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Research Article

Date Seeds and Lactic Acid Fermentation can Increase Antioxidant Capacity of Date Juice; Evaluation of Different Starter Cultures

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Abstract

Introduction

Date fruits have high nutritional values and different forms of date products are consumed daily in the Middle East. Date processing results in substantial amounts of waste material, and during production and storage the bioactivity is declining. A strategy to counteract these problems could be to re-use date seeds and to subject the products for lactic acid fermentation. In the present study, date juice were enriched by milled date seeds and fermented with the use of different starter cultures.

Material and Methods

Date juice was prepared from date syrup. Pure juice and juice supplemented with milled date seeds were prepared. Ferric Reducing Antioxidant Power assay and Folin-Ciocalteau assay were used for evaluation of antioxidant capacity and total phenolic content. Products were inoculated with different starter cultures isolated from various sources and allowed to ferment at 37°C for 72 hours. Different starter cultures of *Lactobacillus* (six isolates) and *Pediococcus* (one isolate) were tested, four were originating from dates. The starter cultures were identified by 16 S rDNA sequencing.

Results

The bioactivity in date juice decreased significantly during incubation, but by the addition of date seeds the total phenolic content and the correlated antioxidant capacity increased at all time points. Throughout the fermentation process, the activity and phenolic content was significantly better preserved. The protective effect differed between the starter cultures, but the process can be performed by lactic acid bacteria isolated from the autochthonous microbiota of date fruits. All the tested starter cultures showed high viability. Best results were achieved with a strain of *L. plantarum* HEAL19 and *P. pentosaceus* D4# isolated from dates.

Conclusion

Supplementation of dates seeds and lactic acid fermentation is a sustainable way to improve and preserve the nutritional value of date juice. However, investigations in a larger scale are required to industrially validate these results.

Keywords: Date Fruits; Fermentation; Antioxidativ Capacity; Phenolic Content

Introduction

Dates are the fruits of the date palm tree (*Phoenix dactylifera L.*) which is one of the oldest cultivated plants, having a history of more than 6000 years [1]. Today more than 2000 varieties of dates are cultivated mainly in the Middle East, North Africa, parts of Central and South America, southern Europe, India and Pakistan and 2010 the worldwide production of dates reached 7.68 million tons, a number that is expected to further increase [2].

The date fruit is composed of flesh, seed, and skin and the flesh has a high content of sugars and therefore also a high total energy content. It also contains a considerable amount of vitamin C and B, selenium, copper, potassium and magnesium. Moreover, it is a good source of dietary fiber, especially insoluble fibers as well as phytochemicals, including phenolic compounds, which provides e.g. antioxidative potential and antibacterial activities [3-6]. Due to the nutritional values date fruits are consumed daily in high amounts especially in the Middle Eastern countries and also many products are made from the fruits such as vinegar, syrup, jam, juice and sugar, with date syrup probably being the most common one [7]. Date syrup is made by heating the flesh mixed with water followed by pressing and filtering. As a subsequent step in the production, the syrup can be appropriately diluted for preparation of date juice [8]. The syrup is produced in small scales in homes but also on a full industrial scale and is used as a food additive.

It has previously been shown that the total phenolic content and antioxidant activity of fruit jams and juices can be affected during processing and storage, which will result in diminished health beneficial potential [2,9,10]. In contrast, fermentation by the use of *Lactobacillus plantarum* as starter culture was shown by Curiel et al. [11] to increase both phenolic content and antioxidative capacity in Myrtle berries. In many cases, bacteria of the genus

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Lactobacillus are used as starters for food fermentation, which can contribute significantly to the storage by preventing the growth risk of harmful organisms, but also to enhancement of flavor, texture and nutritional value [12]. When it comes to spontaneously fermented vegetable products, *Lactobacillus plantarum* is a frequently encountered species. Strains of *L. plantarum* possess tannase activity [13,14], making them tolerant to the antimicrobial effects of polyphenols and also giving them the possibility to degrade tannins and metabolize phenolic acids [14]. Tannins are naturally occurring water soluble polyphenols found in a variety of plants utilized as food, including dates, and are beneficial to health but simultaneously large quantities may result in adverse health effects [15].

