Progress in Nutrition 2014; Vol. 16 N.3: 212-217

Original article

Is Pomegranate juice has a vital role for protective effect on *Saccharomyces cerevisiae* growth?

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Summary. In this study, four groups were composed. i: Control group, ii: H_2O_2 group, iii: Pomegranate juice (PJ) group and iv: PJ + H_2O_2 group. After sterilization, PJ (20%) and H_2O_2 (6% v/v) were inserted to *Saccharomyces cerevisiae* (*S. cerevisiae*) cultures and the cultures were developed at 60°C for 1h, 2h, 4h and 72 hours (overnight). Fatty acids and vitamin values were gauged by HPLC and GC, the total protein was determined by SDS-PAGE. with respect to our studies results; fatty acid synthesis rised in groups to which PJ was taken in proportion to the control group ($p<0,05$). Vitamin ingredient also rised particularly in the group to which PJ was taken in proportion to the control ($p<0,05$). In addition, cell intensity was gauged to be upward of in PJ groups in proportion to the control ($p<0,05$), whereas banding was observed more in PJ groups in proportion to the control. As a result PJ has a protective role for decrease the oxidative damage in *S. cerevisiae*.

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

«Il succo di melograno ha un ruolo protettivo nella crescita di Saccharomyces cerevisiae?»

Riassunto. In questo studio sono stati organizzati 4 gruppi. 1: Gruppo di controllo; 2: gruppo H2O2; 3: gruppo succo di melograno (PJ) e 4: gruppo PJ + H_2O_2. Dopo sterilizzazione sono stati aggiunti a colture di *Saccharomyces cerevisiae (S. cerevisiae),* PJ (20%) e H_2O_2 (6% v/v) e le colture sono state fatte crescere a 60°C per 1h, 2h, 4h e 72 ore (overnight). Gli acidi grassi e le vitamine sono state misurate con HPLC e GC mentre le proteine totali sono state determinate con SDS-PAGE. Lo studio ha mostrato i seguenti risultati: la sintesi degli acidi grassi è aumentata nei gruppi a cui è stato somministrato PJ rispetto al gruppo di controllo ($p<0,05$). Anche le Vitamine sono aumentate in modo particolare nel gruppo a cui è stato somministrato PJ rispetto al gruppo di controllo ($p<0,05$). In aggiunta è aumentata anche la sintesi proteica nei gruppi PJ in modo proporzionale rispetto al controllo ($p<0,05$), sono state infatti osservate delle bande in misura maggiore nei gruppi PJ rispetto al controllo. Concludendo PJ ha un ruolo protettivo nel ridurre il danno ossidativo in *S. cerevisiae*.

Parole chiave: *S. cerevisiae*, succo di melograno, danno ossidativo, SDS-PAGE

Introduction

S. cerevisiae is a substantial yeast that is often used for many studies, recent years, it has been used very new studies as model organism and its value has been very important for academical researchs because of its biological mechanism similarities to animal and humans (1-3). H_2O_2 is a reactive oxigen species (ROS) and it induced oxidative strees in *S. cerevisiae.* ROS in organism, being continuously bred intracellularly as a product of the metabolism in aerobic organisms and otherwise extracellularly during infection in different particular organisms and it has suppressor effect on living microorganism such as *S. cerevisiae*, bacteria, fungi (4-6). According to results of a study, the consumption of H_2O_2 by *S. cerevisiae* is indicated that to cause to change of the plasma membrane fatty acid and total protein synthesis (6, 7). ROS can oxidize nucleic acid, protein, fat and carbohydrates. For example, the oxidative damages to proteins lead to the disrupt of amino acid chains and so decrease the biologic activity. Under normal physiological condition, oxidative damages are prevented by antioxidant defenses system on the other hand under aberrant conditions, antioxidant defense system is inadequate and to be composed of oxidative damage in cell. According to a study carried out, consumption of H_2O_2 at lower dose causes a deadly stress in *S. cerevisiae* thus resulting in a negative effect on the synthesis of vital proteins (6, 8, 9-15). Pomegranate (*Punica granatum* L.) juice has much polyphenol content and a very effective antioxidant capacity; thus it has captivated notice in recent years in terms of human health. It is very important that the anthocyanin amount in its polyphenol content is between the range of 0,2-1,0 g/100 g (16, 17). *Punica granatum* L. (Puniceaceae) has been completely used by conventional drug in America, Europe, Asia and Africa for the remedy of different species of illness (18). Different researchers have pointed out fruit digestion can has a vital role in the suppression of many diseases such as oxidative stress, cancer and antibacterial regulatory effects (16, 19). In this study we studied the effect of PJ on the proportion of the cell growing, total protein, fatty acid and vitamin that the induced with H_2O_2 opposite to oxidative stress growing at 60°C temperature of adding to PJ at different concentrations in *S. cerevisiae* culture.

