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Original Article

The effect of orange juice against to H_2O_2 stress in *Saccharomyces cerevisiae*

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Summary. In this study, seven groups were composed. i: Control group, ii: H_2O_2 group, iii: 5 mM H_2O_2 + orange juice (OJ) group, iv: 10 mM H_2O_2 + OJ group, v: 15 mM H_2O_2 + OJ group vi: 20 mM H_2O_2 + OJ group, vii: 25 mM H_2O_2 + OJ group. After sterilization, fruit juice (25%) and H_2O_2 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 alteration was identified by SDS-PAGE electrophoresis and calculated with biuret method. With respect to our studies results; cell growth rised in fruit juice groups to which OJ was taken in proportion to the positive control (H_2O_2) group at different growing times (1, 3, 5 and 24 hours) ($p<0,05$). As a result orange fruit juices has a protective role for decrease the oxidative damage and increased cell growing and stimulating protein synthesis in *S. cerevisiae*.

Key words: *S. cerevisiae*, orange juice, oxidative damage, protein synthesis, SDS-PAGE

Introduction

Saccharomyces cerevisiae is essential yeast that has been used for a lot of working (1). H_2O_2 is a reactive oxygen species (ROS) in organism, being perennially generated intracellularly as a production of the metabolism in aerobic organisms and otherwise extracellularly during contagion in expert organisms (1-3). The inge-stion of H_2O_2 by *Saccharomyces cerevisiae* is to modify the synthesis of fatty acid and total protein in plasma membrane (1). ROS can oxidate nucleic acid, protein, fat and carbohydrates. for example, the oxidative injury to proteins give rise to breakdown of amino acid chains decreasing the biologic activity. Under normal physiological conditions, oxidative injury are forestalled by antioxidant defenses. then again, under abnormal conditions, antioxidant defense system is deficient and give rise to oxidative injury in cell. According to a study it has been observed that the ingestion of H_2O_2 at lower dose, caused fatal stress in *Saccharomyces cerevisiae* and lead to negative effect on the synthesis of essential proteins (1, 3-5). inherent antimicrobials can be used with different novel protection technologies to simplify the changing of traditional approaches in food conservation. (6). In the last decade, new species of fruit juice products, including strawberry, pomegranate, cherry, grapefruit, lemon juice, orange juice etc. have come into the consumption (7, 8). Fruit and vegetable juices are important for the healthy lives of people at every age. Low sodium, cholesterol, fat; rich polyphenol, flavonoids and vitamin C play key roles in the healthy lives of people (9). The almond very important for human health according to its fatty acid and protein contents (10, 11). OJ is one of the most consumed fruit juices in the world and it has a nice color, aroma and scent. In addition, OJ is also the source of carotenoid, vitamin C and important phenolic compounds. Thanks to this rich content, it has a significant antioxidant potential. A living being that is rich in these compounds is thus more resistant to free radicals

and is stronger against oxidative damage in comparison with his/her peers (12).

In this study we studied the effect of OJ on the proportion of the cell growing, total protein and cell growth that the induced with H_2O_2 opposite to oxidative stress growing at 37°C temperature of adding to OJ in *S. cerevisiae* culture.

Material and Methods

Research groups and growth conditions

Seven groups were composed. i: Control group, ii: H_2O_2 group, iii: 5 mM H_2O_2 + OJ group, iv: 10 mM H_2O_2 + OJ group, v: 15 mM H_2O_2 + OJ group, vi: 20 mM H_2O_2 + OJ group, vii: 25 mM H_2O_2 + OJ group. After sterilization, fruit juice (25%) and H_2O_2 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 alteration was identified by SDS-PAGE electrophoresis and calculated with biuret method for the developed and reproduce of yeast, YEPD (for 50 mL 1,5 g yeast extract, 1 g trypton, 1,5 g glucose) in addition, for the developed and reproduce of *S. cerevisiae*, orange fruit juices was added and improved. After sterilization, yeasts were cultured into media and the samples were incubated for 1h, 3h, 5h, 24 h (overnight, h: hour) at 37°C (3, 13).

Orange juice extract and H_2O_2 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. H_2O_2 was inserted in H_2O_2 and OJ + H_2O_2 groups.

Cell Intensity measurements

In these measurements, 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 600 nm (OD_{600}).

SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Elecrophoresis)

SDS-PAGE was carried out using BIO-RAD Mini-PROTEAN® 3 Cell gel electrophoresis system. 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 (14).

Protein density measurements

The measurement has been carried out using a spectrophotometer at 540 nm (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 (Fig. 4).

Statistical analysis

For statistical analysis SPSS 20.0 software was used. The comparison between experimental groups and the control group was made using one way ANOVA and Post Hoc Hochberg tests. Statistically important differentiation among groups have been stated as $p<0.05$ and the statistically non-significant differences have been specified as $p>0.05$. Standard deviations were point out as ±.

