DEBITTERING OF GRAPEFRUIT JUICE WITH NARINGINASE

ALTINTOP SUYUNDAKİ ACILIĞIN NARİNGİNAZ ENZİMİ İLE GİDERİLMESİ

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ABSTRACT: This study was undertaken to investigate debittering of grapefruit juices from Marsh Seedless and Rio Red varieties. Debittering was carried out with different naringinase concentrations (0.25, 0.50, 0.75 and 1.00 g/L) at different temperatures (25, 35 and 40°C). Increasing naringinase concentration and temperature resulted in a higher naringin reduction. In both varieties, the highest naringin reduction was obtained with 1 g/L naringinase at 40°C for 6 h. The results from organoleptic evaluation showed that the panellists were able to distinguish the control samples from the enzyme-treated samples.

Keywords: Debittering, naringin, naringinase, grapefruit juice

ÖZET: Bu çalışmada Marsh Seedless ve Rio Red çeşiti altıntoplardan elde edilen meyve sularındaki acılığın giderilmesi araştırılmıştır. Acılık giderme işlemi farklı naringinaz konsantrasyonlarında (0.25, 0.50, 0.75 ve 1.00 g/L) ve farklı sıcaklıklarda (25, 35 ve 40°C) gerçekleştirilmiştir. Naringin giderimi, naringinaz miktarı ve sıcaklıktaki artışa paralel olarak artmıştır. Her iki çeşitte de en yüksek naringin giderimi 1 g/L naringinaz konsantrasyonunda 40°C'de ve 6 saatlik inkübasyon sonunda elde edilmiştir. Duyusal değerlendirmede panelistler kontrol örneklerini enzimle muamele edilmiş örneklerden ayırt edebilmişlerdir.

Anahtar Kelimeler: Acılık giderme, naringin, naringinaz, altıntop suyu

INTRODUCTION

Excessive bitterness in grapefruit juice adversely affects the flavour and marketability of the final product. Bitterness in grapefruit juice is primarily caused by naringin which is found in the fruit membrane and albedo and becomes extracted into fruit juice. As the fruit ripens the concentration of naringin decreases (Wilson et al, 1989; Puri et al, 1996). All processed grapefruit juice contains naringin above 50 ppm level along with limonin which acts synergistically with naringin to cause bitterness. The presence of bitterness has been a major limitation in the commercial acceptance of grapefruit juice (Thomas et al, 1958).

To reduce the bitterness of grapefruit juice, naringin can be hydrolysed by naringinase which possesses both a-rhamnosidase (EC 3.2.1.40) and b-D-glucosidase (EC 3.2.1.24) activity. a-rhamnosidase splits naringin into purinin and rhamnose. Purinin is then hydrolysed by b-D-glucosidase to naringenin and glucose. Since the bitterness of purinin is less than one third that of naringin, a-rhamnosidase activity is essential for debittering of grapefruit juice (Jimeno et al, 1987; Tsen et al, 1989; Aksay ve Ünal, 2002).

Although debittering of grapefruit juice is thoroughly investigated, no such research has been carried out on the grapefruit varieties grown in Turkey. The present work was undertaken to investigate enzymatic debittering of the grapefruit juices from Marsh Seedless and Rio Red varieties grown in Adana.

MATERIALS AND METHODS

Materials

Rio Red variety was kindly supplied by the Faculty of Agriculture of the University of Çukurova and Marsh Seedles variety was purchased in the local market. The fruits were kept at 4°C until the experiments.

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Naringinase (*Penicillium* sp) and naringin were purchased from Sigma (St. Louis, USA.). Diethylene glycol and isopropanol were purchased from Merck (Darmstad, Germany).

Juice extraction and enzymatic treatment

The fruits which were washed and halved with a knife were squeezed by using an automatic juice extractor (home style). The fruit juice was filtered through cheesecloth and then frozen in plastic bottles at −18°C until the experiments. The frozen grapefruit juice was thawed and then centrifuged at 1045 x g for 10 minutes. The predetermined amount of enzyme solution was added into the 300 mL of clarified fruit juice. Debittering was carried out with different naringinase concentrations (0.25, 0.50, 0.75 and 1.00 g/L) at different temperatures (25, 35 and 40°C). The control samples contained no enzyme. Experiments were performed in duplicate in a water bath. Naringin content was monitored at two-hour time intervals for a period of 6 h.

