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Faculty of Food Science

Naringinase from probiotic bacteria and its application in production of probiotic citrus juices

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1 INTRODUCTION AND OUTLINE

1.1 Introduction

Citrus family fruits such as grapefruit, orange, lemon, tangerine, etc. are typical fruits growing in tropical and subtropical regions including Vietnam. Nutritionally, these fruits are valuable as they are rich in vitamins (especially vitamin C) and antioxidants, but unfortunately they contain high amounts of bitter compounds. Two main types of bitterness, caused by two different types of compounds, occur in citrus fruits. Flavanone neohesperidosides, as naringin in grapefruit and neohesperidin in sour oranges, provide the typical bitterness of fruits and juices from these species. The other type of bitterness, which constitutes an extremely negative quality factor in some orange juices, is produced by limonin, a triterpene derivative of the limonoid group. Limonin bitterness is known as 'delayed bitterness', since it is not detected in fresh fruits or freshly extracted juices but it is developed during juice storage or by heat treatment. In general, fresh fruits do not contain limonin, but a nonbitter precursor, which converts into limonin after juice preparation. Limonin is detected by taste at concentrations of about 6–8 mg/L in orange juice. Naringin, 4',5,7-trihydroxyflavonone-7-β-L-rhamnoglucoside-(1,2)-α-D-glucopyranoside, is known as the principal component that causes the bitterness in grapefruit. Its amount varies among parts of fruit, one of the main parts containing naringin is the albedo, the fruit membrane. It has been reported that when naringin is present in water solution in concentrations higher than 20 μg/mL, the bitter taste can be detected, however, in grapefruit juices, it is only detectable in concentrations higher than 300–400 μg/mL. Thus, debittering process should be investigated to make these juices to be acceptable by consumers.

Reduction of bitterness has been attempted by many methods, involving changes in cultivation practices (rootstock, fertilization) and juice treatments. Debittering of processed juices seems to be the most promising approach, and some citrus industries are already equipped with debittering devices. Some techniques have been studied and developed for reducing the bitterness in citrus fruit juice, such as using of adsorbents or β-cyclodextrin, by blanching, or using chemicals to remove bitterness. These techniques are classified as physicochemical methods, and they have some limitations on the quality of citrus fruit juice (removal of nutrients, flavor, color, causing turbidity, etc.) leading to unacceptability by consumers. To overcome these limitations, biotechnological methods using enzymatic technology in fruit juice processing should be developed and applied.

Naringinase is an enzyme complex with α-L-rhamnosidase (E.C. 3.2.1.40) and β-D-glucosidase (E.C. 3.2.1.21) activities. This enzyme preparation is commercially attractive due to its potential usefulness in pharmaceutical and food industries. Meanwhile, α-L-rhamnosidase cleaves

terminal α-L-rhamnose specifically from a large number of natural products including naringin, rutin, quercitrin, hesperidin, diosgene, terpenyl glycosides, and many other natural glycosides, whereas the β-D-glucosidase can further hydrolyze glucose molecule from some intermediers such as prunin to produce naringenin. These molecules have a great potential, especially in the food and pharmaceutical industries, due to their recognized antioxidant, anti-inflammatory, anti-ulcer, and hypocholesterolemic effects, whereas naringenin has also shown anti-mutagenic and neuroprotective activities, while prunin has antiviral activity. Moreover, naringinase is commercially used in debittering and clearance of citrus fruit juices as well as enhancement of wine aromas in the food industry. While this enzyme is widely distributed in fungi, its production from bacterial sources is less commonly known. Bioinformatical analysis of genomic data of lactic acid bacteria and bifidobacteria showed that both α-L-rhamnosidase and β-D-glucosidase coding genes are found in the genome, thus these bacteria should synthesize naringinase enzyme.

