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Extended Abstract

The Effect of *Pseudomonas putida* on the Reduction of Limonin in the De-bittering of Orange and Grapefruit Concentrates and Assessment of Physicochemical and Organoleptic Features

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Abstract
The bitterness of citrus juice is one of the most important factors in the reduction of marketability in juice industry. Limonin and Narengin are known as two important compounds in bitter citrus juice during storage. Therefore, various processes, including chemical, microbial and enzymatic methods, have been used to reduce these bitter compounds. The purpose of this study was to investigate the effect of adding *Pseudomonas putida* bacteria on the reduction of the level of limonin and also the measurement of physicochemical and organoleptic properties. *Pseudomonas putida* bacteria with two concentrations of 10^2 and 10^4 cfu/mL as a causative agent of orange and grapefruit concentrate were studied. Also, the physicochemical properties of concentrates were measured at 120 h (growth time and bacterial activity). The results showed that the growth rate of bacteria had a significant effect on the reduction of limonin, pH and turbidity levels ($P<0.05$). Also, it has a significant effect on increasing the acidity of the treatments ($P<0.01$). After pasteurization and inactivation of *Pseudomonas putida* bacteria, to ensure that bitterness is not rejected the specimens were incubated at 40 °C for 45 days and sensory characteristics were measured on days 0, 15, 30 and 45. Among the samples, T_4 (orange concentrate + 10^4 cfu/mL) and T_6 (grapefruit concentrate + 10^4 cfu/mL) treated with the lowest microbial growth rate in 120 h and also, in terms of general acceptance and taste, it has more points than other samples during 45 days of storage were identified as superior samples.

Keywords: Bitter, Grapefruit concentrate, Limonin, Orange concentrate, *Pseudomonas putida*

Introduction
Consumption of fruits and juices in the daily diet due to the presence of vitamins, antioxidants and nutrients reduces the risk of many diseases such as cancer, cardiovascular disease. The beneficial effects of fruit on human health are due to its numerous antioxidant compounds such as vitamin C, polyphenols, flavonoids and carotenoids. Citrus fruits and their products are rich in carbon, minerals, dietary fiber, vitamins, etc., (Shojah, Ghasemnezhad, & Mortazavi, 2011).

The bitterness of citrus fruit extracts is one of the major problems for the producers of these products, because the marketability and economic value of the extracts are greatly reduced. The reason for the bitterness of citrus fruit extract is due to the changes in limonoids and the production of bitter taste of limonin (Syed, Ghatge, Machewad, & Pawar, 2012).

Limonin and narengin are known as two important compounds in bitter citrus juice during storage. Limonin is one of the most important causes of bitterness in citrus juices and is widely found in most citrus fruits (Maier & Dreyer, 1965). Its structure includes a Furan ring, two lactone rings (A and D), five ether rings and an epoxy, and is highly capable of combining with oxygen (Altinok, Kayis, & Capkin, 2006).

The bitterness of the juice is due to the presence of the bitter precursor limonin, limonite-E-lactone ring, which is present in the membranous sacs of the fruit. When the membrane is destroyed during juice extraction, limonite-E-lactone ring in contact with the acidic environment of the juice and by the enzyme limonene-D-ring lactone hydrolase cause the closure of the limonin ring (Sharma & Bansal, 2007).

Therefore, various processes, including chemical, microbial and enzymatic methods, have been used to reduce these bitter compounds. Due to their potential for metabolism and their diverse biological catalysts, microorganisms have the ability to convert biologically, either using complex hydrocarbons and limonin as a source of carbon, or through a common metabolism between plant compounds, reducing the amount of bitter compounds such as terpenoids (Sharma & Bansal, 2007)

Pseudomonas putida has a variety of aerobic metabolism and is able to destroy organic solvents and break them down through oxidative reactions. This bacterium is one of the opportunistic microorganisms in food nutrition and metabolism. *Pseudomonas putida* can be grown by metabolizing a wide range of compounds such as carbon, nitrogen and phosphate (Taneja, 2007).

The purpose of this study was to investigate the effect of adding *Pseudomonas putida* bacteria on the reduction of the level of limonin and also the measurement of physicochemical and organoleptic properties.