Analysis of naringin

Naringin content of grapefruit juice was determined by Davis' method (Davis, 1947). A 6.25 mL aliquot of diethylene glycol solution (90%) was mixed with 0.125 mL of clarified grapefruit juice and 0.125 mL of 4 N NaOH in a test tube. After thorough mixing, it was kept for 10 min at ambient temperature until yellow colour fully developed. The absorbance of the samples was measured at 420 nm. A standard curve of naringin solutions in the range of 100-600 ppm was plotted.

Total Acidity

Total acidity was determined by potantiometric titration method (IFFJP, 1968) and the results were expressed as g/L as citric acid.

Organoleptic evaluation

The enzyme-treated fruit juice samples were evaluated by a nine-member panel. The evaluation was based on the bitterness, which was scaled as follows: 6 = extremely bitter, 5 = very bitter, 4 = bitter, 3 = moderately bitter, 2 = slightly bitter, and 1 = not bitter (Olson et al, 1979).

Statistical analysis

Effects of enzyme concentration and temperature on naringin removal were evaluated by analysis of variance. Means were separated by the Duncan's Multiple Range Test. All the statistical analyses were performed by using SPSS version 10.0 (Barnes, 1994).

RESULTS AND DISCUSSIONS

Composition of grapefruits

The compositions of the grapefruits are given in Table 1. As can be seen from the table, naringin content and total acidity of Rio Red are higher than those of Marsh Seedless.

Debittering of Marsh Seedless juice

The % reduction in naringin content increased with incubation time, temperature and enzyme concentration (Figures 1-3). The highest naringin reduction was 55% with 1 g/L naringinase at 40°C. In a study carried out by Parakash *et al* (2002), the highest naringin reduction in Indian grapefruit juice was 75% with 1 g/L naringinase at 40°C for 4 h, which is higher than the results obtained in this study.

Table 1. General composition of grapefruits

	Marsh Seedless	Rio Red
Naringin (mg/L)	423	551
Brix	10.9	9.5
Total acidity* (g/L)	14.05	16.3
pH	3.33	3.14