Due to historical and technological reasons most of the probiotic foods are based on dairy products. Unfortunately, it may cause inconveniences for some segments of consumers who do not tolerate lactose (lactose intolerance), are allergic to proteins, or simply being vegetarian. Since fruits and vegetables already contain beneficial nutrients such as minerals, vitamins, dietary fibers, and antioxidants, while lacking dairy allergens, they may serve as ideal food matrices for carrying probiotic bacteria. Furthermore, fruit juices have pleasing taste profiles to all age groups, and they are perceived as being healthy and refreshing. Thus, the development of new non-dairy probiotic food products may be very much challenging, as they have to meet the consumer's expectancy for health. In this sense, many studies are carried out to develop novel probiotic fruit or vegetable products mainly focusing on soymilk, carrot juice, noni juice, pineapple etc., but less on other tropical juices. Probiotic bacteria are generally applied in production of fermented functional foods, thus using these bacteria with high naringinase activity for fermentation of citrus juices should have high scientific and innovative impact.

1.2 Outline of dissertation

Recently, applications of naringinase from microbial sources to debitter citrus fruit juice, especially grapefruit juice, are more explored. The various drawbacks when using chemical or physical methods for reducing the bitterness in citrus fruit juice are: (1) the juice must be previously deoiled; (2) the organoleptic properties and quality of juice may be affected by alkali solutions needed for regeneration of the adsorption columns; (3) using chemicals to remove the bitterness could alter the composition of juice or remove nutrients, flavor, color characteristic of citrus juice; (4) the chemicals used in certain cases cannot be recycled. These limitations could be overcome by applying

biotechnological methods. It means treating the juice with enzyme naringinase in citrus fruit juice processing, as it is a viable source, has remarkable reusability, and has a less toxic effect on the environment. Keeping in view, the present work has been carried out under the following objectives:

- Screening probiotic strains for naringinase production
- Study of the factors that influence production of naringinase during fermentation
- Optimization of some factors for enhancement of production of naringinase by probiotic bacteria
- Characterization of crude naringinase
- Application of whole cells of probiotic bacteria for producing probiotic beverage and debittering of grapefruit juice by mono and mixed cultures

2 MATERIALS AND METHODS

2.1 Chemicals

Naringin, diethylene glycol, tri [2-pyridyl]-s-triazine reagent, Folin-Ciocalteu reagent and L-rhamnose were of analytical grade and purchase from Sigma–Aldrich (Hungary). All other chemicals for preparation of media (MRS, TPY, Beeren's) were purchased either from Reanal (Hungary) or VWR (Hungary).

2.2 Screening probiotic bacteria for naringinase production

Bacteria from the fermentation lab of our Research Centre, including 16 strains of *Lactobacillus* and 20 strains of *Bifidobacterium*, were screened for naringinase production.

The *Lactobacillus* spp. were grown in MRS broth without meat extract and lower glucose concentration (3 g/L), supplemented with 0.05% naringin, incubated aerobically at 37 °C for 24 hours. Bifidobacteria were grown in TPY broth modified with 0.05% naringin, and incubated anaerobically (in Bugbox anaerobic chamber, Ruskin Technology) at 37 °C for 24 hours.

The ability of naringinase production was checked for all strains, and the best naringinase producing bacterium was selected for further studies.

2.3 Effect of some factors on naringinase production by *L. fermentum* D13

L. fermentum D13 was chosen to study the effect of some factors (inoculum ratio of bacteria, initial pH, naringin concentration, carbohydrate sources, metal ions) on naringinase production.

To study the effect on enzyme production, different inoculum ratios of bacteria (2%, 4%, 6% and 8%) with the cell culture of 10^9 cfu/mL were added to the MRS medium that was supplemented with 0.05% naringin and the glucose content was adjusted to 3 g/L.

The initial pH of the MRS fermentation medium was adjusted in range from pH 5.0 to pH 8.0 with stepwise of pH 1.0 unit to determine its effect on naringinase production. 1N NaOH and 1N H_2SO_4 solutions were used for adjusting pH of medium.

Different naringin concentration from 0.05% to 0.2% with the stepwise of 0.05 % were added to the MRS medium to check the influence of inducer to naringinase activity.

Nine different carbohydrate sources at the concentration of 1 (w/v) %were used in MRS medium containing 0.05% naringin for studying suitable carbohydrate source for naringinase production.

Different metal ions including Fe^{3+}, Ca^{2+}, Mg^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+} and Ni^{2+} were used in the culture medium to check the influence of different metal ions on naringinase production.

