

Protein expression product alterations in *Saccharomyces cerevisiae*

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Summary. Lemon is very rich in terms of vitamin C and thus it has antioxidant properties. In this study, the effects of lemon juice on cellular development in yeasts and protein expression have been examined. Seven groups were created in this study: 1: Control group; 2: $K_2Cr_2O_7$ group; 3: 5 mM $K_2Cr_2O_7$ + lemon juice (LJ) group; 4: 10 mM $K_2Cr_2O_7$ + LJ group; 5: 15 mM $K_2Cr_2O_7$ + LJ group; 6: 20 mM $K_2Cr_2O_7$ + LJ group; 7: 25 mM $K_2Cr_2O_7$ + LJ group. After sterilization, fruit juice (15%) and $K_2Cr_2O_7$ were added different concentration to *Saccharomyces cerevisiae* (*S. cerevisiae*) cultures and the cultures were developed at 30°C for 1h, 3h, 5h and 24 hours (overnight). *S. cerevisiae* cell growth was analysed by spectrophotometer, total protein alterations was identified by SDS-PAGE electrophoresis and measured with biuret method. As a results: cell growth and protein expression amount increased in LJ groups to which LJ was taken in comparison to the positive control ($K_2Cr_2O_7$) group at different growing times (1, 3, 5 and 24 hours) ($p < 0,05$). These results indicated LJ has a protective effect for reduce the oxidative damage and raised cell growing and encourage protein synthesis in *S. cerevisiae* culture.

Key words: *S. cerevisiae*, protein expression, lemon juice, chromium, SDS-PAGE

Introduction

Chromium which is a transition element is very toxic for plants and microorganisms. Chromium which has oxidative effects is used widely in the industry and is one of the major pollutants while also having carcinogenic effects. *Saccharomyces cerevisiae*, has been used as principal yeast in many biological studies. Their use continues to spread every day since their genetic structures and cellular properties are well known and accordingly, it is one of the most preferred microorganisms in scientific studies. In recent years, fruit extracts known to be rich in antioxidants, minerals and vitamins are being used on yeasts in many studies (1-4). Oxidative damage can be completely or partially removed thanks to various foods with strong

antioxidant effects (5-9). Lemon juice has disinfectant effect against microorganisms as well as antioxidant effects on the cellular level. Thus, it is used against some diseases and oxidative damages. Lemon is very rich in terms of vitamin C and thus it has antioxidant properties. Lemon juice has an acidity of around 5% and thus its pH value varies between 2-3 (10, 11). Many studies carried out put forth that different fruit content increases cellular development in yeasts, encourages protein synthesis and displays protective features against oxidative stress (12-14). In this study, seven different groups were formed to examine the oxidative damage caused by $K_2Cr_2O_7$ oxidant material on *Saccharomyces cerevisiae* and the effects of lemon juice on cellular development in yeasts and protein expression have been examined.

Material and Methods

Research groups

Seven groups were composed. After sterilization, fruit juice (15%) and $K_2Cr_2O_7$ were added different concentration to *Saccharomyces cerevisiae* (*S. cerevisiae*) cultures and the cultures were improved at 30°C for 1h, 3h, 5h and 24 hours (overnight). Occurrence media of *S. cerevisiae*: for the developed and reproduce of yeast, YEPD (for 50 mL 1.75 g yeast extract, 1.6 g trypton, 1.5 g glucose) besides, for the growth and reproduce of *S. cerevisiae*, lemon juices was added and improved. After sterilization, samples were incubated for 1h, 3h, 5h, 72 h (overnight, h: hour) at 30°C (7).

Lemon juice extract and $K_2Cr_2O_7$ chemical

Fruit (From center county of Elazığ city) was crushed in water and added in to *S. cerevisiae* media cultures and inserted 15% (v/v) ratio in at the duplicating for 30°C. $K_2Cr_2O_7$ was added in $K_2Cr_2O_7$ and LJ+ $K_2Cr_2O_7$ groups.

