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Original Article

The inhbition of chromium effect in *Saccharomyces cerevisiae* thrive from grapefruit

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Summary. In this study, seven groups were composed. i: Control group, ii: $K_2Cr_2O_7$ group, iii: 5 mM $K_2Cr_2O_7$+ grapefruit juice (GFJ) group, iv: 10 mM $K_2Cr_2O_7$ + GFJ group, v: 15 mM $K_2Cr_2O_7$ + GFJ group, vi: 20 mM $K_2Cr_2O_7$ + GFJ group, vii: 25 mM $K_2Cr_2O_7$ + GFJ group. After sterilization, fruit juice (25%) and $K_2Cr_2O_7$ were inserted different concentration to *Saccharomyces cerevisiae* (*S. cerevisiae*) cultures and the cultures were developed at 37°C for 1h, 3h, 5h and 24 hours (overnight). *S. cerevisiae* cell growth was determined by spectrophotometer, total protein changes was detected by SDS-PAGE electrophoresis and reckoned with biuret method. According to our studies results; cell growth rised in GFJ groups to which GFJ was taken in comparison to the positive control ($K_2Cr_2O_7$) group at different growing times (1, 3, 5 and 24 hours) ($p<0,05$). As a result GFJ has a protecting for decrease the oxidative damage and increased cell growing and induced protein synthesis in *S. cerevisiae* culture.

Key words: S. *cerevisiae*, grape fruit juice, oxidative damage, SDS-PAGE

Introduction

The need for fruit juice continues to increase in recent years due to consumer demands. The reasons for this increase in demand are the facts that these fruits are sources of antioxidants, minerals and vitamins. In addition, prevention of elements that threaten the health of individuals such as cardiovascular diseases, cancer or diabetes is another factor (1). Chromium is a transition element and is located in the B group in the periodic table of elements. Even though it can be present at different oxidation steps, it is frequently found in the three valence and six valence forms. Chromium is very toxic for microorganisms and plants. It is also quite effective on environmental pollution since it is commonly used in the industry. It can also be effective on the cell since it is carried in the prokaryote and eukaryote cells (2). It has been emphasized by various studies that grapefruit is particularly rich in terms of polyphenol content and especially catechin and epicatechin content. It is also stated that grapefruit is particularly a strong antifungal, antiviral, antibacterial fruit. In addition, it has also been put forth that it stimulates apoptosis in *Saccharomyces cerevisiae* (3). Many microorganisms are used as models in scientific studies, especially *Escherichia coli* and *Saccharomyces cerevisiae* are frequently preferred because their genetic structures are known (4). Results of many different studies also emphasize the facts that different fruit content increase cell development, protects the yeast from apoptosis, encourages protein synthesis thus increasing the chance of the yeast to remain strong against metabolic products in *Saccharomyces cerevisiae* that has been subject to chemical agents (oxidative stress) (5, 6). In this study, chromium substance prepared at different concentrations has been transferred to *Saccharomyces cerevisiae* culture after which the effects of grapefruit plant in this living thing against metabolic activity have been examined.

Material and Methods

Research groups

Seven groups were formed. i: Control group, ii: $K_2Cr_2O_7$ group, iii: 5 mM $K_2Cr_2O_7$+ GFJ group, iv: 10 mM $K_2Cr_2O_7$ + GFJ, v: 15 mM $K_2Cr_2O_7$ + GFJ group vi: 20 mM $K_2Cr_2O_7$ + GFJ group, vii: 25 mM $K_2Cr_2O_7$ + GFJ group. After sterilization, fruit juice (25%) and $K_2Cr_2O_7$ were inserted different concentration to *Saccharomyces cerevisiae* (*S. cerevisiae*) cultures and the cultures were developed at 37°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 1.5 g yeast extract, 1.5 g trypton, 1.5 g glucose) in addition, for the growth and reproduce of *S. cerevisiae*, fruit juices was inserted and developed. After sterilization, samples were incubated for 1h, 3h, 5h, 72 h (overnight, h: hour) at 37°C (7, 8).

Grape fruit juice extract and $K_2Cr_2O_7$ Chemical

Fruit (From center county of Elazığ city) was squashed in water and added in to *S. cerevisiae* media cultures and added 25% (v/v) ratio in at the reproducing for 37°C. $K_2Cr_2O_7$ was added in $K_2Cr_2O_7$ and GFJ+ $K_2Cr_2O_7$ groups.

Cell intensity measurements

In these calculations, culture samples that were developed at 37°C for 1, 3, 5 hours and overnight (24 hours) have been analyzed. The measurement has been carried out using a spectrophotometer at 600nm (OD_{600}).

SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide gel electrophoresis)

The samples of *Saccharomyces cerevisiae* cultures were prepared for SDS-PAGE after which they were loaded to sample loading wells 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 (9).