Research treatments

The specifications of the treatments used in this research are presented in Table (1):

Table 1. Coding of research treatments

Treatments	Code
Orange Concentrate	T_1
Grapefruit Concentrate	T_2
100mL Orange Concentrate + 10^2cfu/mL	T_3
100mL Orange Concentrate + 10^4cfu/mL	T_4
100mL Grape fruit Concentrate+ 10^2cfu/mL	T_5
100mL Grape fruit Concentrate +10^4cfu/mL	T_6

Materials and methods

The fruit juice was prepared manually and then the pulp was separated by small pores with a size of 0.02 mm. After preparing the juice, to prepare the concentrate, a microwave device with a maximum power of 350 watts is used up to the orange juice brix was 45 and grapefruit was 42 (Zoghi, Khosravi-Darani, Sohrabvandi, Attar, & Alavi, 2019). *Pseudomonas putida* was prepared as a pure strain culture. The bacterial strain was cultured in TBS medium and after heating, the 0،5 McFarland standard was prepared. *Pseudomonas putida* bacteria suspension was infused at 2 mL with concentrations of 10^2 and 10^4 cfu/mL in grapefruit and orange concentrate samples under sterile conditions and under Laminar hood (Yoon, Kang, Lee, & Oh, 2005). Also, the physicochemical properties of concentrates such as pH, brix, acidity, limonin, turbidity, colour (L,a,b) and bacterial growth were measured at 120 h.

Pasteurization and Incubator of samples

The pasteurization of grapefruit and orange concentrates was performed after physicochemical tests in Ben Marie at 90 °C for 10 min to inactivate *Pseudomonas putida* bacteria. After pasteurization, the samples was incubated at 40 °C for 45 days. The purpose of this step is to ensure that the bitterness of the concentrate didn't return and to assess the sensitivity of the samples during storage. Therefore, the sensory characteristics of the samples were examined on the days of 0, 15, 30 and 45.

Statistical Analysis

The test was performed with 6 samples at 4 different times, a total of 24 treatments and 3 replications. In this experiment, *Pseudomonas putida* bacteria with two different concentrations were inoculated with two concentrate samples and its effect on reducing the bitterness of the samples and physicochemical and sensory properties during different time periods was investigated. Statistical analyses were performed using SAS software version 9.1 and drawing charts with Microsoft Excel (Version 2013), 3 replications were used for the measurement of concentrates samples.

Results and discussion

pH

According to the results presented in the pH analysis analysis table, the effect of treatment and storage time on the pH index of the samples was quite significant ($P<0.01$).

Brix

According to the results presented in the Brix variance analysis table, the effect of treatment type on Brix samples of orange concentrate and grapefruit was quite significant ($P<0.01$).

Acidity

According to the results presented in the analysis of acidity analysis table, the effect of storage time, treatment type and bilateral interaction on the acidity of orange and grapefruit concentrate samples was quite significant ($P<0.01$).

Limonin content

According to the results presented in the analysis table of limonin variance, the effect of treatment type, maintenance time effect and bilateral interaction on limonin samples of orange and grapefruit concentrate samples were quite significant ($P<0.01$).

Turbidity

According to the results presented in the analysis of turbidity variance table, the effect of treatment type, effect of storage time and bilateral interaction on turbidity of orange and grapefruit concentrate samples were quite significant ($P<0.01$).

Color

According to the results presented in the color L* variance analysis table, the effect of treatment type, maintenance time effect and bilateral interaction on color L* of orange and grapefruit concentrate samples were quite significant ($P<0.01$). According to the results presented in the analysis table of a* color variance, it was found that the effect of treatment type, effect of storage time and bilateral interaction on color a* of orange and grapefruit concentrate samples were quite significant ($P<0.01$). According to the results presented in Table b* color variance analysis table, the effect of treatment type, effect of storage time and bilateral interaction on color b* of orange and grapefruit concentrate samples were quite significant ($P<0.01$).

Bacterial growth

According to the results presented in the analysis table of variance count of *Pseudomonas putida* bacterium, it was found that the effect of treatment type, effect of storage time and bilateral interaction on the counting of *Pseudomonas putida* bacterial samples of orange and grapefruit concentrate samples were quite significant (P<0.01).

Sensory evaluation

According to the results presented in the total Acceptance Sensitivity Analysis , the effect of treatment type, maintenance time effect and bilateral interaction on sensory evaluation of the total acceptance index of orange and grapefruit concentrate samples were quite significant (P<0.01).

Conclusions

Bacterial growth and storage time had a significant effect on pH, acidity, and brix (P<0.01). With increasing concentrate storage time, the pH, brix and turbidity of the drink decreased and the acidity of concentrate increased significantly (P<0.01). As the storage time increases, the amount of limonene in microbial-free treatments increases, however, in treatments containing microbial concentrations, the amount of limonene is significantly reduced. In contrast, the growth of the bacterium *Pseudomonas putida* has increased with the use of limonin (P<0.01). Storage time has had a positive and significant effect on organoleptic characteristics of treatments during storage. But the color and flavor scores increased due to the reduction of bacteria. Among the samples, T_4 (orange concentrate+10^4 cfu/mL) and T_6 (grapefruit concentrate+10^4 cfu/mL) treated with the lowest microbial growth rate in 120 h and also, in terms of total acceptance and taste, it has more points than other samples during 45 days of storage were identified as superior samples.

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