A consequence of industrial date processing is a substantial amount of waste. These waste-products, mostly the flesh, have been tried out as substrates for production of ethanol, organic acids and fructose [8,16,17]. Most of the date seeds are milled and used as animal feed. The seeds make up approximately 12 % of the total fruit weight and they contain higher amounts of protein, fat, fiber and resistant starch than the flesh [3,18]. Furthermore, date seeds have a higher total phenolic content and antioxidant capacity than the flesh, syrup or pressed cakes, therefore their potential to be used as a food ingredient should be evaluated, which would also contribute to development of a sustainable date production [4,7].

The aim of this pilot study was to evaluate the effect of (*i*) date seed supplement and (*ii*) lactic acid fermentation on antioxidative capacity and total phenolic content of date juice. Seven different starter cultures isolated from dates and other sources were compared.

Material and methods

Preparation of Juices

Date juice was prepared by diluting commercially available date syrup (obtained by boiled and pressed date flesh, 200 g of dates have been used for production of 100 g date syrup, Sevan AB, Sweden) with distilled water. Half of the juices prepared by syrup were remained as a simple juice and for the other half milled date seeds (Shahd Babe Pars Co, Iran) with a diameter of approximately 1 mm was also added, resulting in two types of juice: pure date juice without any supplements (D-juice) and date juice supplemented with date seeds (DS-juice). Both juices were autoclaved at 121°C for 20 minutes and kept in 4°C until inoculation.

The amount of syrup added to prepare both juices was dependent on the sugar concentration (10 % for D-juice and 7 % for DS-juice) and calculated according to Pearson square. In DS-juice, about 3 % of syrup was replaced by the milled seeds. The percentage of seed weight in an average date (12 %), and the amount of dates used to make the syrup were considered in calculations of the amount of seed replacing the syrup.

Starter Cultures

Isolation of Lactic Acid Bacteria from Date Fruits

To isolate lactic acid bacteria to be used as starter cultures in the fermentation process, mature date fruits (maturity stage: Tamar; Shahd Babe Pars Co, Iran) were homogenized in physiological saline (0.85% NaCl and 0.1 % bacteriological peptone (Oxoid, Basingstoke, UK)) for 2 minutes at high speed and in room temperature on a Laboratory Blender Stomacher 400 (Seward Medical, London, UK). Homogenate samples (1 ml) were serially diluted in dilution liquid (sodium chloride [Merck], 8.5 g/l; Bacteriological peptone [Oxoid, Unipath LTD, Basingstoke, Hampshire, England], 1 g/l; Tween 80 [Merck], 1 g/l; L-Cystine hydrochloride monohydrate [Merck], 0.2 g/l) and 0.1 ml of the samples from appropriate dilutions were spread plated. Viable counts were obtained from De Man-Rogosa-Sharpe (MRS) agar (Merck, Germany) that was incubated anaerobically at 37°C for 72 h. Colonies were randomly picked from the plates with positive cultures, re-streaked to purity prior to identification by sequencing of the 16 S ribosomal RNA gene. Selected isolates are marked #.

Also tested as starter cultures were the following strains: *Lactobacillus plantarum* 299v (=DSM 9843), isolated from human intestinal mucosa and commercially available as probiotics (Probi AB, Lund, Sweden); *L. plantarum* HEAL19 (=DSM 15313) also isolated from human mucosa and has been used with probiotic intentions in scientific studies [19] (Probi AB, Lund, Sweden); *L. plantarum* 56 isolated from lactic acid fermented red sorghum (Department of Food Technology, Engineering and Nutrition, Lund University).

16SrDNA Sequencing

As template for the polymerase chain reaction, crude cell extract was prepared from selected isolates [20].

