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Original article

Cell culture developing and the imaging of total protein product changing with SDS-PAGE in *Saccharomyces cerevisiae*

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Summary. Five groups were created in this work. i: Control group, ii: H_2O_2 group, iii: H_2O_2 + %10 Mulberry Juice (MBJ) group, iv: H_2O_2 + %15 MBJ, v: H_2O_2 + %25 MBJ group. After sterilization, H_2O_2 and fruit juice were inserted 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 computed by spectrophotometer, total protein alteration was analysed by SDS-PAGE electrophoresis and reckoned with bradford method. our studies results indicated that; cell developing increased in MBJ groups 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 MBJ has a preservative role for reduce the oxidative damage and expanded cell developing and encourage protein synthesis in *S. cerevisiae*.

Key words: *S. cerevisiae*, mulberry juice, hidrogen peroxide, protein expression, SDS-PAGE

Introduction

Saccharomyces cerevisiae (*S. cerevisiae*) is important yeast and it has been employed recent researchs (1). The uptake of H_2O_2 by *S. cerevisiae* is to change the production of total protein and fatty acid in plasma membrane (1). ROS can oxidize protein, nucleic acid, fat and carbohydrates. for example, the oxidative abuse to proteins bring about to collapse of amino acid shacles diminishing the biologic activity (1, 3-5). Many works executed assert that unlike fruit content expands cellular growth in yeasts, supports protein expression and shows preservative properties towards oxidative stress (6-8). In reference to a study it has been detected that the intake of H_2O_2 at lower dose, lead to lethal stress in *S. cerevisiae* and bring on negative effect on the expression of significant proteins (1, 3-5). Native antimicrobials can be used with varied new conservation technologies to make easy the modification of conventional attitudes in food prevention (9). In the last years, new kind of fruit juice products, including pomegranate, strawberry, mulberry, grapefruit, lemon juice, etc. have be come very important for human health (10, 11). Fruit and vegetable juices are useful for the people live every time. Low sodium, cholesterol, fat; rich polyphenol, flavonoids and vitamin C acting essential roles in the salutary lives of people (12) in addition for example almond very distinguished for human health with regard to its protein and fatty acid contents (13, 14). Mulberry (MB) is one of the most consumed fruit in the world and it has a nice color, aroma and it leaves have been used as treatment of different illness. In addition, MB is also the source of quercetin, rutin, isoquercetin, and astragalin and significant phenolic compounds, this compounds has preservative effect against H_2O_2-induced oxidative damage, antidiabetic, anti-inflammatory activity and inhibit oxidative injury (15-18). In this work we studied the effect of MB on the rate of the cell developing, total protein expres-

sion and cell proliferation that the induced with H_2O_2 against to oxidative injury growing at 30˚C temperature of adding to MB in *S. cerevisiae* culture.

Material and Methods

Research groups and growth conditions

In this research five groups were composed. . i: Control group, ii: H_2O_2 group, iii: H_2O_2 + %10 Mulberry Juice (MBJ) group, iv: H_2O_2 + %15 MBJ, v: H_2O_2 + %25 MBJ group. After sterilization, H_2O_2 and fruit juice were inserted 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 proliferation was calculated by spectrophotometer, total protein expression was indicated by SDS-PAGE electrophoresis and reckoned with bradford 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 developing and reproduce of *S. cerevisiae*, mulberry fruit juices was added and cultivated. After sterilization, yeasts were cultured into media and the samples were incubated for 1h, 3h, 5h, 24 h (overnight, h: hour) at 30˚C (7).

Mulberry 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 20% (v/v) ratio in at the reproducing for 30˚C. H_2O_2 was inserted in H_2O_2 and MBJ + H_2O_2 groups.

Cell concentration measurements

In these measurements, culture samples that were examined 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)

SDS-PAGE was made using BIO-RAD Mini-PROTEAN® 3 Cell gel electrophoresis system. The samples of *S. cerevisiae* cultures were organized for SDS-PAGE after which they were loaded to sample loading wells to be subject to electrical current and after this process, their images were taken and the intergroup protein bandings were used as data in the study (19).

Protein density measurements

The measurement has been accomplished using a spectrophotometer at 600 nm (OD_{600}) with regard to bradford method. BSA (bovine serum albumin) protein standards at different concentrations were obtained using BSA protein. Accordingly, the total protein amount in *S. cerevisiae* groups corresponding to this standard valuation was calculated (Figures 3, 4).