Material and methods

Research groups

Four groups were composed; I: control group, ii: H_2O_2 group, iii: PJ group and iv: PJ+H_2O_2 group, occurrence media of *S. cerevisiae:* for the developed and reproduce of yeast, YEPD (for 50 mL, 2 g yeast extract, 2 g trypton, 2 g glucose). Then, for the developed and reproduce of *S. cerevisiae*, PJ was added and improved. After sterilization, yeasts were cultured into media and the samples were incubated for 2 h, 4 h, 6 h, 72 h (overnight) at 60°C (20, 21).

Pomegranate juice extract and H_2O_2 Chemical

Pomegranate fruit (from Alacakaya 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 60°C. H_2O_2 was inserted in H_2O_2 and PJ+H_2O_2 groups for 6% (v/v) ratio.

Table 1. 2, 4, 6 hours and overnight (72 hours) cell density amounts for improving at 60°C and a PJ adding of 20% (v/v) to the culture environment calculated with spectrophotometer at 600 nm (OD_{600}).

	2 h	4 h	6 h	Overnight
Control	1,53±0,03[a]	1,56±0,00[a]	1,55±0,00[a]	1,68±0,06[a]
H_2O_2	1,52±0,01[a]	1,53±0,00[b]	1,52±0,00[b]	1,47±0,00[b]
PJ	1,66±0,00[b]	1,68±0,00[c]	1,66±0,00[c]	1,70±0,00[a]
PJ + H_2O_2	2,15±0,00[c]	2,08±0,00[d]	2,09±0,01[d]	1,74±0,00[a]

***a,b,c,d; among the groups which bearing of different letter are significant (p<0.05).*
Anova Post Hoc LSD Test.

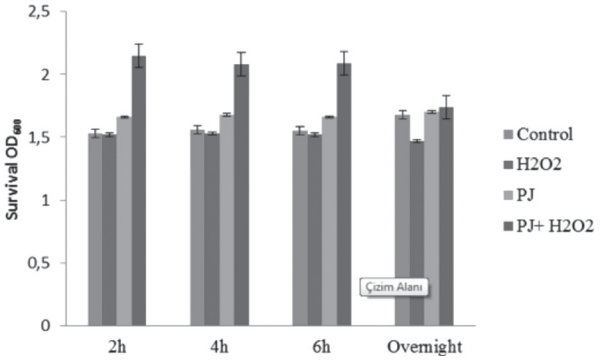

Figure 1. 2, 4, 6 hours and overnight (72 Hours) cell density amounts for improving at 60°C and a PJ adding of 20% (v/v) to the culture environment calculated with spectrophotometer at 600 nm (OD_{600}).

Fatty acids analyses

Cell pellets whose moisture weights were resolved and were homogenized with 3/2 (v/vG1) Hexaneisopropanol mixture. The homogenate was centrifuged at 5000 rpm for 5 min at 4°C and cell pellet junk was expedited. The supernatant piece was used in the ADEK vitamin and fatty acid analysis (21).

