

## The Effects of Different Sugar Sources on Fatty Acid Biosynthesis in the *Saccharomyces Cerevisiae* Cell Culture

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**Abstract:** Molasses contains a variety of ions, vitamins and sugar, it is considered as a good source of nutrient. In this study, we aimed to investigate the effects of molasses samples (produced from 2 different crop supplies) on the *Saccharomyces cerevisiae* FMC-16 culture. For this purpose, control yeast cell cultures that contained mulberry and grape molasses were prepared. In the control group, glucose was used as a source for sugar and in the other group, molasses samples were used. In our result, it was found that the  $\alpha$ -tocopherol decreased in the grape molasses group. Ergosterol decreased in the group to which, mulberry molasses was added, when compared to the control group. Linoleic acid increased in the groups, in which molasses was added when compared to the control group. Palmitic acid ascended in mulberry molasses culture media but decreased in the grape molasses media. Palmitoleic acid decreased in the grape molasses group and oleic acid, a decrease in both molasses media was observed when compared to the control group. Consequently, it was found that molasses samples added to the development media of *Saccharomyces cerevisiae* FMC-16 instead of glucose caused differences on the synthesis of some vitamins and fatty acids.

**Key words:** Mulberry molasses, grape molasses, *Saccharomyces cerevisiae*, fatty acids, lipophilic vitamins, carbon source

### INTRODUCTION

Molasses is a good source of carbohydrate in terms of its organic acids, mineral substances and high sugar content and has been commonly used in the human diet for centuries. As a result, molasses is one of the main nutrition substances that should be used in the prevention of disorders that may emerge as a result of a deficiency of mineral substances, which are known as trace elements in the human body. Additionally, although it is poor in protein content, it helps the provision of the amino acid balance in the body due to the vitamins it contains. With these properties, molasses is one of the suitable media that is especially used for the development and reproduction of microorganisms in scientific studies (Cazetta *et al.*, 2007; Kopsahelis *et al.*, 2006; Swain *et al.*, 2007; Cha *et al.*, 2005; Donmez, 2001). *Saccharomyces cerevisiae*, which is one of these microorganisms, is a eukaryotic organism, which is commonly used in scientific studies that particularly investigate substance synthesis (Dawson and Boopathy, 2007; Kumari and Abraham, 2006; Cipak *et al.*, 2005; Ciafardini *et al.*, 2005; Abrodo *et al.*, 2004).

In this study, carried out Weete (1980), used *Saccharomyces cerevisiae* yeast for fatty acid synthesis

and reported that 3% miristic, 18% palmitic, 52% palmitoleic, 2% stearic and 20% oleic acid was synthesized from *Saccharomyces cerevisiae*. In another study, carried out by Martin *et al.* (2006), the methods for unsaturated fatty acid synthesis from *Saccharomyces cerevisiae* were indicated. In the study of Bronzetti *et al.* (2001) it was reported that vitamin A and E was synthesized from *Saccharomyces cerevisiae*. In addition, mutagenic and antimutagenic effects of both vitamin A and vitamin E and 2 distinct Se compounds were investigated and it was observed that Se compounds had mutagenic and antimutagenic effects in different concentrations but vitamin A and E did not have toxic or antimutagenic effects. In another study carried out, by Dilsiz *et al.* (1997), fatty acid analysis was made for 2 different species of *Saccharomyces cerevisiae* and the effects of vitamin E on fatty acids were analyzed.

In our study, by means of benefiting from the features of *Saccharomyces cerevisiae*, we investigated vitamin and fatty acid analysis in mulberry and grape molasses media. We indicated the comparisons between the groups through the evaluation of experiment results with the statistics program. Because, fatty acids are essential compounds in the cell and are fats that are vital for life but they are also, substances that can not be

produced by the human body (Tehlivets *et al.*, 2007). Today, due to improper diet habits, these fats and vitamins are consumed as low as to threaten health and therefore, vital disorders occur in the body. Although, the fatty acid and vitamin content of mulberry and grape molasses, which are commonly used in our traditional diet are not known exactly, a study towards analyzing these differences has not been conducted as yet. In our study our objective was to put forth and indicate the (A, D, E, K vitamins, fatty acid) differences caused by the contents of mulberry and grape molasses that have a significant role and importance in our nourishment, in line with the findings of the results.