For sequencing, primers ENV1 (59-AGA GTT TGA TII TGG CTC AG-39, Escherichia coli numbering 8–27) and ENV2 (59-CGG ITA CCT TGT TAC GAC TT-39, E. coli numbering 1511–1492) [21] were used for amplification of the 16 S rRNA genes. The PCR reaction mixture contained 0.2 mM of both primers, 5 ml of template DNA, 5 ml of 106PCR reaction buffer with 1.5 mM MgCl2 (Roche Diagnostics GmbH, Mannheim, Germany), 200 mM of each deoxyribonucleotide triphosphate, and 2.5 U of Taq DNA polymerase (Roche Diagnostics, Mannheim, Germany). Water was added to a final volume of 50 ml. PCR was performed in a PCR Mastercycle 5333 (Eppendorf) with the following profile: 1 cycle at 94°C for 3 min, followed by 30 cycles of 96°C for 15 s, 50°C for 30 s, and 72°C for 90 s, with an additional extension at 72°C for 10 min. The amplification products (5 ml) were checked by running the products on 1.5 % (wt./vol.) agarose gel in 16 TBE buffer (89 mM Tris, 89 mMboric acid, 2.5 mMEDTA, pH 8.3), after ethidium bromide staining. Amplicons were sent to MWG (Biotech, Ebersberg, Germany) for single strand sequencing. 16 S rDNA sequences (mostly around 500 bp) were searched against Genbank (National Centre for Biotechnology Information, Bethesda, MD) using the Basic Local Alignment Search Tool (BLAST) accessible from the homepage at the National Centre for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/) [22] or aligned to 16 S rDNA encoding sequences downloaded from the Ribosomal Data Base (RDP-II) [23] for an approximate phylogenetic affiliation.

Fermentation

Selected starter cultures were propagated anaerobically in De Man-Rogosa-Sharpe (MRS) broth (Merck, Germany) for 24 hours in 37ºC and inoculation tubes containing 10^8 CFU dissolved in 1 ml freezing media (4·28 mM-K_2HPO_4, 1·31 mM-KH_2PO_4, 1·82 mMNa-Citrate, 0·87 mM-$MgSO_4 \cdot 7H_2O$ and 1·48 mM-98 % glycerol) were prepared and saved in -80ºC until starting the fermentation process in which they were used as inoculums.

The juices were inoculated with the individual starter cultures, without any other supplementation and the initial cell density of the bacteria was 10^6 CFU/ml. Fermentation was allowed at 37°C for 72 hours, without stirring conditions. Juice without bacterial inoculums was incubated under the same conditions and used as the reference. Samples for bacterial counts were collected before fermentation and after 48 and 72 hours respectively and samples for analysis of total antioxidant capacity and total phenolic content were collected along with the first and last sampling occasions, when also reference samples were taken. All samples were stored in -80ºC until analysis.

Extraction Technique

A simple extraction method was used to prepare the extract of the liquid juice to be used in the Ferric Reducing Antioxidant Power- and Folin-Ciocalteau assays. Samples were thawed and vortexed (Scientific industries-USA) for 2 minutes followed by a sonication step in a sonication bath (Millipore-USA) of ice-water, 4ºC for 5 minutes. Thereafter, the samples were centrifuged (Eppendorf 5804-Hamburg) at 11,000 rpm for 10 minutes. The supernatant was transferred to 1.5 ml tubes and was saved in -80ºC until further analysis.

Ferric Reducing Antioxidant Power (FRAP) Assay

Total antioxidant power of the extracted juice samples were measured using the FRAP assay according to Benzie and Strain [24] with partial modifications [25].

The FRAP reagent was prepared just before the experiment by mixture of respectively 25 ml sodium acetate buffer (pH 3.6, Sigma Aldrich-USA), 2.5 TPTZ (10mmol/l, Flukar Analytical-Switzerland) and 2.5 ml ferric chloride (20 mmol/l, Sigma Aldrich-USA).

A standard curve was made with trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma Aldrich-German) and ethanol in 5 different concentrations; 100, 250, 500, 750 and 1000 µl/L. Samples for standard measurements were done by mixing 900 µl of FRAPP reagent, 90 µl of distilled water and 30 µl of different trolox concentrations and as a blank 900 µl FRAP reagent and 120 µl distilled water was used.

For analysis, 30 µl of the extract from the juices were mixed with 900 µl of FRAP reagent and 90 µl of distilled water. After 10 minutes of incubation in room temperature, the absorbance was read at 593 nm by Simple read software and UV-Vis spectrophotometer (Varian Cary 50 Bio- Netherland). Data were expressed as µmol trolox equivalents per milliliter. The samples were analyzed 6 times at different time points and the reproducibility of the method was analyzed.