Gas chromatographic analysis

Methyl esters were analyzed with the SHIMADZU GC 17 Ver. 3 gas chromatography (Kyoto, Japan). During the analysis, the colon temperature was held at 120-220°C, injection temperature was held at 240°C and the detector temperature was kept at 280°C (21).

ADEK Vitamins and Sterol analysis

The 5 mL supernatant was inserted to 25 mL tubes with caps and 5% KOH solution was added. After it was vortexed, it was kept at 85°C for 15 min. The tubes were then taken and chilled to room temperature and 5 mL of natural water was added and mixed. The Hexane phase vaporized with nitrogen flow. It was dispersed in 1 mL (50+50%, v/vG1) acetonitril/methanol mixture and then was taken to auto sampler vials and was analyzed. The analysis was made with the Shimadzu brand HPLC device (Shimadzu, Kyoto Japan) (21).

SDS-PAGE analysis

After extraction of proteins from cell cultures, protein samples were used for SDS-PAGE and after SDS-PAGE, image of gel was taken and was evaluated for total protein bands among the groups (22).

Living Cell Intensity measurements

In these measurements, culture samples that were developed at 60°C for 2, 4, 6 hours and overnight (72 hours) have been analyzed. The measurement has been carried out using a spectrophotometer at 600 nm (OD_{600}).

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 and LSD tests. Statistically significant differences among

Table 2. Fatty acid amounts for improving at 60°C and a PJ adding of 20% (v/v) to the culture environment calculated with spectrophotometer at 600 nm (OD_{600}).

	Control	PJ	H_2O_2	PJ + H_2O_2
Palmitic acid (16:0)	584,75±0,04[a]	648,72±0,00[b]	519,87±0,56[c]	711,76±0,02[d]
Palmitoleic acid (16:1)	1004,14±1,00[a]	924,59±0,00[b]	846,82±0,02[c]	548,93±0,58[d]
Stearic acid (18:0)	471,17±0,05[a]	403,16±0,00[b]	390,21±0,01[c]	297,37±0,00[d]
Monoenoleic acid (18:1)	1013,42±0,02[a]	1125,48±0,58[b]	885,45±0,00[c]	809,49±0,00[d]
Dienlinoleic acid (18:2 n6c)	61,52±0,02[a]	53,53±0,05[b]	54,21±0,019[c]	38,23±0,20[d]
Dienlinoleic acid (18:2 n6t)	38,71±0,02[a]	224,48±0,48[b]	56,54±0,57[c]	195,92±0,56[d]

***a,b,c,d; among the groups which bearing of different letter is significant (p<0.05).*
Anova Post Hoc LSD Test.

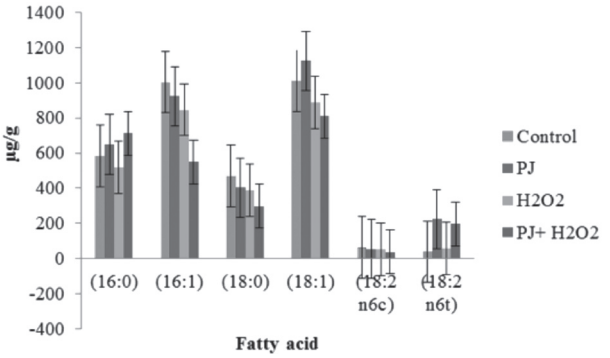

Figure 2. Fatty acid amounts for improving at 60°C and a PJ adding of 20% (v/v) to the culture environment calculated with spectrophotometer at 600 nm (OD_{600}).