2.4 Optimization of medium components for naringinase production

Optimal conditions for naringinase production by *L. fermentum* D13 was established via Response Surface Methodology (RSM). The central composite design (CCD) was used to investigate the effects of three independent variables (concentration of sucrose, naringin content and pH) based on the results of the preliminary experiments. The central composite design consisting of 17 experimental points including three replications at the central point (0) was applied to find the optimal levels of each variables.

Both linear and quadratic effects of the three variables as well as their interaction were calculated. Second-order polynomial model was applied to fit to experimental data (Eq. 1).

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2 + a_{12}X_1X_2 + a_{13}X_1X_3 + {}_{23}X_2X_3 \quad \text{(Eq. 1)}$$

where a_0, a_1, a_2, a_3, a_{11}, a_{22}, a_{33}, a_{12}, a_{13}, a_{23} are the regression coefficients for intercept, linear, quadratic, and interaction terms, respectively, and X_1, X_2, X_3 are independent variables pH, naringin and sucrose contents.

Regression analysis was applied to estimate the coefficients of model. The fit of the models was evaluated by the determination coefficients (R^2) and adjusted R^2 (R^2-adj).

2.5 Characterization of crude naringinase enzyme

After 24 hours of fermentation, the culture broth was centrifuged at 10000 rpm for 10 min at 4 °C. The supernatant was collected for lyophilization at 0.25 MPa, 17 °C. Effect of pH, temperature and different metal ions on naringinase activity of crude enzyme were investigated.

The effect of pH on the activity of naringinase was studied at 40 °C in eight different buffers (0.1 M) with a range of pH values (3.6–7.0): acetate buffer (pH 3.6–5.5), sodium phosphate buffer (pH 6.0–7.0).

The optimum temperature for naringinase activity was determined by incubating the enzyme at different temperatures (30–55 °C) in 0.1 M acetate buffer (pH 4.0).

The influence of metal ions on naringinase activity was investigated in 0.1 M acetate buffer of pH 4.0 at 40 °C. Crude naringinase was incubated in the presence of metal ions Cu^{2+}, K^{+}, Mn^{2+}, Zn^{2+}, Fe^{3+}, Al^{3+}, Ca^{2+} and Mg^{2+} with concentrations of 5 mM. Control sample was prepared without any metal ions.

2.6 Application of probiotic lactic bacteria for debittering of grapefruit juice

Pasteurized grapefruit juices were purchased from the supermarket. The initial pH of the grapefruit juice was adjusted to pH 6.3 with 4N NaOH before fermentation. High naringinase production probiotic lactic acid bacteria (*Lactobacillus plantarum* 01*, Lactobacillus rhamnosus* B01725*, Lactobacillus fermentum* D13*, Bifidobacterium bifidum* B7.5) were used for simultaneous fermentation and debittering of the grapefruit juice.

Grapefruit juice was inoculated in 100 mL flask with *Lactobacillus* and/or *Bifidobacterium* strain and kept under aerobic conditions (in case of mono culture of *Lactobacillus*) or anaerobic condition by using Bugbox anaerobic chamber (in case of mixed cultures or *B. bifidum B7.5*) at 37 °C for 24 hours. Samples were taken during fermentation process every 6 h and every 2 weeks during the storage of 3 months at 4 °C. The number of the colony-forming units (cfu) of bifidobacteria and lactobacilli were counted. In addition, the pH, antioxidant capacity and total polyphenol content of fermented grapefruit juice were also measured during fermentation process and 12 weeks of storage at 4 °C. The carbohydrate concentrations, organic acid contents and naringin content of the juices were determined during the fermentation.

2.7 Analytical methods

Naringinase activity was assayed by Davis method (Davis, 1947). A yellow chalcone produced by the reaction between the substrate (naringin), the hydrolyzed products (pruning, naringenin) and diethylene glycol in alkaline solution (4N NaOH).The absorbance of yellow chalcone was measured at the wavelength of 420 nm. One unit of naringinase activity was defined as the amount of enzyme that could hydrolyze 1 μg of naringin per mL and minute at the assay conditions.

The protein content was determined by the Bradford dye-binding procedure using Bio-Rad Protein Assay Kit (Bio-Rad, USA).

