国連大学生研究成果

# Purification of Benzoic acid from Cranberry juice using Nanofiltration Technique

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Benzoic acid ($C_6H_5$-COOH) has been used widely in food industry and cosmetic industry as a preservative because of its antibacterial efficiency. Cranberry is rich in Benzoic acid, and the content of Benzoic acid is about 100ppm in the cranberry juice (10%Brix). The concentration implies that cranberry juice contains excess amount of Benzoic acid which can be utilized as natural preservative. Membrane separation technologies such as microfiltration, ultrafiltration and reverse osmosis have many advantages over other separation technologies because they require less energy and no heat treatment. Their application in food industries has been widely developed. If the excess Benzoic acid in cranberry juice can be separated with low cost by use of membrane separation technologies, it will be a promising material for natural preservative.

In previous research of reaction and separation engineering laboratory, NFRI, Benzoic acid rich solution could separate from Cranberry juice by using nanofiltration. However, puerility of Benzoic acid was still low and it's required more purification for use a preservative of food and cosmetics. And NFT-50 membrane which was most suitable nanofiltration membrane for separate Benzoic acid became the abolition from manufacture.

In this study, the efficient conditions of nanofiltration for purification of Benzoic acid from cranberry juice were investigated. At first, rapid analysis method using HPLC with UV detector for organic acids and RI detector for monosaccharide (sugar) was conducted. And eleven commercial nanofiltration membranes were tested with test cell (C60F, Nittodenko, Japan) for checking Benzoic acid separation ability under the high pressure (more than 3 MPa) from the model solution and straight cranberry juice. The effect of pretreatment by ultrafiltration (UF) also tested in separation test using straight cranberry juice. Furthermore, these results were checked with a pilot scale membrane separation system (DDS Lab Module Type 20 plate and frame system).

According to results of model solution separation test, DL, DK, "NF" and UTC-60 considered suitable for purified by second NF treatment. In NF treatment of cranberry juice, GR40PP which is a UF membrane showed good performance for pretreatment of NF because of keeping Benzoic acid (permeability of Benzoic acid) and high permeate flux on next NF test. Benzoic acid was purified by twice nanofiltration. The optimum conditions were using DK membrane at 5 MPa and pH 4.5 on 1st nanofiltration, and at 3MPa and pH 2.5 on 2nd nanofiltration. The concentration of each components after twice NF treatment were as follows; Benzoic acid 2.46 (3.34* ,*before NF)mM Malic acid 1.78 (37.9*) mM, Citric acid 0.14 (65.2*) mM, Fructose 0.13 (43.1*) mM, Glucose 0.44 (188.1*) mM. The purity of Benzoic acid benzoic acid was increased from 0.86 %(mol/mol) to 49.7 % (mol/mol) by twice NF at these conditions. The pilot scale test with Lab Module Type 20 was conducted with no pH adjustment on 1st NF for concentrated cranberry juice to use as other food materials such as juice, jam and etc. Under this condition, purity of Benzoic acid after twice nanofiltration became32.5 % (mol/mol)

This study was investigated Benzoic acid was separated and purified from cranberry juice by twice nanofiltration. Moreover, no pH adjustment on 1st NF might be suitable for recover excess Benzoic acid and produce cranberry foods.

# ENCAPSULATION OF CLOVE OIL USING MICROCHANNEL EMULSIFICATION AND A HOMOGENIZATION METHOD

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Clove oil is an essential oil extracted from clove, and it has been widely applied in pharmaceutical, fragrance, and flavor industry. The oil has several functionalities such as antioxidant agent, free-radicals scavenger, anti-microbial agent, etc. Clove oil is generally recognized as safe (GRAS) to be applied in foods with a considered safety limit (< 1500 ppm). Encapsulating clove oil in the form of oil-in-water (O/W) emulsion is a possibility to preserve the functionalities. The release of clove oil from the emulsion could also be controlled via its formulation to ensure its safety during application. Microchannel (MC) emulsification and homogenization method were used in this research to prepare clove oil-in-water emulsion. MC emulsification is a novel emulsification that requires low energy input and produces monodisperse emulsion droplets. Homogenization method involves high intensive energy to prepare the emulsion and usually results in polydisperse emulsion droplets. Clove oil emulsion droplets produced by MC emulsification were initially monodisperse with coefficient of variance of below 10%. However, the droplets shrunk immediately when surrounded by the continuous phase. This phenomenon took place because the nature of the clove oil that is slightly hydrophilic. Clove oil droplets tend to diffuse to the continuous phase regardless the surfactant applied, resulting in instable emulsion system over time. Instability was also observed when the emulsion was prepared by homogenization method. However, different instability phenomena involved, such as coalescence, Ostwald ripening, and sedimentation. Shrinkage of oil droplets was difficult to be determined with this method. In addition, the instability was contributed by the nature of clove oil that changes in color over time. Stable emulsion until 31 days was obtained when a high concentration (10% w/w) of whey protein isolate (WPI) as the surfactant was applied. However, high concentration of surfactant is hardly applied in food formulation. Instability of clove oil emulsion droplets with a low WPI concentration (2.5% w/w) prepared by homogenization method might be enhanced by increasing viscosity of the continuous phase. A mixture of sodium alginate and WPI with a ratio of at least 1:2 enhanced the stability of the emulsion for at least 10 days.