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

According to in this study, we believe that significant results have been obtained. When looking at these findings, further studies can be carried out and the common and effective mechanism of PJ can be tested on different living organisms. For that purpose, we hope that our study has substantial additive to scientific literature. According to our results, there is a range of variety in membrane structure in H_2O_2 groups in comparison with control group. Bienert et al (2013) and Folmer et al (2008) have also carried out a study with similar results (6, 23). Almeida et al (2000) indicated that H_2O_2 induced the lethality of *Escherichia coli* in low iron conditions (24) Santos et al (2012) have indicated that in given PJ groups in comparison with control group intensity of cell population the survival rate increased (19). According to Yan et al (2011) H_2O_2 has inverse effects on the β- caroten synthesis in yeast which the repressive effect of oxidative damage (25) in our datas indicated that in PJ given groups, in comparison with conrol group there are significant rising and so in PJ given groups, in comparison with in given H_2O_2 group there are essential rising at cell density (p<0.05) (Table 1, Figure 1). In addition, when the analysis results obtained by taking 1 hour, 2 hours, 4 hours measurements of cell densities are examined, it has been monitored that in common PJ given groups had higher cell density in compared to the control (Table 1, Figure 1). In our sudy it was indicated that in fatty acid composition from *S. cerevisiae* yeasts; there were 16:0, 16:1, 18:0, 18:1 and 18:2 n-6c, 18:2 n-6t (in growing at 60°C culture media), and it was indicated that the fatty acid amount various according to changes in the media. We achieved that the synthesis of these fatty acids was due firstly to the enzymes that played a role in the synthesis expressed in *S. cerevisiae*. In different studies, it was showed that the enzymes, which made fatty acid synthesis from *S. cerevisiae* yeast and other yeasts species were affected by a lot of ingredient in the culture (21). And also when we look at the present of various fatty acid synthesis in proportion to in given H_2O_2 group,

Table 3. Vitamin amounts for improving at 60°C and a PJ adding of 20% (v/v) to the culture environment calculated with spectrophotometer at 600 nm (OD_{600}).

	Control	PJ	H_2O_2	PJ + H_2O_2
K vit. (1)	15,93±0,01[a]	20,17±0,00[b]	13,89±0,00[c]	17,67±0,00[d]
K vit. (2)	0,67±0,00[a]	1,63±0,00[b]	0,95±0,01[c]	0,76±0,00[d]
D vit. (2)	10,73±0,00[a]	10,02±0,03[b]	8,88±0,00[c]	6,11±0,00[d]
α-Tocopherol	33,55±0,00[a]	65,63±0,01[b]	53,47±0,00[c]	38,67±0,00[d]
γ-Tocopherol	0,51±0,01[a]	0,17±0,00[b]	0,58±0,00[c]	0,24±0,00[d]
Ergosterol	170,49±0,05[a]	178,17±0,00[b]	150,18±0,00[c]	139,95±0,00[d]
Cholesterol	34,56±0,01[a]	5,27±0,00[b]	20,65±0,01[c]	8,01±0,00[d]
Stigmasterol	694,56±0,05[a]	759,77±0,05[b]	610,22±0,02[c]	438,54±0,00[d]
β-sitosterol	0,33±0,00[a]	60,16±0,01[b]	38,97±0,01[c]	98,21±0,00[d]

***a,b,c,d; among the groups which bearing of different letter is significant (p<0.05).*
Anova Post Hoc LSD Test.

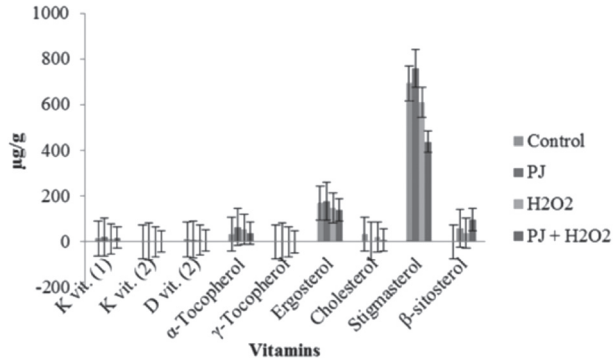

Figure 3. Vitamin amounts for improving at 60°C and a PJ adding of 20% (v/v) to the culture environment calculated with spectrophotometer at 600 nm (OD_{600}).