* As citric acid

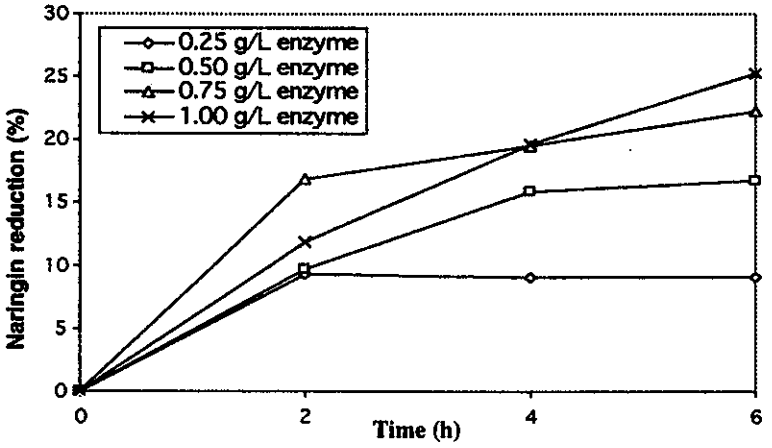

Figure 1. Effect of enzyme concentration on naringin reduction at 25^{0}C

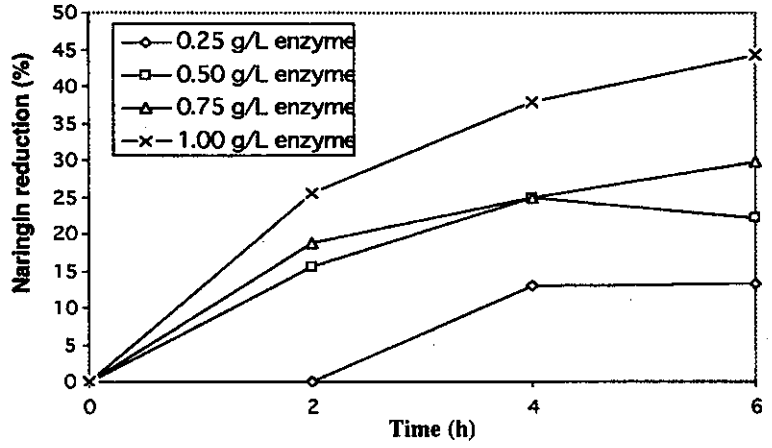

Figure 2. Effect of enzyme concentration on naringin reduction at 35^{0}C

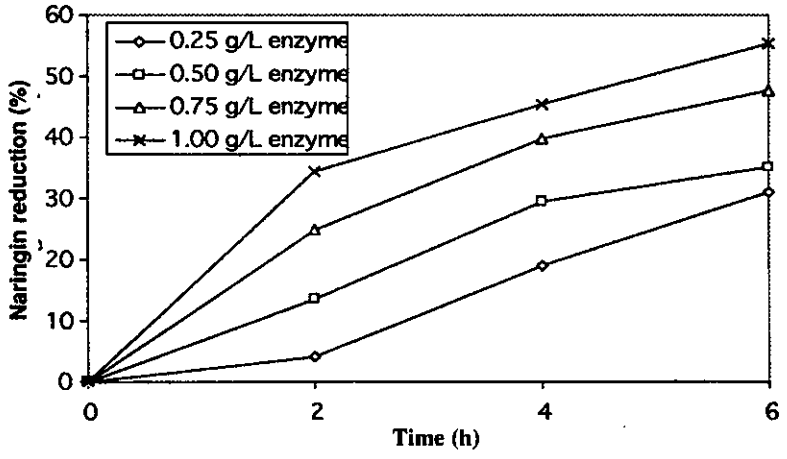

Figure 3. Effect of enzyme concentration on naringin reduction at 40°C

Debittering of Rio Red juice

The experiments were conducted under the same conditions as with the Marsh Seedless variety and the results are depicted in Figures 4-6. The results were similar to those with Marsh Seedless variety. The reduction in naringin content increased with the increasing temperature, enzyme concentration and incubation time. The highest naringin reduction was 59% with the use of 1 g/L naringinase at 40°C. Tsen and Tsai (1989) found that optimum pH and temperature for naringinase from *Penicillium* sp. immobilised on cellulose triacetate were 3.7 and 55°C, respectively. This explains why the naringin reduction increased as the temperature was increased from 25°C to 40°C. Yalım (2002) investigated the determination and reduction of naringin in various citrus products and the highest naringin reduction varied between 30-40% with 300 mg/L naringinase at 60°C.

Statistical evaluation

Effects of temperature and enzyme concentration on naringin removal were found to be statistically significant ($p<0.01$) in both varieties. The differences between various temperatures and enzyme concentrations in terms of naringin removal were tested by Duncan Multiple Range Test and the results are