Protein density measurements

The calculation has been realised using a spectrophotometer at 540nm (OD_{540}) according to biuret method. BSA protein standards at different concentrations were obtained using BSA protein. Accordingly, the total protein amount in *Saccharomyces cerevisiae* groups corresponding to this standard value was calculated (Figure 4).

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 stated as $p<0.05$ and the statistically non-significant differences have been stated as $p>0.05$. Standard deviations were indicated as ±.

Results and Discussion

We hope that the results obtained from this study will provide a significant reference for future studies. When the results in Table 1 and Figure 1 are examined, it is observed that there is a statistically significant difference between groups with different development times ($p<0.05$). It can be observed that the grapefruit extract transferred to the culture environment protects cell development against the negative effect of chromium and at times even increase it. When the biuret protein results given in Table 2 and Figure 2 are examined, we can state that grapefruit extract triggers protein synthesis in the yeast. It is especially observed that protein intensity has increased significantly in 5 mM $K_2Cr_2O_7$ + grape fruit and 20 mM $K_2Cr_2O_7$ + GFJ in comparison with the control. When the SDS-PAGE gel image in Figure 3 is examined; it is observed that protein band intensity has increased statistically significantly in groups to which grapefruit content has been administered in comparison with the control group. We had acquired similar results in previous studies carried out on *Saccharomyces cerevisiae* with fruits such as pomegranate juice, apple juice, cherry, sour cherry etc. We had observed in the study during

Table 1. *Saccharomyces cerevisiae* cell growth in Grapefruit juices

OD_{600} 37°C	1h	3h	5h	Overnight
Control	1,78 ± 0.002[b]	1,60 ± 0.002[c]	1,83 ± 0.002[d]	1,59 ± 0.002[g]
$K_2Cr_2O_7$	1,76 ± 0.002[c]	1,85 ± 0.002[b]	1,83 ± 0.002[d]	1,85 ± 0.002[f]
5 mM $K_2Cr_2O_7$ + grape fruit	1,73 ± 0.002[d]	1,89 ± 0.002[a]	1,90 ± 0.002[b]	1,88 ± 0.002[c]
10 mM $K_2Cr_2O_7$ + grape fruit	1,78 ± 0.002[b]	1,86 ± 0.002[b]	1,91± 0.002[b]	2,01 ± 0.002[c]
15 mM $K_2Cr_2O_7$ + grape fruit	1,79 ± 0.002[b]	1,85 ± 0.002[b]	1,89 ± 0.002[b]	2,04 ± 0.002[b]
20 mM $K_2Cr_2O_7$ + grape fruit	1,81 ± 0.002[a]	1,90 ± 0.002[a]	1,95 ± 0.002[a]	2,08 ± 0.002[a]
25 mM $K_2Cr_2O_7$ + grape fruit	1,70 ± 0.002[e]	1,74 ± 0.002[c]	1,87 ± 0.002[c]	1,98 ± 0.002[d]

*[a,b,c,d,e,f] *among the groups which bearing of different letter are significant (p<0.05). Anova Post Hoc Hochberg and Games-Howell Test*

which pomegranate juice was given that pomegranate juice increased *Saccharomyces cerevisia*e development and thus was protective against oxidative damage in the yeast despite the negative effects of the hydrogen peroxide radical. We also determined that pomegranate juice was protective in the yeast in *Saccharomyces cerevisia*e against the damages caused by hydrogen peroxide even at 60°C which is above the normal development temperature (5). Cao et al. (2012) have put forth that grapefruit is an antioxidant and that it also protects fruits and vegetables against yeast while stimulating apoptosis in the yeasts and thus taking the cell towards necrosis (cell death) (3). Contrary to the results acquired in different studies, it has been emphasized that *Saccharomyces cerevisiae* development in grapefruit under high pressure regresses which can be due to the change in the energy requirement mechanism of the yeast as a result of yeast metabolism under the effect of high pressure and temperature (10). Dilsiz et al. (1997) have emphasized that vitamin e and selenium have antioxidant capacity in *Saccharomyces cerevisiae*, that they encourage total protein synthesis and that it stops the formation of some fatty acids by increasing the synthesis of certain fatty acids (11). Ozsahin et al. (2009) have emphasized that different sources of sugar cause changes in the synthesis of certain vitamins and fatty acids in *Saccharomyces cerevisiae* (8). Aslan et al. (2014) have emphasize that milk thistle extract is effective in apoptosis mechanism in rats, that it encourages the synthesis of apoptotic proteins and protects the cell against DNA damage (12). Tuzcu et al. (2010) have emphasized that tomato powder is effective against liver cancer in rats, that it is protective against DNA damage and that it encourages the synthesis of some proteins (13). Sahin et al. (2010) have emphasized that EGCG' (Epigallocatechin-3-gallate) is effective against nephrotoxicity in rats and that it protects the tissue by activating the apoptotic mechanism (14). Karatay et al. (2014a) and