Plate counter method was used for counting the number of bacteria in fermented grapefruit juices. The MRS agar was used to determine the cell number of lactobacilli in grapefruit juices

fermented by mixed cultures and monoculture. Beeren's agar medium was used to determine the cell number of bifidobacteria in grapefruit juices fermented by mixed cultures. TPY agar medium was used to determine the cell number of bifidobacteria in grapefruit juices fermented by mono culture. The incubation was done anaerobically in the case of bifidobacteria and aerobically in the case of lactic acid bacteria.

Carbohydrates and organic acids were analyzed by HPLC.

Antioxidant capacity was measured by the ferric-reducing antioxidant power (FRAP) assay (Benzie & Strain, 1996).

Total polyphenol content was determined by using the Folin-Ciocalteu colorimetric method (Singleton *et al*, 1999).

Naringin concentration was evaluated by HPLC method. The Surveyor HPLC system (Thermal Scientific Corporation, USA) consisted of a quadruple pump, an autosampler, a photodiode array (PDA) detector, and a column of Supelcosil^{TM} LC-18 (250 x 4.6 mm, 5 μm) was applied. The mobile phase consisted of acetonitrile (A) and water (B). Separation was performed using a gradient program: 0-8 min 23% A; 8-15 min 23-65% A linear; 15-20 min 65-70% A linear; 20-21 min 70-23% A linear; 21-22 min 23% A. The chromatogram was taken at 280 nm.

3 RESULTS AND DISCUSSION

3.1 Screening probiotic bacteria for naringinase production

All investigated strains (16 strains of lactic acid bacteria and 20 strains of bifidobacteria) grew well in their typical medium, and most of them exhibited extracellular naringinase activity. Among the investigated strains, *L. fermentum* D13 showed the highest naringinase activity.

3.2 Effect of some factors on naringinase production by *L. fermentum* D13

There was a significant effect on naringinase activity of inoculum sizes. Increase in the inoculum size from 2 (v/v) % to 4 (v/v) % had positive effect on naringinase production. After 24 hours of fermentation, the highest yield of naringinase was observed at inoculum size of 4 (v/v) %.

The naringinase activity by *L. fermentum* D13 at varying pH levels showed that the relative enzyme activity increased from pH 5.0 to 6.0. Further increase in pH from 6.0 to 7.0 and 8.0 resulted in decrease of activity of naringinase. The highest naringinase activity (4.8 U/mL) was obtained when the pH medium was 6.0.

Adding different naringin concentrations to the fermentation medium, affected naringinase production. *L. fermentum* D13 grew well in naringin modified medium even at high concentration

(0.2 w/v %). The optimized naringin concentration was 0.1 (w/v) % and application of this concentration resulted 4.4 U/mL enzyme activity.

The highest relative enzyme activity was obtained when *L. fermentum* D13 strain was grown in the medium containing 1% (w/v) sucrose. Other carbohydrates such as molasses, glucose, lactose and galactose also were good carbon sources for naringinase production. Rhamnose and maltose were not suitable substrates for production of naringinase by *L. fermentum* D13 strain, as very low enzyme activities were measured in the fermentation broth after 24 h fermentation. There was a suppression of naringinase production at the sucrose concentrations of 15 g/L and 20 g/L after 24 h fermentation. The maximal relative enzyme activity was obtained at 5 g/L sucrose concentration.

While Cu^{2+} and Zn^{2+} stimulated naringinase synthesis, the other metal ions (Fe^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+} and Ni^{2+}) inhibited naringinase activity at concentration of 5 mM. The maximum naringinase yield (4.6 U/mL) was observed when adding Cu^{2+} into the MRS fermentation medium.

3.3 Optimization of medium components for naringinase production

Based on preliminary experiments, three factors, namely pH, naringin and sucrose, were selected for optimization of naringinase production by *L. fermentum* D13 strain. Central Composite Design (CCD) method with experimental set consisting of 17 runs was applied to determine the optimal levels of these factors. Full second-order polynomial model was applied to describe the changes of naringinase activity. The fit of model to the experimental results was first checked by analysis of variance (ANOVA). The fisher F-test with a low probability value ($P>F$=0.006) demonstrates a high significance of the regression model. The goodness of fit of the model was checked by the determination coefficient (R^2).