in PJ given group, the fatty acid synthesis were substantially rised ($p<0.05$) (Table 2, Figure 2). According to Yan et al (2011) addition of H_2O_2 can significantly induce the β-carotene production and given H_2O_2 amount more and more, in culture media calculated β-carotene quantity was decline in similar with reported that the catalase enzyme activity was reduced (25). According to Matias et al (2007), H_2O_2 can change the plasma membrane fatty acid composition of *S. cerevisiae* (7). In accordance with Guvenc et al (2010) adding various sugar source at nutrition media that was raised of fatty acid production (26). Thus these conditions have led us to think that particularly H_2O_2 has repressive effect on fatty acid synthesis of genes (5, 21, 27). It was indicated in our study that vitamin synthesis stemmed from the *S. cerevisiae*, It was monitored that the produced vitamin ratios were α-tocoferol, "γ-tocoferol, β-sitosterol cholesterol, stigmasterol ergesterol, D, and K 1,2 the rates of these vitamins in changing media were determined by calculation with statistical methods (21). Once again, we observed that our vitamin results, in given PJ group which was the vitamins were upwards in proportion to control group ($p<0.05$) (Table 3, Figure 3). We observed that our results of the SDS-PAGE total protein band profiles in carry out 60°C culture media was been indicated that in given PJ group whose protein density upwards than, in proportion to the H_2O_2 group (Figure 4). According to Landolfo et al (2008) ROS inhibited or decreased some protein synthesis in *S. cerevisiae* (15).

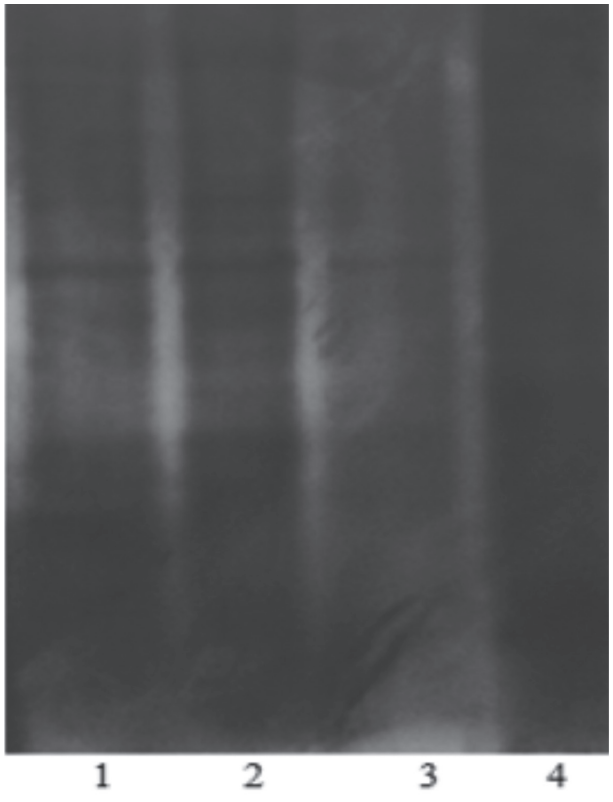

Figure 4. SDS-PAGE total protein bands profiles for development at 60°C and a PJ adding of 20% (v/v) to the culture environment. Lane 1, Control; lane 2, PJ; lane 3, H_2O_2; lane 4, PJ+ H_2O_2.

Conclusion

In conclusion, with respect to our results, PJ has made encourage effect on the vitamin, fatty acid, and protein synthesis of the yeast cell mechanism of the Saccharomyces species when PJ added to the culture environment and to this end has increased fatty acid, vitamin and protein synthesis. Accordingly, we believe that it has particularly reduced oxidative damage in this way making a positive influence on yeast improving, with respect to our results, we suppose that PJ possibly a like impact on human health when we imagine its effect mechanism on yeasts.

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