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 Duncan and Games howell 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 think that the results of this study will provide important contributions to the present literature. The results of table 1 and figure 1 show that mulberry has essential effects on *S. cerevisiae* proliferation. It is indicated that mulberry juice (MBJ) maintains its live cell amount in spite of the growing hydrogen peroxide densities. A dissimilarity is detected between the yeast proliferation amounts for 1h in comparison with the control ($p<0.05$). It is observed that MBJ preserves the cell almost as much as the control opposite hydrogen peroxide which is the great radical origin in the 25% MBJ + H_2O_2 group and 15% MBJ + H_2O_2 group. When 3h values are investigated; it is obtained that MBJ has increased yeast development in the 25% MBJ + H_2O_2 group, in spite of the inverse effects of the hydrogen peroxide radical comparatively the control and H_2O_2 group ($p<0.05$). When the 5h values are investigated; it is obtained that MBJ has rised yeast improving at a maximum level in the 10% MBJ + H_2O_2 group despite the inverse effects of the hydrogen peroxide radical

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

OD_{600} 30°C	1h	3h	5h	Overnight
Control	1,488±0,00[b]	1,395±0,00[c]	1,755±0,00[c]	2,096±0,00[c]
H_2O_2	1,510±0,00[c]	1,413±0,00[d]	1,776±0,00[d]	1,876±0,00[a]
H_2O_2 + 10% mulberry	1,073±0,00[a]	1,286±0,00[a]	1,774±0,00[d]	2,092±0,00[c]
H_2O_2 + 15% mulberry	1,577±0,00[d]	1,364±0,00[b]	1,556±0,00[a]	2,102±0,00[d]
H_2O_2 + 25% mulberry	1,605±0,00[e]	1,464±0,00[e]	1,741±0,00[b]	2,071±0,00[b]

***a,b,c,d,e; among the groups which bearing of different letter are significant (p<0.05). one way ANOVA and Post Hoc Duncan and Games howell tests*

comparatively the control (p<0.05). When the overnight (24 h) values are investigated; it is obtained that MBJ has rised yeast growth in the 10% MBJ + H_2O_2, 15% MBJ + H_2O_2 and 25% MBJ + H_2O_2 groups, in spite of the opposite effects of the hydrogen peroxide radical in comparison with the control and H_2O_2 group; besides it can also be obtained that yeast growth has rised at a statistically significant degree in all other groups comparatively the control and H_2O_2 groups (p<0.05) (Table 1). Stinco et al (2015) indicated that orange juice activates the antioxidant defensive system towards free radicals for yeast development (20). Aslan et al (2014a) have indicated that pomegranate juice is protective against oxidative injury in *S. cerevisiae* (1). Again Aslan (2015) indicated that as a result of the work performed with several fruit juices and their mixtures that different fruit juices and their mixtures are preservative against oxidative injury in *S. cerevisiae* and that they rise yeast development (10). Tserennadmid et al (2011) have stated that apple juice has a protective role for growing in yeasts (21). Krivoruchko and Nielsen (2015) have stated that resveratrol and flavonoids act protective roles towards oxidative injury in bacteria and yeasts (22). Zhang et al (2017) showed that the mulberry extract has protective effect in human cell culture against oxidative stress (17). Rynko et al (2016) indicated that the leave of mulberry has antidiabetic activity, anticancer activity, antibacterial activity (15). Riche et al (2017) demonsrated that mulberry leaf extract decrease the human blood glucose level (18). Gregorio et al (2011) have stated that mulberry extract has antioxidant activity in *S. cerevisiae* (23). Chen et (2015) have put forth that mulberry fruit has antioxidant and hyperglycemic activity in vitro (16). When the SDS-PAGE results are investigated; it is obtained that protein band intensity rise in pellet gel images is greater in groups to which MBJ is applied

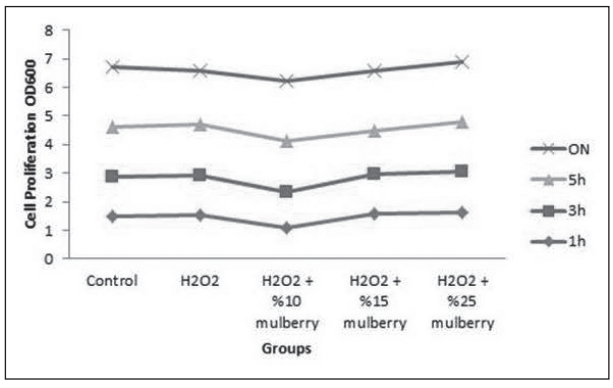

Figure 1. The growing of *Saccharomyces cerevisiae* in mulberry juices at different hours.