## MATERIALS AND METHODS

**Development media of *Saccharomyces cerevisiae*:** For the growth and reproduction of yeast, YEPD (for 100 mL 1 g yeast extract, 2 g bactopecton, 2 g glucose) media was prepared in 5 parallels to make 250 mL (Dilsiz *et al.*, 1997). Then, for the growth and reproduction of *Saccharomyces cerevisiae* instead of glucose, two group of media in 5 parallels to which grape and mulberry molasses (from Adiyaman province) was added and prepared. After sterilization, yeasts were cultivated into media and the samples were incubated for 72 h at 25°C. At the end of incubation, the samples were centrifuged in a cooling centrifuge (5000 rpm +4°C de 5 min). The pellets obtained by centrifuge were weighed and separated for vitamin and fatty acid analysis. The pellets were washed by the 50 mM KH<sub>2</sub>PO<sub>4</sub> solution.

**Extraction of lipids:** Cell pellets whose wet weights were determined were homogenized with 3/2 (v v<sup>-1</sup>) Hexane-isopropanol mixture. The homogenate was centrifuged at 5000 rpm for 5 min at 4°C and cell pellet remnant was precipitated. The supernatant part was used in the ADEK vitamin and fatty acid analysis (Hara and Radin, 1978).

**Preparation of fatty acid methyl esters:** An aliquot was taken from the supernatant part of the cell pellet and 5 mL of 2% methanolic sulphuric acid was added. The mixture was vortexed and then kept at 50°C for 12 h. Then, after being cooled to room temperature, 5 mL of 5% sodium chloride was added and then it was vortexed. Fatty acid methyl esters were extracted with 2×5 mL hexane. Fatty acid methyl esters were treated with 5 mL 2% KHCO<sub>3</sub> solution and then the hexane phase was evaporated by the nitrogen flow and then by dissolving in 1 mL fresh hexane (Christie, 1992) they were taken to auto sampler vials.

### **Gas chromatographic analysis of fatty acid methyl esters:**

Methyl esters were analyzed with the SHIMADZU GC 17 Ver. 3 gas chromatography (Kyoto, Japan). For this analysis, 25 m of long Machery-Nagel (Germany) capillary column with an inner diameter of 0,25 µm and a thickness of 25 micron film was used. During the analysis, the column temperature was kept at 120-220°C, injection temperature was kept at 240°C and the detector temperature was kept at 280°C. The column temperature program was adjusted from 120-220°C and the temperature increase was determined to be 5°C min<sup>-1</sup> until 200 and 4°C min<sup>-1</sup> from 200-220°C. It was kept at 220°C for 8 min and the total duration was set as 35 min and nitrogen gas was used as the carrier gas. During the analysis, before the analysis of fatty acid methyl esters, mixtures of standard fatty acid methyl esters were injected and the residence time of each fatty acid was determined. After this process, the necessary programming was made and the fatty acid methyl esters mixtures of the samples were analyzed (Christie, 1992).

### **HPLC analysis of ADEK vitamins and sterol amount:**

The 5 mL supernatant was taken 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 cooled to room temperature and 5 mL of pure water was added and mixed. Lypophilic molecules that did not saponify were extracted with 2×5 mL hexane. The Hexane phase was evaporated with nitrogen flow. It was dissolved in 1 mL (50 + 50%, v v<sup>-1</sup>) acetonitril/methanol mixture and then was taken to auto sampler vials and was analyzed.

The analysis was made with the Shimadzu brand HPLC device. In the device as the pump LC-10 ADVP UV-visible, as the detector SPD-10AVP, as column oven CTO-10ASVP, as auto sampler SIL-10ADVP, as degasser unit DGU-14A and Class VP software (Shimadzu, Kyoto Japan) was used and during the mobile phase the acetonitril/methanol (60+40% v v<sup>-1</sup>) mixture was used. The mobile phase flow rate was determined to be 1 mL A UV detector was used for the analysis and as a column the Supelcosil LC 18 (15×4.6 cm, 5 µm; Sigma,USA) column was used. For vitamin A and beta-caroten, detection of wave length 326 nm, for vitamin E, 202 nm and for vitamin D and K, 265 nm was used (Katsanidis and Addis, 1999).