The regression equation obtained after the analysis of variance, is presented as a function of pH, naringin content, and sucrose concentration. The model obtained in terms of the coded factor is:

$$Y = 4.532 + 0.272X_1 + 0.185X_2 + 0.269X_3 – 0.394X_1^2 – 0.352X_2^2 – 0.282X_3^3$$

The optimum values of pH, naringin and sucrose concentration for optimization of naringinase production were 6.1, 0.1% (w/v) and 6.0 g/L, respectively. The naringinase activity at the optimal conditions was estimated to be about 5.0 U/mL.

3.4 Characterization of crude naringinase enzyme

Among eight different buffers (0.1 M) with a range of pH values (3.6–7.0): acetate buffer (pH 3.6–5.5), sodium phosphate buffer (pH 6.0–7.0), the enzyme showed maximum activity at pH 4.5. It was still relatively active at pH 4.0, having lost only 16% of its maximum activity. At pH 3.6 and pH

5.0, its activity was about 70% of the maximum activity. The activity of the enzyme decreased dramatically above pH 5.5. The optimum pH for the hydrolysis of naringin by *L. fermentum* D13 naringinase was pH 4.5.

The optimum temperature of the crude enzyme at pH 4.0 was found to be 40 °C. The activity of enzyme lost about 15 % of its maximum activity at 45 °C. Only 50% of the maximum activity was observed at 30 °C, 50 °C and 55 °C. The optimal temperature for the activity of the naringinase as well as α-L-rhamnosidase from various species of microorganism has been obtained to be range from 40 °C to 60 °C.

The presence of 5 mM Cu^{2+} had a significant positive effect on the enzymatic activity. It is interesting that this result is similar to the result of the experiment on checking the effect of metal ions present in fermentation medium on naringinase production by *L. fermentum* D13. No effect was observed in the case of K^{+} on the naringinase activity. The crude naringinase was inhibited strongly (about 50% reduction of activity) in the case of 5mM Al^{3+}. The enzyme activity was slightly restricted by Ca^{2+} and Mn^{2+}.

3.5 Application of probiotic lactic bacteria for debittering of grapefruit juice

The grapefruit juice was fermented by mono culture of *L. plantarum* 01, *L. rhamnosus* B01725, *L. fermentum* D13 and *B. bifidum* B7.5; and by mixed cultures of *Lactobacillus* and *Bifidobacterium*. In all cases, probiotic bacteria showed to be capable of growing well on sterilized grapefruit juice without any nutrition supplementation. These strains did not only able to survive, but also to utilize the carbohydrates in grapefruit juice for their cell synthesis and metabolism. After 24 h of fermentation, the microbial population achieved about 10^9 cfu/mL, the cell counts were 4.22×10^9 cfu/mL, 2.71×10^9 cfu/mL and 1.47×10^9 cfu/mL in the case of *L. fermentum* D13, *L. plantarum* 01 and *B. bifidum* B7.5, respectively. In the case of mixed culture, all combinations showed higher total viable counts than monocultures in the fermentation. After fermentation, significantly high cell numbers were observed in cases of *L. plantarum* 01 strain and *L. fermentum* D13 strain in combination with *B. bifidum* B7.5 strain, 1.67×10^9 and 3.82×10^9 cfu/mL, respectively. The total colony forming units were 2.07×10^9 cfu/mL, 3.85×10^9 cfu/mL, 7.26×10^9 cfu/mL for the 3 combinations between *B. bifidum* B7.5 and *L. rhamnosus* B01725 or *L. plantarum* 01 or *L. fermentum* D13, in order.

After 12 weeks of storage at 4 °C, the surviving cell counts in the fermented grapefruit juice still remained 10^8–10^9 cfu/mL in most cases which is above the required level of 10^7 cfu/mL. Exception of juice fermented by monoculture *L. fermentum* D13 strain, where the viable cell count

was 9×10^7 cfu/mL, 5-12 times lower. Overall, grapefruit juice can be a potential growth medium for lactic acid bacteria, and functional beverage can be produced from grapefruit juice containing high number of probiotic cells after fermentation and maintaining probiotic content during storage.