Results and Discussion

We believe that the results obtained from this study will make significant contributions to the current literature. When the results in Table 1 and Figure 1 are examined, it is observed that OJ has significant effects on *Saccharomyces cerevisiae* development. It is observed that orange juice preserves its live cell amount despite the increasing hydrogen peroxide concentrations. A difference is observed between the yeast development amounts for 1h in comparison with the control ($p<0.05$). It is observed that OJ protects the cell almost as much as the

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

OD_{600} 37 °C	1h	3h	5h	Overnight
Control	1,78 ± 0.002[a]	1,60 ± 0.002[e]	1,83 ± 0.002[c]	1,59 ± 0.002[d]
H_2O_2	1,74 ± 0.002[b]	1,51 ± 0.002[f]	1,88 ± 0.002[b]	1,99 ± 0.002[a]
50 µl H_2O_2 + orange juice	1,66 ± 0.002[d]	1,84 ± 0.002[b]	1,84 ± 0.002[c]	1,89 ± 0.002[c]
100 µl H_2O_2 + orange juice	1,70 ± 0.002[c]	1,93 ± 0.002[a]	1,90 ± 0.002[a]	2,00 ± 0.002[a]
150 µl H_2O_2 + orange juice	1,73 ± 0.002[b]	1,68 ± 0.002[d]	1,79 ± 0.002[d]	2,01 ± 0.002[a]
200 µl H_2O_2 + orange juice	1,54 ± 0.002[e]	1,72 ± 0.002[c]	1,74 ± 0.002[e]	1,94 ± 0.002[b]
250 µl H_2O_2 + orange juice	1,74 ± 0.002[b]	1,68 ± 0.002[d]	1,67 ± 0.002[f]	1,98 ± 0.002[a]

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

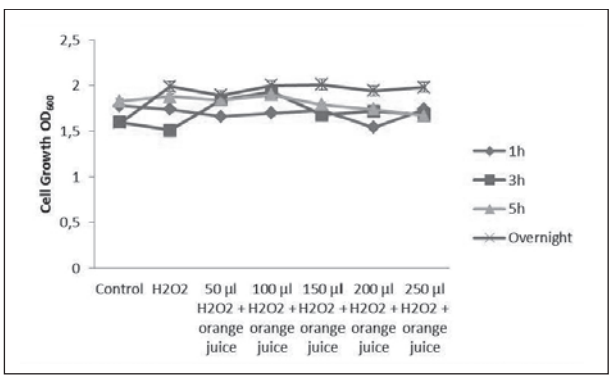

Figure 1. The growing of *Saccharomyces cerevisiae* in orange fruit juices at different hours.

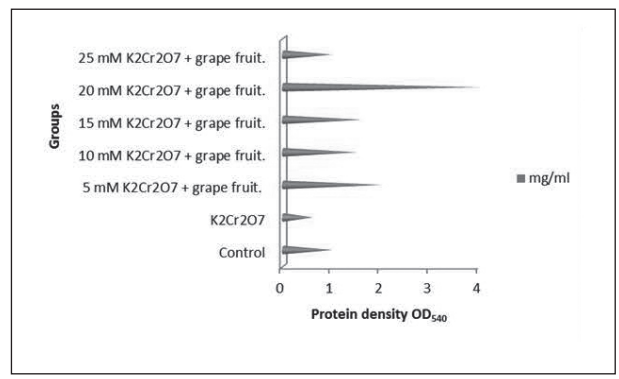

Figure 2. Protein density at between groups

control against hydrogen peroxide which is the largest radical source in the 250 µl H_2O_2 + OJ group. When 3h values are examined; it is observed that OJ has increased yeast development in the 100 µl H_2O_2 + OJ group despite the adverse effects of the hydrogen peroxide radical in comparison with the control ($p<0.05$). When the 5h values are examined; it is again observed that OJ has increased yeast development at a maximum level in the 100 µl H_2O_2 + OJ group despite the adverse effects of the hydrogen peroxide radical in comparison with the control ($p<0.05$). When the overnight (24 h) values are examined; it is observed that orange juice has increased yeast development in the 150 µl H_2O_2 + OJ group despite the adverse effects of the hydrogen peroxide radical in comparison with the control; in addition it can also be observed that yeast development has increased at a statistically significant level in all other groups in comparison with the control ($p<0.05$) (Table 1). Stinco et al (2015) have put forth that OJ activates the antioxidant defense system against free radicals thereby making a positive impact on yeast development (12). Aslan et al (2014a) have indicated that pomegranate juice is protective against oxidative damage in *Saccharomyces cerevisiae* (1). Again Aslan et al (2015) have put forth as a result of the study carried out with different fruit juices and their mixtures that different fruit juices and their mixtures are protective against oxidative damage in *Saccharomyces cerevisiae* and that they increase yeast development (7). Tserennadmid et al (2011) have put forth that apple juice has a protective role for development in yeasts (15). Krivoruchko and Nielsen (2015) have stated that resveratrol and flavonoids play protective roles against oxidative damage in bacteria and yeasts (16). When the SDS-PAGE results are examined; it is observed that protein band density in-