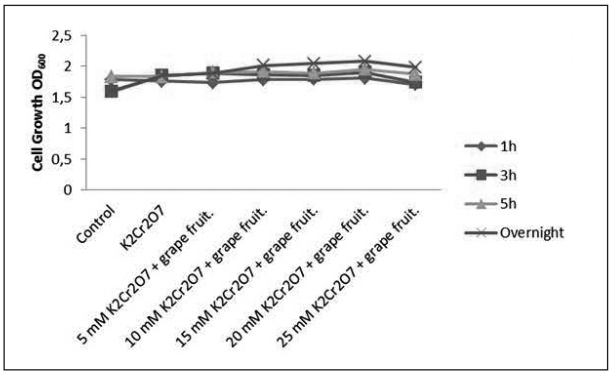

Figure 1. The growing of *Saccharomyces cerevisiae* in GFJ at different hours.

Table 2. Biuret protein density

OD_{600} 37 °C	Mg/ml
Control	1
$K_2Cr_2O_7$	0.6
5 mM $K_2Cr_2O_7$ + grape fruit.	2
10 mM $K_2Cr_2O_7$ + grape fruit.	1,5
15 mM $K_2Cr_2O_7$ + grape fruit.	1,6
20 mM $K_2Cr_2O_7$ + grape fruit.	4
25 mM $K_2Cr_2O_7$ + grape fruit.	1

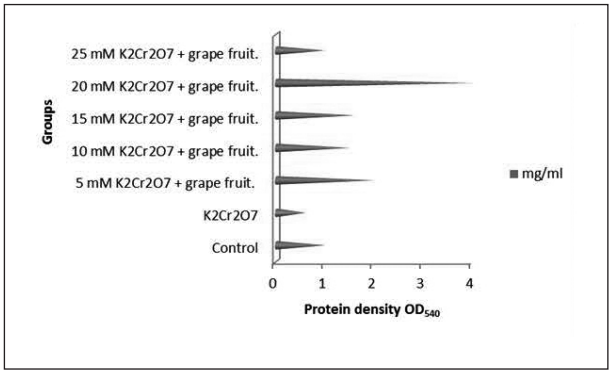

Figure 2. Protein density at between groups

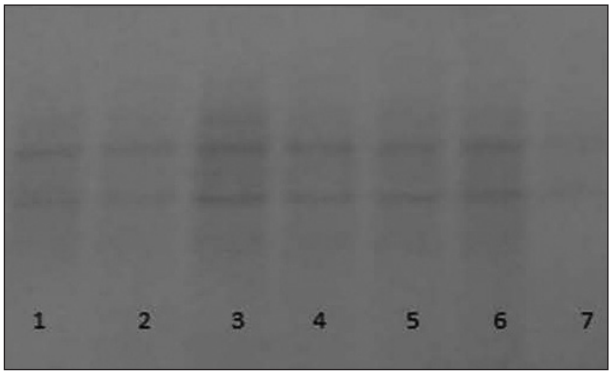

Figure 3. SDS-PAGE supernatant total protein bands profiles for development at 37°C. Lanes, 1: Control; 2: $K_2Cr_2O_7$; 3: 5 mM $K_2Cr_2O_7$ + GFJ; 4: 10 mM $K_2Cr_2O_7$+ GFJ; 5: 15 mM $K_2Cr_2O_7$ + GFJ; 6: 20 mM $K_2Cr_2O_7$ + GFJ; 7: 25 mM $K_2Cr_2O_7$ + GFJ

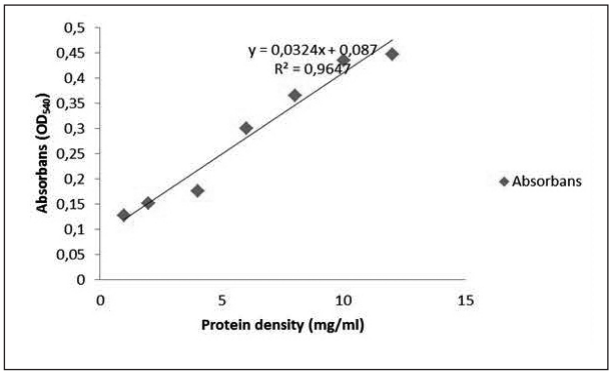

Figure 4. Biuret BSA (bovine serum albumin) standart graph

Karatay et al. (2014b) have emphasized that the almond very important for human health according to its fatty acid and protein contents (15, 16). As can be seen, the positive effects of fruit or vegetable extracts have been observed in studies carried out on yeasts as well as rats.

Conclusion

These results put forth the antioxidant capacity of grapefruit thus making us think that it can have similar effects on humans like its effects on *Saccharomyces cerevisiae*. To this end, 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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