The fermentable sugars in grapefruit juice were glucose, fructose, and sucrose with the initial concentration of 3.6 g/100 mL, 3.1 g/100 mL and 2.5 g/100 mL, respectively. During the fermentation of grapefruit juice, the concentration of sucrose decreased at the same rate in all cases (from 2.01 g/100 mL to 2.17 g/100 mL). The contents of glucose and fructose were utilized at different rates by mono and mixed cultures. Significant utilization of glucose and fructose were observed in grapefruit juice fermented by *L. fermentum* D13 strain (2.31 g/100 mL) and *L. rhamnosus* B01725 strain (2.03 g/100 mL), respectively.

The lactic acid and acetic acid were produced in grape fruit juice in the concentration range 9.6 – 190 mM and 8.9 – 1074 mM, respectively, in all cases. Significant difference between *Lactobacillus* and *Bifidobacterium* was detected. In the case of grapefruit juice fermented by *Lactobacillus*, the significant higher acetic acid production was observed, while the equal amounts of lactic acid and acetic acid were produced by *B. bifidum* B7.5. The presence of citric acid in the grapefruit juice with the initial concentration 123 mM may lead to the formation of acetic acid by *Lactobacillus* species through the citric cycle (Krebs cycle). The production of organic acids in lactic fermentation could be depended on the media and strains.

The antioxidant capacity and the TPC of the fermented grapefruit juice slightly decreased during fermentation. They continued to decrease during the storage period. At the 12^{th} week of storage, reduction of antioxidant capacity and TPC were about 43-50% and 12-24%, respectively, compared to the initial values.

The initial naringin content in the grapefruit juice was about 2.5 g/L. The maximum decrease of naringin (about 28%) was obtained after 24 h of fermentation by monoculture *L. plantarum* 01 strain as starter. This result might be explained by the origin of the *L. plantarum* strain, as it was isolated from plant. In view of the frequent occurrence of lactobacilli on decaying plant material and fermented vegetable substrates, one could anticipate that their genomes carry one or more genes encoding enzymes capable of utilizing rhamnosilated compounds. Our experiments could not achieve efficient debittering of grapefruit juice by naringinase produced by lactic acid bacteria as in other studies in the literature with purified enzyme supplementation. However, this may be the first time that reduction of naringin in grapefruit juice is reported by living probiotic bacteria, when fermentation of grapefruit juice is combined with debittering.

4 NOVEL CONTRIBUTIONS

1. Sixteen strains of genera *Lactobacillus* and twenty strains of genera *Bifidobacterium* ssp were screened for naringinase activity. These strains secreted extracellular naringinase in presence of naringin as producer after 24 hours of fermentation and *L. fermentum* D13 strain was the best producer.
2. Different strategies were applied to investigate effects of medium compositions and physical parameters on production of naringinase. Sucrose was the best carbon source for enzyme production. The optimum amount of inoculum, pH, naringin concentration and sucrose concentration were determined to be 4 (v/v) %, pH 6.1, 0.1 (w/v) % and 6.0 g/L, respectively. The presence of 5 mM Cu^{2+} in the MRS fermentation medium stimulated naringinase synthesis. The maximum naringinase activity was 5 U/mL under optimal conditions.
3. The maximum activity of crude naringinase from *L. fermentum* D13 strain was achieved at pH 4.5 and temperature 45 °C. The presence of Cu^{2+} has a significant positive effect on the enzymatic activity.
4. Strategy for simultaneous debittering and production of probiotic grapefruit juice was designed and carried out. Four strains *L. plantarum* 01, *L. rhamnosus* B01725, *L. fermentum* D13 and *B. bifidum* B7.5 were selected for monocultural and mixed cultural fermentation of grapefruit juice. Lactic acid bacteria were able to grow well on grapefruit juice without any nutrition supplements. After 24 h of fermentation, the microbial population achieved about 10^9 cfu/mL. The maximum decrease in naringin content (about 28%) was obtained after 24 h of monocultural fermentation by *L. plantarum* 01 strain.