Folin-Ciocalteau Assay

Total phenolic content of the extracted juice samples were analyzed using Folin-Ciocalteau reagent according to Singleton et al., [26] with some modifications [27].

In order to prepare the measurement solutions, 3.16 ml of distilled water, 200 µl of Folin-Ciocalteau reagent (Sigma Aldrich-Germany), 40 µl of extracted juice samples (or gallic acid (Sigma Aldrich-Germany), for standard curve and distilled water for blank) were mixed and after 1 min, 600 µl of Na_2CO_3 (20 % w/v, Sigma Aldrich-Germany) was added.

To obtain the standard curve, gallic acid was diluted in distilled water to achieve the following concentrations: 50, 100, 150, 250, 500 mg/liter. The measurement solutions were incubated in room temperature for 120 minutes and the absorbance was read at 765 nm using Simple read software and UV-Vis spectrophotometer (Varian Cary 50 Bio). Data were expressed as mg/ml gallic acid equivalents and samples were analyzed 4 times at different time points to ensure reproducibility of the method.

Bacterial Enumeration

Viable count of the DS-juice was performed at day 2 and 3 of the fermentation process. As previously described, the samples were serially diluted in dilution liquid and spread plated. Viable counts were obtained from MRS agar after 48 and 72 hours of fermentation and the results were expresses as CFU/ml.

Statistical Calculations

Samples were analyzed in duplicates which were measured six times in different time points for FRAP analysis and four times for total phenolic content. For all statistical analysis SigmaPlot version 11.0, (SYSTAT Software, Point Richmond, USA) was used. Kruskal-Wallis One Way Anova on Ranks was used to compare all groups. For pair-wise comparison, Mann-Whitney rank sum test was used. The correlation between expectations of benefit was ascertained using Pearson Product Moment Correlation. Results are presented as medians ± interquartile range and p-values less than 0.05 were considered significant.

Results

Identification of Isolates from Date Fruits

All identified isolates were designated to the genus *Lactobacillus* or *Pediococcus* and showed 100% 16S rDNA similarity to the type strain of the established species; the dominating species were *L. plantarum*, *L. paracasei* subsp. *paracasei, L. salivarius* and *P. pentosaceus* and amongst these isolates one from each species was randomly chosen for evaluation of their capacity to ferment date-juice and to affect antioxidative capacity and total phenolic content (Table 1-3).

Total Antioxidant Capacity

The total antioxidant capacity (TAC) values according to the FRAP method in date juice without and with supplementation are shown in table 1.

In D-juice, a significant difference was found between the TAC

value of the reference sample before compared to after incubation (5.5±0.2 µmol trolox/ml and 4.72±0.19 µmol trolox/ml respectively) ($p<0.01$). No significant increase was found after fermentation of D-juice (Table 1).

In DS-juice, a significant difference was found between the TAC value before incubation compared to after incubation of the reference sample (6.24±0.18 µmol trolox/ml and 5.05±0.23 µmol trolox/ml respectively) ($p<0.01$)

Significantly higher TAC values were also found in DS-juice fermented with *L. plantarum* 299v (6.13±0.16 µmol trolox/ml) ($p<0.01$) *L. plantarum* HEAL19 (6.46±0.50 µmol trolox/ml) ($p<0.01$), *L. plantarum* 56 (5.91±0.15 µmol trolox/ml) ($p<0.01$*), L. paracasei* subsp. *paracasei* D2# (5.28±0.14 µmol trolox/ml) ($p<0.05$), *L. salivarius* D3# (5.75±0.31 µmol trolox/ml) ($p<0.01$) and *P. pentosaceus* D4# (6.22±0.28 µmol trolox/ml) ($p<0.01$) compared to the reference sample after incubation (5.05±0.23 µmol trolox/ml) (Table 1).

Comparing the total antioxidative capacity between the juices, it was generally higher in DS-juice than in D-juice, for all samples, both fermented and reference samples (Table 1). Significantly higher values were found between the reference samples of DS-juice compared to D-juice, before as well as after incubation (6.24 ± 0.18 µmol trolox/ml compared to 5.55 ± 0.20 µmol trolox/ml and 5.05 ± 0.23 µmol trolox/ml compard to 4.72 ± 0.19 µmol trolox/ml respectively) ($p<0.01$) (Table 1).

