical parameters of unfermented (control) and fermented YOW samples with lactobacilli and streptococci after 48 hours at 30 °C and 37 °C, respectively.

Parameters	Control	*L. fabifermentans*	*L. plantarum*	*S. macedonicus*	Probability
Viable cells (CFU/ml)	–	$3.20 \cdot 10^8 \pm 2.62 \cdot 10^8$	$4.26 \cdot 10^8 \pm 2.52 \cdot 10^8$	$5.45 \cdot 10^8 \pm 8.18 \cdot 10^7$	NS
pH	5.92 ± 0.03^{a}	3.81 ± 0.01^{c}	3.91 ± 0.01^{b}	3.90 ± 0.01^{b}	***
Sugars (g/L)					
Fructose	12.00 ± 0.08^{a}	11.72 ± 0.83^{ab}	7.68 ± 0.48^{c}	10.02 ± 0.81^{b}	***
Galactose	0.69 ± 0.06^{b}	4.95 ± 0.29^{a}	4.69 ± 0.21^{a}	5.12 ± 0.46^{a}	***
Glucose	17.82 ± 2.37	16.79 ± 0.30	17.81 ± 0.12	17.05 ± 1.52	NS
Inulin	7.97 ± 0.30	7.71 ± 0.60	7.69 ± 0.48	7.50 ± 0.69	NS
Lactose+Sucrose†	82.26 ± 1.67^{a}	$69.44 \pm 0.91^{b††}$	68.80 ± 2.21^{b}	68.72 ± 3.09^{b}	***
Total sugars	120.74 ± 4.33^{a}	110.62 ± 0.85^{b}	106.68 ± 1.25^{b}	108.40 ± 6.43^{b}	**

†Sugars eluted at the same retention time (9.02 min) in the HPLC analysis

†† Presence of lactosucrose based on MS/MS analysis.
'NS' $P > 0.05$, '**' $P \leqslant 0.01$, '***' $P \leqslant 0.001$.
a, b Each value is expressed as mean ± SD (n = 3). Means within each row and with different lowercase letters are statistically different ($P \leqslant 0.05$).

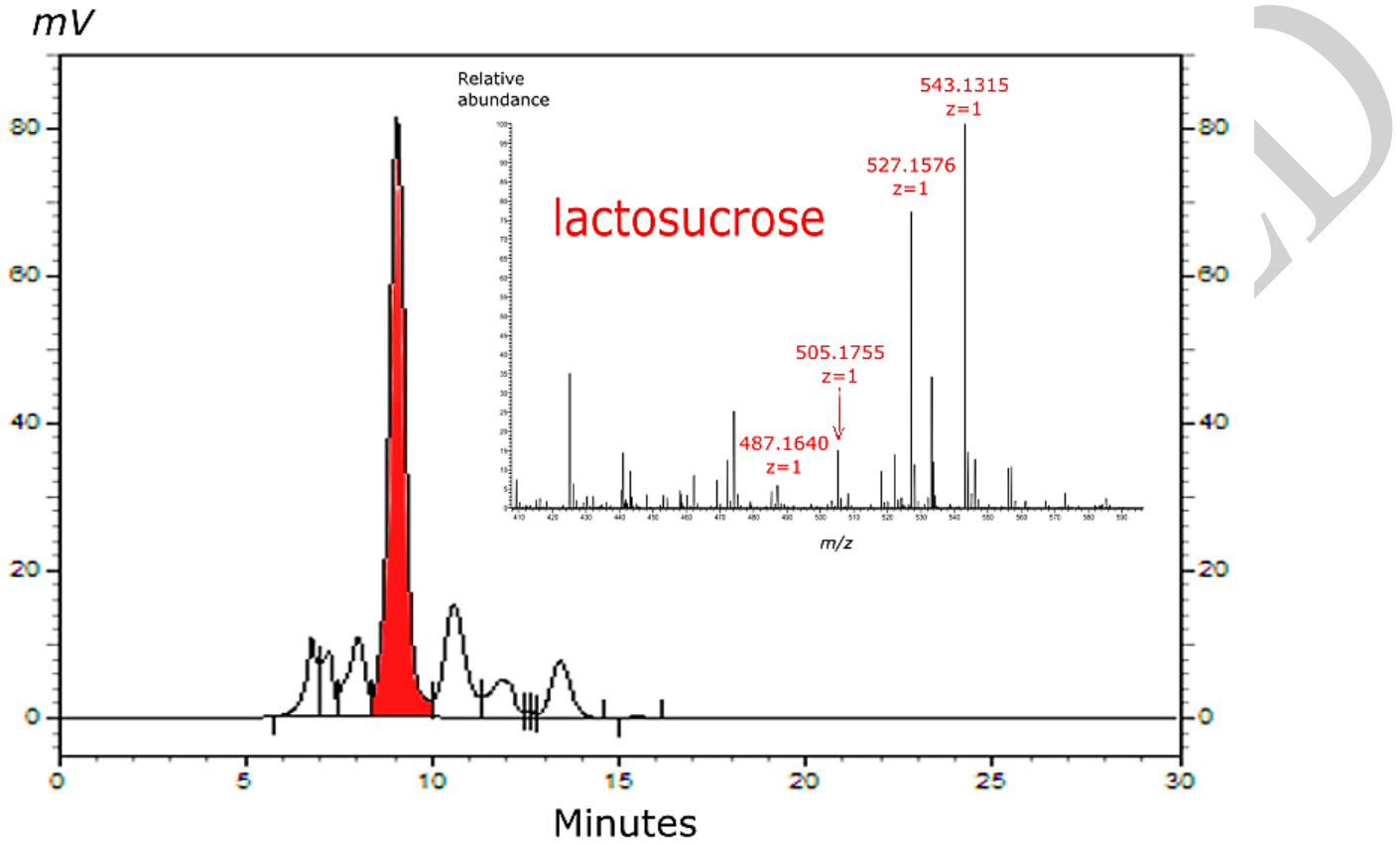

Fig. 1S – HPLC chromatogram of *L. fabifermentans* fermented YOW sample and MS/MS identification of lactosucrose corresponding to the chromatographic peak with retention time of 9.02 min.