5 PUBLICATIONS

Articles in reviewed journals

1. Anh M. Tran, Toan Bao Nguyen, Vuong Duc Nguyen, Erika Bujna, Mai S. Dam, Quang D. Nguyen (2019). **Changes in bitterness, antioxidant activity and total phenolic of grapefruit juice fermented by *Lactobacillus* and *Bifidobacterium* strains**. *Acta Alimentaria* (accepted, in press)
2. Vuong D. Nguyen, Gabriella Styevkó, Erzsébet Madaras, Gökce Haktanirlar, Anh T.M. Tran, Erika Bujna, Mai S. Dam, Quang D. Nguyen (2019). **Immobilization of β-galactosidase on chitosan-coated magnetic nanoparticles and its application for synthesis of lactulose-based galactooligosaccharides**. *Process Biochemistry* (in press). DOI: 10.1016/j.procbio.2019.05.021
3. Vuong D. Nguyen, Gabriella Styevkó, Linh P. Ta, Anh M. Tran, Erika Bujna, Petra Orbán, Mai S. Dam, Quang D. Nguyen (2018). **Immobilization and some properties of**

commercial enzyme preparation for production of lactulose-based oligosaccharides. *Food and Bioproducts Processing*, 107: 97-103. DOI: 10.1016/j.fbp.2017.11.004.

4. Erika Bujna, Nikoletta Annamária Farkas, Anh Mai Tran, Mai Sao Dam, Quang Duc Nguyen (2018). **Lactic acid fermentation of apricot juice by mono – and mixed cultures of probiotic *Lactobacillus* and *Bifidobacterium* strains**. *Food Science and Biotechnology*, 27:547-554. DOI: 10.1007/s10068-017-0269-x.

Poster presentations

1. Anh M. Tran, Erika Bujina, Judit Krénusz, Mai S. Dam, Quang D. Nguyen. **Naringinase activity of some probiotic bacteria strains**. *Fiatal Biotechnológusok Országos Konferenciája (FIBOK2016 Conference)*, 2016, Godollo-Hungary.
2. Quang D Nguyen , Erika Bujna, Szilard Kun, Gabriella Styevko, Mai Anh Tran, Vuong D Nguyen, Judit M Rezessy-Szabo. **Development of probiotic fruit and vegetable drinks: problems and challenge**. *The 3rd Asia-Pacific Probiotic Workshop 2016.* December 2016, Iran.
3. Anh T. M. Tran, Erika Bujna, Judit Krénusz, Mai S. Dam, Quang D. Nguyen. **Effect of inducer, different carbohydrates and metal ions on production of naringinase from *Lactobacillus fermentum* D13**. *Chemical Engineering Conference.* April 2017, Veszprem – Hungary.
4. Anh M. Tran, Erika Bujna, Judit Krénusz, Mai S. Dam, Quang D. Nguyen. **Srceening some probiotic bacteria strains for naringinase activity**. *EuroFoodChem XIX Conference.* 2017, Budapest – Hungary.
5. Anh M. Tran, Erika Bujna, Mai S. Dam, Quang D. Nguyen. **Optimization of medium components for naringinase production from *Lactobacillus fermentum* D13**. *Fiatal Biotechnológusok Országos Konferenciája (FIBOK2018 Conference).* 2018, Budapest-Hungary.
6. Bao Toan Nguyen, Erika Bujna, Anh M. Tran, Quang D. Nguyen. **Fermentation of pineapple juice by some probiotic *lactobacillus* sp.** *Fiatal Biotechnológusok Országos Konferenciája (FIBOK2018 Conference).* 2018, Budapest-Hungary.
7. Anh M. Tran, Erika Bujna, Toan B. Nguyen, Mai S. Dam, Quang D. Nguyen. **Change of naringin during fermentation of grapefruit juice by some probiotic bacteria**. *3th International Conference on Food Science and Technology*. 2018, Budapest.
8. Bao Toan Nguyen, Erika Bujina, Anh M. Tran, Quang D. Nguyen. **Storage stability of pineapple juice fermented by probiotic bacteria *Lactobacillus* sp**. *3th International Conference on Food Science and Technology*. 2018, Budapest.