Total Phenolic Content

The total phenolic content (TPC) of D-juice and DS-juice analyzed according to Folin-Ciocalteau method is shown in table 2.

In D-juice, a significant difference was found between the TPC value of the reference sample before compared to after incubation (0.81±0.03 mg gallic acid/ml and 0.77±0.01µmol trolox/ml respectively) ($p<0.01$). No significant increase was found after fermentation of D-juice.

In DS-juice, a significant difference was found between the TPC value before incubation compared to after incubation of the reference sample (1.17 ± 0.08 mg gallic acid/ml and 0.83 ± 0.03 mg gallic acid/ml respectively) ($p<0.01$).

Significantly higher TPC values were also found in juice fermented with *L. plantarum* 299v (0.90±0.03 mg gallic acid/ml) ($p<0.01$) *L. plantarum* HEAL19 (1.03±0.02 mg gallic acid/ml) ($p<0.01$), *L. plantarum* 56 (0.87±0.03 mg gallic acid/ml) ($p<0.01$) and *P. pentosaceus* D4# (0.95±0.07 mg gallic acid/ml) ($p<0.01$) compared to the reference sample after incubation (0.83±0.03 mg gallic acid/ml).

Comparing the total phenolic content between the juices, it was generally higher in DS-juice than in D-juice, for all samples, both fermented and reference samples (Table 2). Significantly higher values were found between the reference samples of DS-juice compared to D-juice, before as well as after incubation (1.17 ± 0.08 mg gallic acid/ml compared to 0.81 ± 0.03 and 0.83 ± 0.03 mg gallic acid/ml compared to 0.77 ± 0.01 mg gallic acid/ml respectively) ($p<0.01$) (Table 2).

Correlation between TAC and TPC Measurements

The correlation between data obtained by FRAP and Folin-Ciocalteau methods were calculated and in DS-juice a positive correlation was found ($r = 0.67$, $p = 0.047$). In D-juice no linear relationship was observed.

Viable Lactic Acid Bacteria during Fermentation

The viable count of lactic acid bacteria in DS-juice inoculated with the different starter cultures, at different time-points (0, 48 h and 72 h) are shown in Table 3. After 48 hours all starter cultures increased the count, with highest values found after fermentation using *L. plantarum* 299v (mean 8.7 CFU/ml) and *L. plantarum* HEAL19 (mean 8.2 CFU/ml). After 72 hours, the viable count had decreased in all samples except for samples fermented with *L. salivarius* D3# (mean 7.9 CFU/ml) and *P. pentosaceus* D4# (7.0 CFU/ml), which were continuously increasing (Table 3).

Discussion

Dates are especially popular in the Middle East countries and due to its nutritional value, the fruit itself or processed to e.g. syrup is consumed in high amounts [3-7]. The date seeds are however considered a waste product and are at present mainly used as animal feed. The high worldwide production of dates gives rise to several hundred thousand tones of date seeds that could be utilized to improve the income of date cultivation. Such re-use could also be applied to improve the nutritional value of date products, since the seeds serve as a good source of natural antioxidants [4].

In the present study the seeds were milled and added to date juice resulting in significantly increased TAC as well as increased TPC values in the juice. It has previously been shown that processing and heat treatments of fruits and storage of the products unfortunately quickly affects the antioxidative capacity and also the phenolic content and freezing of date juice seems so far to be the single option for stabilizing the content of bioactive compounds [9,10,28,29]. In our study a decrease was actually shown after only 3 days of incubation after juice preparation and significantly lower TAC- and TPC- values were found in both juice types. In DS-juice the values decreased to almost the same level as the starting values of D-juice, indicating the significance of establishing higher baseline values in the product (Table 1 and Table 2).