Cell concentration results

In these measurements, culture samples that were grown at 30°C for 1, 3, 5 hours and overnight (24 hours) have been analyzed. The calculation has been accomplished using a spectrophotometer at 600 nm (OD_{600}).

SDS-PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis)

The samples of *Saccharomyces cerevisiae* cultures were made for SDS-PAGE after which they were charged to sample loading wells as 18 microliters and to be subject to electrical current and after this process

the gels were dyed, their images were taken and the intergroup protein bandings were used as data in the study (15).

Protein concentration measurements

The protein content was analysed by a modified biuret method. In this method briefly, we prepared BSA standards. BSA protein standards at different concentrations (1-15 mg/ml) were obtained using BSA protein. 1 ml of the protein samples was added 4 ml of biuret reagent and they were vortexed. After vortex they were incubated in room temperature at 25°C, after this process protein values were calculated at 540nm (OD_{540}) (16). Hence, the total protein amount in *Saccharomyces cerevisiae* groups value was computed.

Statistical analysis

For statistical analysis the SPSS 20.0 software was used. The comparison between experimental groups and the control group was made using one way Anova Post Hoc Hochberg and Games-Howell test. Statistically significant differences among groups have been indicated as $p < 0.05$ and the statistically non-significant differences have been indicated as $p > 0.05$. Standard deviations were indicated as \pm .

Results and Discussion

We hope that the results obtained in this study will be an important reference for future studies. When the results put forth in Table 1 and Figure 1 are examined, it can be observed that there are statistically significant ($p < 0.05$) differences among groups at

Table 1. The growing of *Saccharomyces cerevisiae* in lemon juice at different hours.

OD_{600} 30°C	1h	3h	5h	Overnight
Control	1,59 \pm 0.002 ^a	1,60 \pm 0.002 ^b	1,83 \pm 0.002 ^b	1,59 \pm 0.002 ^b
$K_2Cr_2O_7$	1,60 \pm 0.002 ^a	1,56 \pm 0.002 ^a	1,67 \pm 0.002 ^a	1,50 \pm 0.002 ^a
5 mM $K_2Cr_2O_7$ + lemon juice	1,88 \pm 0.002 ^b	1,83 \pm 0.002 ^c	1,90 \pm 0.002 ^c	1,75 \pm 0.002 ^c
10 mM $K_2Cr_2O_7$ + lemon juice	2,01 \pm 0.002 ^d	1,85 \pm 0.002 ^e	1,83 \pm 0.002 ^b	2,04 \pm 0.002 ^f
15 mM $K_2Cr_2O_7$ + lemon juice	2,04 \pm 0.002 ^e	1,87 \pm 0.002 ^e	1,90 \pm 0.002 ^c	1,98 \pm 0.002 ^d
20 mM $K_2Cr_2O_7$ + lemon juice	2,08 \pm 0.002 ^f	1,77 \pm 0.002 ^d	1,89 \pm 0.002 ^c	2,08 \pm 0.002 ^e
25 mM $K_2Cr_2O_7$ + lemon juice	1,98 \pm 0.002 ^c	1,69 \pm 0.002 ^c	1,90 \pm 0.002 ^c	2,00 \pm 0.002 ^c

^{**}a,b,c,d,e,f,g; among the groups which bearing of different letter are significant ($p < 0.05$).