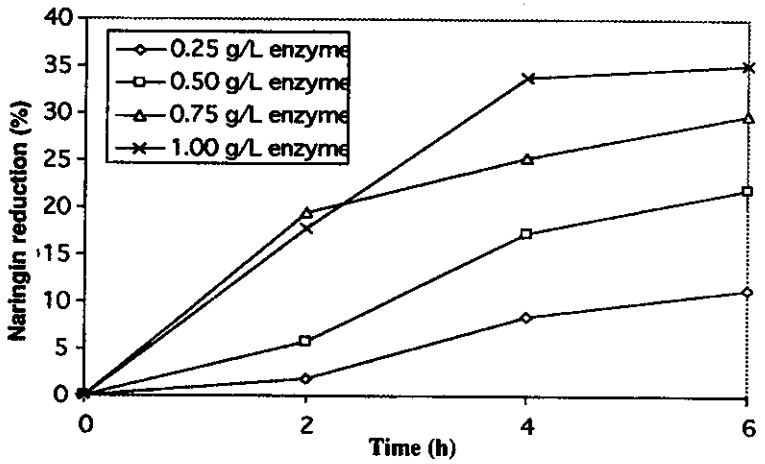

Figure 4. Effect of enzyme concentration on naringin reduction at 25°C

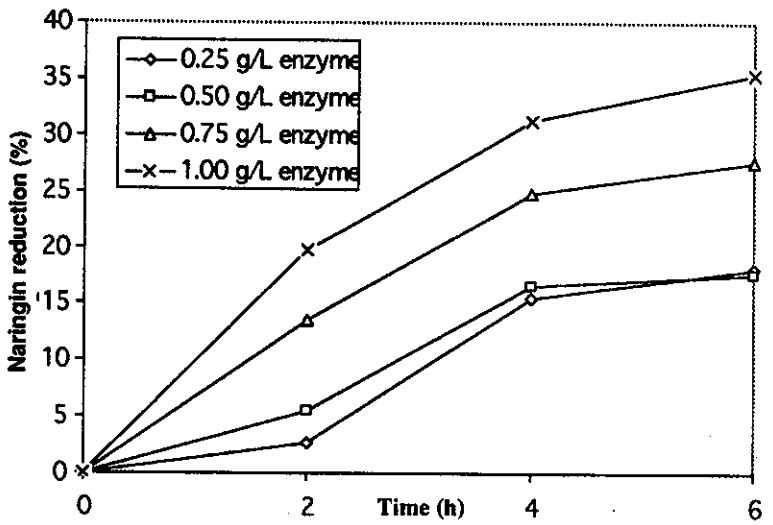

Figure 5. Effect of enzyme concentration on naringin reduction at 35°C

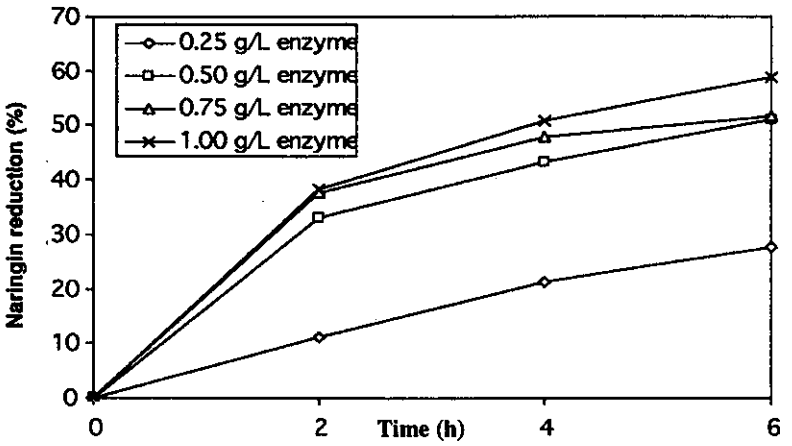

Figure 6. Effect of enzyme concentration on naringin reduction at 40°C

Table 2. Effect of temperature on naringin reduction

Temperature (°C)	Marsh Seedless*	Rio Red*
40	28.71^{a}	47.16^{a}
35	27.46^{a}	24.74^{b}
25	18.36^{b}	24.64^{b}

*Means followed by the same letter are not statistically significant at p<0.01

Table 3. Effect of enzyme concentration on naringin reduction

Enzyme concentration (g/L)	Marsh Seedless*	Rio Red*
1.00	33.78^{a}	43.13^{a}
0.75	27.40^{ab}	36.42^{ab}
0.50	21.51^{bc}	30.20^{b}
0.25	16.68^{c}	18.97^{c}

*Means followed by the same letter are not statistically significant at p<0.01

tabulated in Table 2. The difference between 35°C and 40°C in Marsh Seedless was found to be insignificant at p<0.01 whereas the difference between 25°C and 35°C /40°C were significant. As for Rio Red, the difference between 25°C and 35°C was insignificant whereas the differences between 40°C and 25°C/35°C were significant (p<0.01). In both varieties, the differences between enzyme concentrations were statistically significant at $p < 0.01$ (Table 3).

Organoleptic Evaluation

The results from organoleptic evaluation are tabulated in Table 4. In experiments with Marsh Seedless, the panellists seem to distinguish the control samples from the enzyme-treated samples. The sensory score of the enzyme-treated samples varied between not bitter and moderately bitter. As for Rio Red, the panellists were able to distinguish the control samples from the enzyme-treated samples except for the control samples at 25°C.

Table 4. Sensory evaluation of the samples

Temperature (°C)	Enzyme concentration concentration (g/L)	Marsh Seedless Average bitterness score*	Rio Red Average bitterness score*
25	Control	3.00^{ab}	2.66^{bc}
	0.25	2.55^{abcd}	2.77^{abc}
	0.50	2.55^{abcd}	2.77^{abc}
	0.75	1.55^{d}	2.11^{bc}
	1.00	1.00^{bcd}	1.77^{c}
35	Control	3.44^{a}	3.00^{ab}
	0.25	2.88^{abc}	2.44^{bc}
	0.50	2.11^{bcd}	2.44^{bc}
	0.75	2.77^{abc}	2.66^{bc}
	1.00	2.33^{abcd}	2.66^{bc}
40	Control	3.44^{a}	3.77^{a}
	0.25	2.77^{abc}	2.44^{bc}
	0.50	2.33^{abcd}	1.78^{c}
	0.75	1.77^{cd}	2.66^{bc}
	1.00	2.11^{bcd}	2.55^{bc}

*Means followed by the same letter are not statistically significant at $p<0.05$

CONCLUSION

The following conclusions can be drawn from this study: (i) Bitterness in grapefruit juice can be reduced by enzymic hydrolysis. (ii) Naringin reduction depends on temperature, incubation time and amount of enzyme employed. (iii) Reduction in bitterness was detected by the panellists.

ACKNOWLEDGEMENTS

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