Fermentation has also been pointed out as a method to maintain and eventually also improve antioxidant capacity and phenolic content in products and in a study by Curiel et al. [11], his was found to be true for Myrtle berries. Spontaneous lactic acid fermentation is usually carried out by the indigenous microbiota existing on the fruit or vegetables; however the composition of microbiota varies depending on the quality of the raw material, harvesting conditions and post-harvest treatments. This type of traditional fermentation may therefore be an uncontrolled process which may result in a final product harboring undesirable microorganisms. Nevertheless, due to diverging properties, specific species are associated with specific food products and *L. plantarum* is often found in fermented foods of plant origin [30]. Thermal

Table 1: Total antioxidant capacity

Total antioxidant capacity (TAC) values found in pure date juice (D-juice) and juice supplemented with date seeds (DS-juice) after fermentation for 72 hours using different starter cultures, as well as before and after incubation for 72 hours of the reference sample. Data are expressed as µmol trolox/ml.

Starter culture	TAC values D-juice	TAC values DS-juice
Lactobacillus plantarum 299v	4.66 ± 0.07	6.13 ± 0.16^{e}
Lactobacillus plantarum HEAL19	4.24 ± 0.22	6.46 ± 0.50^{e}
Lactobacillus plantarum 56	4.35 ± 0.08	5.91 ± 0.15^{e}
Lactobacillus plantarum D1#	4.20 ± 0.19	5.26 ± 0.19
Lactobacillus paracasei subsp. *paracasei* D2#	4.65 ± 0.21	5.28 ± 0.14^{f}
Lactobacillus salivarius D3#	4.37 ± 0.25	5.75 ± 0.31^{e}
Pediococcus pentosaceus D4#	3.59 ± 0.18	6.22 ± 0.28^{e}
Reference sample before incubation	5.55 ± 0.20^{a,c}	6.24 ± 0.18^{b,c}
Reference sample after incubation	4.72 ± 0.19^{a,d}	5.05 ± 0.23^{b,d,e,f}

*indicates isolated from date fruits

^{a,b,c,d,e}denotes $p<0.01$

^{f}denotes $p<0.05$

Table 2: Total phenolic content

Values of total phenolic content found in pure date juice (D-juice) and juice supplemented with date seeds (DS-juice) after fermentation for 72 hours using the starter cultures as well as before and after incubation for 72 hours of the reference sample. Data are expressed as mg Gallic acid /ml.

Starter culture	TPC D juice	TPC S juice
Lactobacillus plantarum 299v	0.71 ± 0.03	0.90 ± 0.03^{e}
Lactobacillus plantarum HEAL19	0.54 ± 0.04	1.03 ± 0.02^{e}
Lactobacillus plantarum 56	0.66 ± 0.02	0.87 ± 0.03^{e}
Lactobacillus plantarum D1#	0.55 ± 0.01	0.78 ± 0.05
Lactobacillus paracasei subsp. *paracasei* D2#	0.51 ± 0.02	0.80 ± 0.03
Lactobacillus salivarius D3#	0.64 ± 0.04	0.66 ± 0.05
Pediococcus pentosaceus D4#	0.75 ± 0.06	0.95 ± 0.07^{e}
Reference sample before incubation	0.81 ± 0.03^{a,c}	1.17 ± 0.08^{b,c}
Reference sample after incubation	0.77 ± 0.01^{a,d}	0.83 ± 0.03^{b,d,e}

#indicates isolated from date fruits

^{a,b,c,d,e}denotes $p<0.01$

processing of date juice results in microbial inactivation and no spontaneous fermentation by wild-type strains can be performed. Hence, fully mature date fruits were analyzed for indigenous lactic acid bacteria, well adapted to the natural habitats of the date fruits and with the potential application as starter cultures of the fermentation process. In comparison, already identified and partly

Table 3: Viable count of lactic acid bacteria during fermentation with different starter cultures

Number of lactic acid bacteria in fermenting date juice supplemented with date seeds (DS-juice) after different time-points, as determined by viable count on MRS agar. Data are expressed as Log CFU/ml juice.