Table 2. Biuret protein density

OD_{600} 37°C	Mg/ml
Control	1
H_2O_2	0.5
50 µl H_2O_2 + orange juice	2
100 µl H_2O_2 + orange juice	3
150 µl H_2O_2 + orange juice	1
200 µl H_2O_2 + orange juice	1
250 µl H_2O_2 + orange juice	1

crease in supernatant and pellet gel images is greater in groups to which OJ is administered in comparison with the control (Figure 3a and 3b). Aslan et al (2014b) have put forth that pomegranate juice has a protective effect in *Saccharomyces cerevisiae* against oxidative damaged caused by the administration of hydrogen peroxide and that protein band density increase is greater in pomegranate administered groups in comparison with hydrogen peroxide administered groups (3). When the biuret results in Figure 2 and Table 2 are examined; greater protein amount has been measured in OJ (100 µl H_2O_2 + OJ and 50 µl H_2O_2 + OJ) administered groups in comparison with control and H_2O_2 groups (Table 2, Figure 2). On the other hand there are a lot of study in vivo on rat about fruit and vegetable mechanism. For example these, Tuzcu et al (2012) have stated that tomato powder is protective in rats against colorectale cancer (17). Aslan et al (2014c) have stated that the milk thistle extract is protective against lung damage in rats (18). Sahin et al (2010) have stated that EGCG increases antioxidant defense in rats (19). According to these results, OJ has a positive effect on *Saccharomyces cerevisae* cell growth and reduced the oxidative damage effect.

Conclusion

When these results are examined; we can stated that OJ is quite effective against the hydrogen peroxide induced oxidative damage in *Saccharomyces cerevisiae*, that it protects cell development and even increases cell development; thus encouraging protein synthesis in yeast cells.

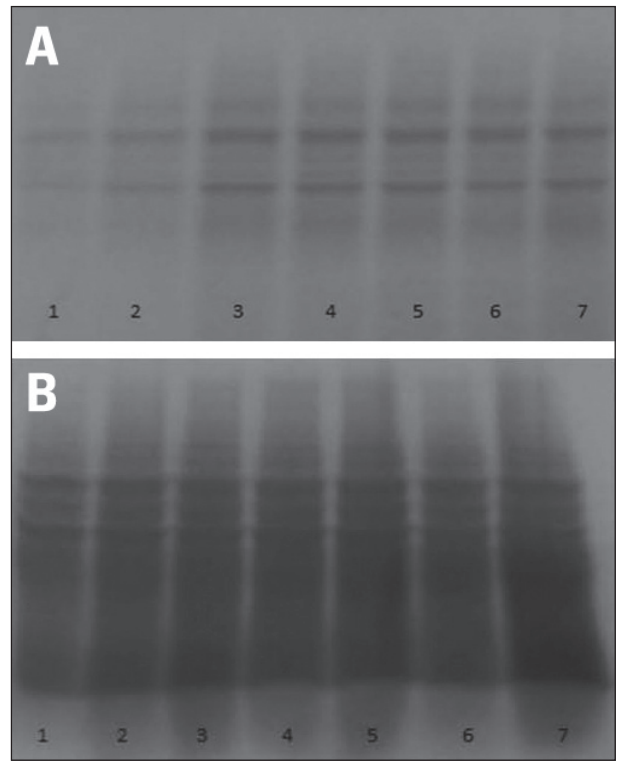

Figure 3. A) SDS-PAGE pelet total protein bands profiles for development at 37°C. Lanes 1: Control; 2: H_2O_2; 3: 50 µl H_2O_2 + orange juice; 4: 100 µl H_2O_2 + orange juice; 5: 150 µl H_2O_2 + orange juice; 6: 200 µl H_2O_2 + orange juice; 7: 250 µl H_2O_2 + orange juice. B) SDS-PAGE supernatant total protein bands profiles for development at 37°C. Lanes 1: Control; 2: H_2O_2; 3: 50 µl H_2O_2 + orange juice; 4: 100 µl H_2O_2 + orange juice; 5: 150 µl H_2O_2 + orange juice; 6: 200 µl H_2O_2 + orange juice; 7: 250 µl H_2O_2 + orange juice

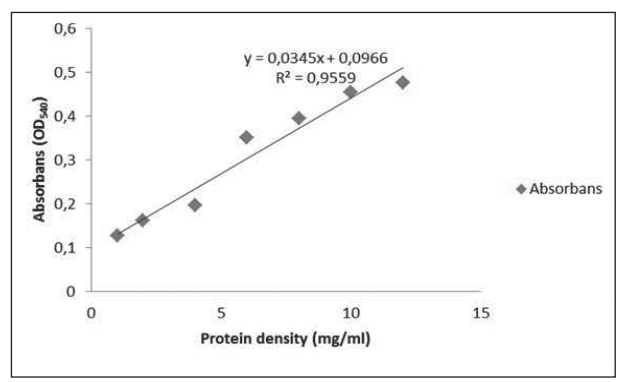

Figure 4. Biuret BSA (bovine serum albumin) standart graph

In the light of these findings, we hope that our study will encourage other studies to try OJ in animal experiments and that in this regard OJ will be consumed more by people based on the positive results that will be obtained.

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