Anova Post Hoc Hochberg and Games-Howell Test

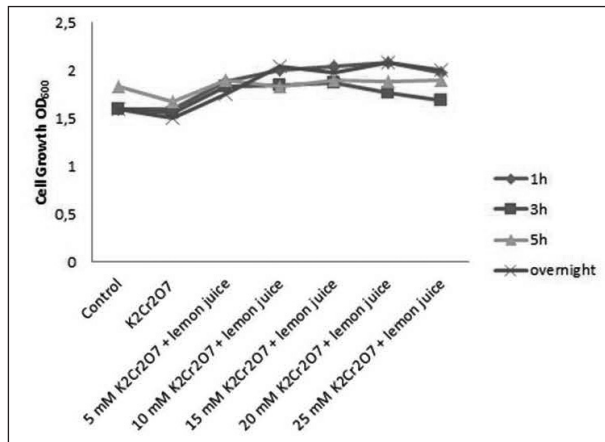


Figure 1. The growing of *Saccharomyces cerevisiae* in LJ at different hours

different development times. It can be seen that lemon juice added to the cultures protects cellular development against the negative effects of chromium and indeed in some cases increased it. When the biuret protein results given in Table 2 and Figure 2 are examined, we can state that lemon juice triggers protein synthesis in yeasts. It is observed that the protein density is greater in yeast groups with added lemon juice in comparison with the positive control group. When the SDS-PAGE supernatant and pellet total protein bands in Figure 3 and Figure 4 are examined, it can be observed that lemon juice has a positive effect on protein expression in *Saccharomyces cerevisiae*. We had obtained similar results in our previous studies with fruit juices such as pomegranate juice, apple juice and cherry on *S. cerevisiae*. It was determined during the study we carried out using pomegranate juice that the

Table 2. Biuret protein density

OD ₆₀₀ 30°C	mg/ml
Control	1
K ₂ Cr ₂ O ₇	0.6
5 mM K ₂ Cr ₂ O ₇ + lemon juice	1
10 mM K ₂ Cr ₂ O ₇ + lemon juice	4
15 mM K ₂ Cr ₂ O ₇ + lemon juice	3,5
20 mM K ₂ Cr ₂ O ₇ + lemon juice	3,5
25 mM K ₂ Cr ₂ O ₇ + lemon juice	3

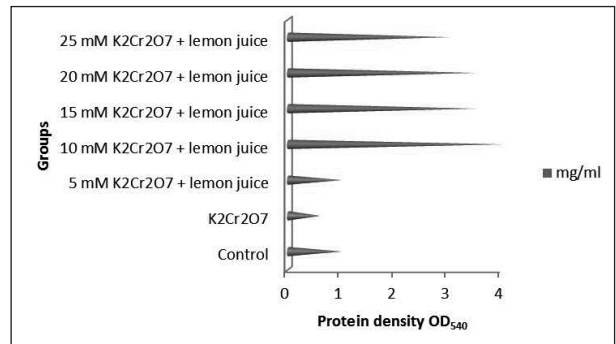


Figure 2. Protein densities of among the groups.

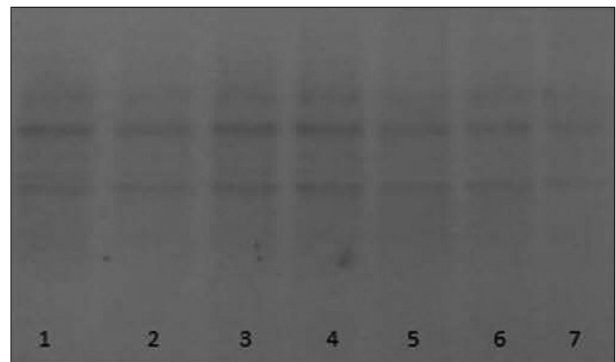


Figure 3. SDS-PAGE supernatant total protein bands profiles for development at 30°C. Lanes, 1: Control; 2: K₂Cr₂O₇; 3: 5 mM K₂Cr₂O₇ + LJ; 4: 10 mM K₂Cr₂O₇ + LJ; 5: 15 mM K₂Cr₂O₇ + LJ; 6: 20 mM K₂Cr₂O₇ + LJ; 7: 25 mM K₂Cr₂O₇ + LJ