Starter culture	Log CFU/ml 0h	Log CFU/ml 48h	Log CFU/ml 72h
Lactobacillus plantarum 299v	6.0	8.7	8.0
Lactobacillus plantarum HEAL19	6.0	8.2	7.0
Lactobacillus plantarum 56	6.0	7.4	7.0
Lactobacillus plantarum D1#	6.0	6.7	6.2
Lactobacillus paracasei subsp. *paracasei* D2#	6.0	6.8	6.3
Lactobacillus salivarius D3#	6.0	7.3	7.9
Pediococcus pentosaceus D4#	6.0	6.5	7.0

#indicates isolated from date fruits

commercially available *L. plantarum* strains were also included as starter cultures. By the addition of milled seeds to the juice, the fermentation process resulted in higher TAC values for all bacterial strains used compared with the reference juice after incubation, except for *L. plantarum* D1# (Table 1). Indeed, the TAC-value after fermentation by *L. plantarum* HEAL19 was even higher than the corresponding value of the reference sample before incubation and almost as high was the value found after fermentation by *P. pentosaceus* D4#, a strain isolated from the date fruits (Table 1). Higher values than the reference samples, irrespective of time points, could not be found in fermented date juice without seeds. A similar pattern as found for TAC was also found after analysis of TPC (Table 2). By fermentation using *L. plantarum* 299v, *L. plantarum* HEAL19, *L. plantarum* 56, and *P. pentosaceus* D4#, significantly higher TPC-values were found in comparison to the reference samples after incubation, with the highest values shown for *L. plantarum* HEAL19 and subsequently *P. pentosaceus* D4# (Table 2). To verify the relationship between total phenolic content and the antioxidative capacity of the product, the Pearson Product Moment Correlation was calculated and a positive correlation was found in the DS-juice.

Date syrup has demonstrated antibacterial activity against both gram-positive and gram-negative bacteria, an inhibition that may be attributed to bioactive compounds including plant-derived phenolic molecules, since the high sugar content naturally present in the syrup did not contribute to this effect [6]. Due to the amplification of phenolic compounds by the addition of seeds, an enumeration and viability verification was performed for all strains used for fermentation after 48 and 72 hours in DS-juice (Table 3). After 48 hours of fermentation, *L. plantarum* 299v reached the highest count, which was slightly declining after additionally 24 hours. *L. salivarius* D3# and *P. pentosaceus* D4#, both isolated from date fruits, were the only strains not decreasing in viable count after 72 hours compared to 48 hours of fermentation. On the other

hand, all strains increased their viable count compared to starting values of the inoculum, indicating possibilities to survive and multiply despite high concentrations of phenolic compounds.

Among dates and their by-products, seeds have the highest contents of total phenolics and antioxidant activity [4]. Analysis revealed that this part of the fruit also contained high concentrations of moderately polymerized condensed tannins [31]. Previously it has been found that tannin molecules with higher molar mass have stronger anti-nutritional effects and also lower biological activities. In contrast, smaller molecules are suggested to have fewer anti-nutritional effects and can be more readily absorbed [15]. Some microbes are resistant to the antimicrobial effects of tannins and have different abilities to degrade tannins into gallic acid, ellagic acid, glucose, or alcohols. Strains of the closely related species *L. plantarum*, *L. pentosus* and *L. paraplantarum* often possess tannase activity, which makes it possible to metabolize polyphenols [13] and thereby producing substituted phenyl propionic acids [32], substances that are commercially used as the anti-inflammatory drug Ibuprofen®. The proven viability in the present study suggest the tannase activity to be involved in bacterial survival and also multiplication, this needs however to be analysed in more detail.

In conclusion, date juice, produced from date syrup, is quickly loosing bioactivity capacity after processing. To improve the nutritional value of the juice, date seeds, a waste product from date production, can be used to increase total phenolic content and thereby also antioxidant capacity. By fermentation using lactic acid bacteria with possibilities to maintain viability in the harsh antimicrobial environment, which is a consequence of high tannin content, the bioactivity is even better preserved. For this reason, a microbial tannase activity may be considered, which would also beneficially affect the phenolic profile. Furthermore, new bacterial strains isolated from the autochthonous microbiota of date fruits may be adapted to the conditions and can therefore be used as starter cultures for the fermentation process. To our knowledge, neither the possibilities of combining date seeds and fermentation in date juice nor the potential of finding indigenous bacterial strains on date fruits useful for this purpose has been previously evaluated. Even though this is a small pilot study, the results support an ideal way to generate a new date-based product with health beneficial effects, however more and larger scale investigations are required to validate these results.

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