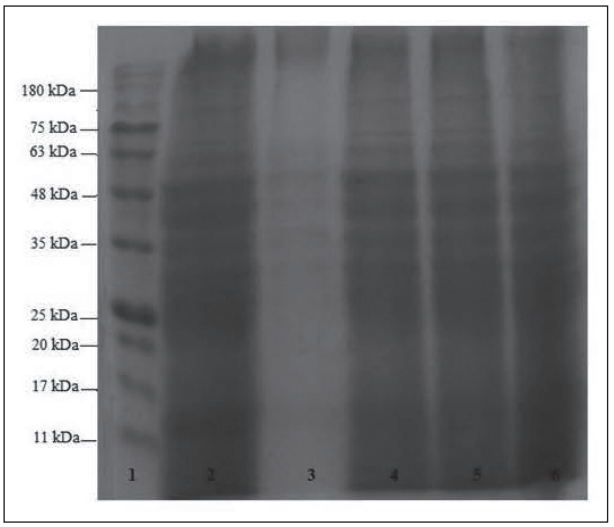

Figure 2. SDS-PAGE pelet total protein bands profiles for development at 30°C. Lanes 1: Marker; 2: Control; 3: H_2O_2; 4: H_2O_2 +10% MBJ; 5: H_2O_2 +15% MBJ; 6: H_2O_2 +25% MBJ

in comparison with the control (Figure 2). Aslan et al (2014b) have stated that pomegranate juice has a preservative effect in *S. cerevisiae* towards oxidative injury reasened by the applying of hydrogen peroxide and that protein band intensity rise is bigger in pomegranate applied groups comparatively hydrogen peroxide applied groups (3). When the bradford results in Figure 3 and figure 4 are analyzed; large protein quantity has been calculated in MBJ (H_2O_2+10% MBJ, H_2O_2+15% MBJ, H_2O_2+25% MBJ) applied groups comparatively to control and H_2O_2 groups (Figure 3,4). However, there are a lotof research in vivo on rat about fruit and vegetable mechanism. For example these, Aslan et al (2014c) and Aslan et al (2016a) have indicated that the milk thistle extract is preservative towards lung damage in rats (24, 25), Aslan and Can (2017a) have stated that lemon juice has a protective effect for diminish the oxidative injury, increased cell growing and protein synthesis in *S. cerevisiae* culture (26), Aslan et al (2016b) demonsrated that black cumin extract may be a drug for lung damage in rats (27), Aslan et al (2015) indicated that Nigella sativa extracts has a preservative effects against to rats lung damage (28), Ozsahin et al (2009) expressed that different sugar extracts induce fatty acid biosynthesis in the *S. cerevisiae* cell culture (29), Aslan et al (2017b) indicated that kiwi fruit juice has a protective effect against to hydrogen peroxide damage in *S. cerevisiae* (30).. With respect to these results, MBJ has a positive effect on *S. cerevisiae* cell proliferation and decreased the oxidative injury effect.

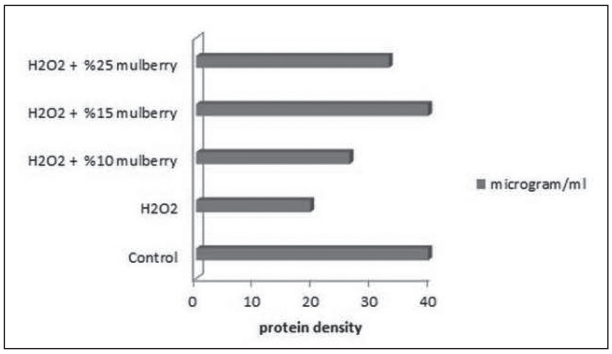

Figure 3. Protein density at between groups

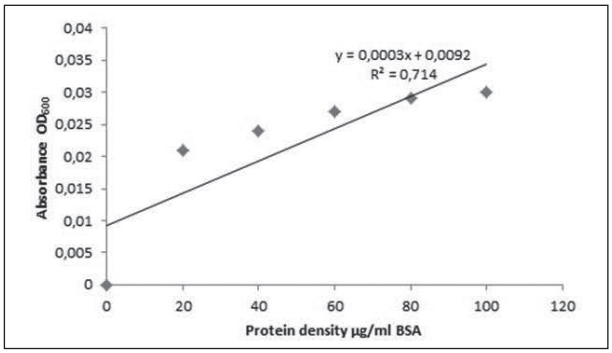

Figure 4. Bradford BSA (bovine serum albumin) standart graph

Conclusion

When these results are evaluated; we can said that MBJ is quite effective towards the hydrogen peroxide induced oxidative injury in *S. cerevisiae*, that it safeguards cell improve and even rises cell thrive; thus supporting protein expression in yeast cells. With respect to these findings, we expect that our study will support other studies to try MBJ in animal experiments and that in this respect MBJ will be digested more by people based on the positive results that will be gained.

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