# The study on a bacterial adaptation by gene amplification

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The reiteration of a chromosomal DNA segment, called gene amplification, occurs spontaneously during replication in all organisms. Gene amplification plays an important role in bacterial adaptation to antibiotics. The aim of this work is to understand the gene amplification-mediated adaptive mechanism under antibiotic stress condition in *Escherichia coli* and *Bacillus subtilis,* which are well-characterized bacteria as the model organisms.

Approximately 4% of ampicillin resistant *E. coli* was found to have duplicated copy of the genomic region including a multidrug resistance gene *acrA*. These *acrA*-amplified strains exhibited resistance to many antimicrobial agents including chloramphenicol, tetracycline, erythromycin, kanamycin, novobiocin, cefotaxime and rifampicin.

*B. subtilis* also can acquire a higher tolerance to tetracycline by increasing the gene dosage of its resistance gene *tetB*. Here we showed that approximately one-third of total tetracycline-resistant cells had multiple copies of *tetB* gene ranging 2 to 230. Four direct repeats flanking *tetB* gene apparently contributed to the *tetB* amplification. Furthermore, the disruption of *recA* gene resulted in a 30-fold decrease in the frequency of *tetB* amplification. Our results indicate that the direct repeats and RecA have an important role for Tc-tolerance development in *B. subtilis*.

# Genetic improvement of secondary metabolite production of an industrial bacterial strain

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*Bacillus subtilis* (natto) is an industrial fermentation strain that significantly increases the nutritional value of soybeans and develops a unique flavor and texture. *B. subtilis* (natto) produces extracellular poly-gamma-glutamate (γ-PGA), a very viscous polymer of DL-glutamic acid linked by gamma peptide bonds. In *B. subtilis* (natto), γ-PGA is synthesized by *pgsBCA* operon. The expression of the *pgs* operon is regulated by quorum-sensing components, ComPA, DegQ, DegS, DegU and cell motility related SwrA. Disruption of *degQ* gene causes loss of ability of γ-PGA production, which is restored by mutations in *degS* as well as other unknown target genes. By whole genome sequencing analysis for the unknown targets revealed several candidate genes responsible to the mucoid colony phenotype, including a single point mutation occurred in *yxyZ* gene leading to alternation of an amino acid in the protein. We obtained evidence that single amino acid alteration of wild-type *yxyZ* plays an important role in restoring γ-PGA production that was abolished by disruption of *degQ*. In addition, it is noted that disruption of *yxyZ* gene not only effects on colony morphology relative to bacteria swarming mobility on solid surface but also reduces in *pgs* operon expression and exoprotease production. Furthermore, recombinant wild-type and the mutant YxyZ protein produced in *E. coli* cells behaved quite differently; wild-type YxyZ was stably expressed and effectively purified due to its good solubility whereas the mutant was very sensitive with changes of fermentation condition.

# Effect of packaging material on shelf life, quality and gene expression of tomatoes

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The effect of packaging material and storage temperatures on the shelf life and quality changes of tomato 'KEK-I' (called as super tomato fruit containing 9° Brix) during storage. Tomatoes with three packaging conditions, Modified Atmospheric Packaging (MAP), MAP+ hinokitiol (MH), and perforated film package as control were compared for their quality change. Packaging material used were Low Density Polyethylene (LDPE) film (40μm) and fresh sheets of hinokitiol. Hinokitiol is a volatile oil and reported to have a strong chelating agent, inhibits microbial spoilage, antifungal, inhibits enzymatic browning and was approved as food additive by Japanese Government.

Tomato is a climateric fruit which shows a dramatic increase in respiration rate and ethylene production rate at the onset of ripening on vine or postharvest. Ripening process is characterized by softening of fruit, degradation of chlorophyll, and synthesis of acids, sugar and lycopene associated with increased expression of ACC Synthase (ACS) gene,. Packaged 'KEK-I' tomatoes stored at 15℃ or 25℃ were analyzed for the ripening related parameters and quality control factors during the intervals of storage. Gas composition was maintained at the recommended level of 3 to 5 % $O_2$ upto $9^{th}$ day of storage at 15℃ or 25℃. Control tomato has significant difference of color, texture, and pigments in both the temperatures whereas those under MAP and MH showed little change. A linear relation was found between a* and lycopene and between b* and β carotene of all samples throughout the storage period.

Expression levels of genes related to the ethylene production, textural and fermentative changes of tomato were evaluated through quantitative Real Time PCR (qRT-PCR). qRT-PCR results for *LeACS*?, *LeADH*, *LePDC* and *LeTBG4* showed the drastic change of *LeACS*? in the control sample but not in the other samples and genes.

Green of the tomatoes was maintained after $20^{th}$ and $15^{th}$ day of storage under MAP and MH at 15℃ and 25℃ respectively. Packaging condition and storage temperatures used in this research study are effective for MAP and MH in terms of shelf life extension of 'KEK-I' tomatoes when compared to control. This study would provide valuable information of shelf life extension of 'KEK–I' tomato by using MAP treatment and would be useful for future research on the quality control of fresh tomatoes.

Key words: Modified Atmosphere Packaging, Hinokitiol, 'KEK–I', ACS, ADH, PDC and TBG4