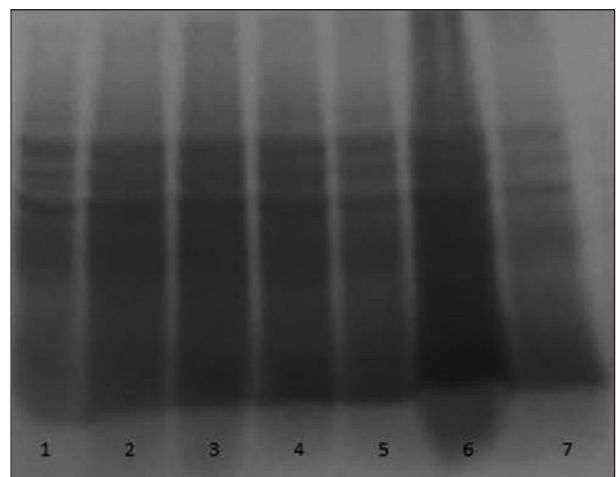


Figure 4. SDS-PAGE pellet total protein bands profiles for development at 30°C. Lanes, 1: Control; 2: K₂Cr₂O₇; 3: 5 mM K₂Cr₂O₇ + LJ; 4: 10 mM K₂Cr₂O₇ + LJ; 5: 15 mM K₂Cr₂O₇ + LJ; 6: 20 mM K₂Cr₂O₇ + LJ; 7: 25 mM K₂Cr₂O₇ + LJ

negative effects of the hydrogen peroxide radical are eliminated by pomegranate juice and that pomegranate juice increases cellular development in yeasts while at the same time providing a protective effect against oxidative damage. It was also determined that pomegranate juice has a protective role against H_2O_2 damage in yeasts even above normal development temperatures ($60^\circ C$) (7). It was also determined in the study in which we examined the protective effects of grapefruit juice against the negative effects of chromium in yeasts that grapefruit juice has positive effects on cellular development in *S. cerevisiae* and that it decreases chromium damage (13). Auesukaree et.al. (2015) put forth that *Moringa oleifera* leaves have a cellular development providing effect against cadmium mediated oxidative stress in *S. cerevisiae* thus stating that some plant, vegetable and fruit contents have a protective effect against cellular stress in yeasts (17). Vicario et.al. (2015) carried out a study examining the chemical and cellular effects of orange juice on *S. cerevisiae* in which they indicated that vitamin C rich orange has many therapeutic effects and that it provides an antioxidant cellular defense against oxidative stress in yeasts while also putting forth based on HPLC and other chemical analyses results that fruits with high vitamin C content have significant protective effects in yeasts (18). Farcasanu et. al. (2014) carried out a study examining the effect of blueberry extract against cadmium effect in *S. cerevisiae* in which they used many biochemical parameters and emphasized the protective role of blueberry extract against cadmium toxicity in yeast cells while also putting forth that it increases cellular viability and development (19). And also Aslan and Can

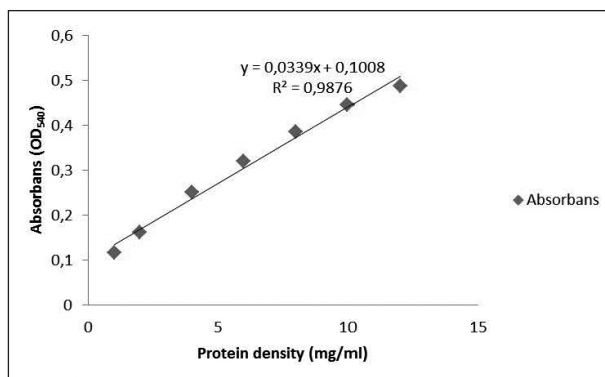


Figure 5. Biuret BSA (bovine serum albumin) standart graph

(2014b) and Aslan et al. (2016b) indicated that milk thistle has a protective effect on rat animal model (6, 20). In addition, Tuzcu et al (2010) suggest that Zinc picolinate can protect the kidney against to oxidative damage in rats (21).

Conclusion

These results indicate the preventive effect of lemon juice against to *Saccharomyces cerevisiae* cell death thus making us think that it can have similar effects on humans like its effects on *Saccharomyces cerevisiae*. So, we are of the opinion that similar results can be obtained for humans when fruits and their juices are consumed regularly.

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