**Statistical analysis:** For statistical analysis the SPSS 10.0 software program was used. The comparison between experimental groups and the control group was made using ANOVA and LSD tests.

## RESULTS

**Lypophilic vitamins:** It was observed that there was not a significant difference in the  $\delta$ -Tocopherol amount between the control group and the mulberry molasses group but the amount decreased relatively in the groups to which grape molasses was added ( $p < 0.05$ ). It was found that vitamin  $D_2$  increased significantly in mulberry molasses media when compared to the control group ( $p < 0.001$ ) but the amount decreased in grape molasses media when compared to the control group ( $p < 0.01$ ). Also, it was found that the vitamin  $D_3$  amount in mulberry molasses media decreased when compared to grape molasses group but the amount moderately increased in the grape molasses group ( $p < 0.001$ ). It was observed that the  $K_1$  vitamin amount decreased both in mulberry and grape molasses media when compared to the control group but this decrease was greater in mulberry molasses group ( $p < 0.001$ ). It was found that the  $\alpha$ -Tocopherol amount did not change between the control group and mulberry molasses group and that the amount decreased in the grape molasses group ( $p < 0.05$ ). As for the ergosterol amount, it was observed that this amount decreased in the mulberry molasses group when compared to the control group but its amount in grape molasses media was parallel to the amount in control group ( $p < 0.05$ ) (Table 1).

**Fatty acids:** According to the fatty acid analysis results; it was found that the lauric acid (12:0) amount did not change in mulberry media when compared to the control group, but its amount in grape molasses increased when compared to the control group ( $p < 0.01$ ).

It was found that myristic acid (14:0) and linoleic acid (18:2, n-6) amounts increased in groups to which molasses was added when compared to the control group ( $p < 0.05$ ). It was observed that the lauroleic acid (12:1) amount increased in mulberry molasses media but decreased in the grape molasses media when compared to the control group ( $p < 0.05$ ). It was found that the myristoleic acid (14:1) amount decreased in grape molasses group but its amount in mulberry molasses media did not change when compared to the control group ( $p < 0.05$ ). It was found that the changes in palmitic acid (16:0) amount increased in mulberry molasses culture media ( $p < 0.05$ ) but the amount decreased in grape molasses culture media ( $p < 0.01$ ). It was observed that stearic acid (18:0) amount decreased both in mulberry and grape molasses media when compared to the control group. It was found that palmitoleic acid (16:1, n-7) amount did not change in mulberry molasses group when compared to the control group, but decreased in grape molasses group ( $p < 0.05$ ). It was found that in

Table 1: Vitamins analysis results

Vitamins	Control	Mulberry molasses	Grape molasses
$\delta$ -Tocopherol	3.15 $\pm$ 6.02	3.12 $\pm$ 0.19	2.55 $\pm$ 0.29 <sup>a</sup>
$D_2$	5.57 $\pm$ 1.02	13.31 $\pm$ 0.85 <sup>c</sup>	2.82 $\pm$ 0.24 <sup>b</sup>
$D_3$	4.57 $\pm$ 0.99	1.69 $\pm$ 0.23 <sup>c</sup>	7.54 $\pm$ 0.25 <sup>c</sup>
$K_1$	4.35 $\pm$ 0.30	0.44 $\pm$ 0.27 <sup>c</sup>	3.94 $\pm$ 0.80
$\alpha$ -Tocopherol	2.56 $\pm$ 0.35	2.27 $\pm$ 0.39	1.66 $\pm$ 0.44 <sup>a</sup>
Ergosterol	10.44 $\pm$ 0.44	8.48 $\pm$ 4.93 <sup>a</sup>	10.60 $\pm$ 0.20

Table 2: Fatty acid analysis results

Fatty acids	Control	Mulberry molasses	Grape molasses
12:0	5.30 $\pm$ 7.12	5.91 $\pm$ 0.40	7.33 $\pm$ 0.47 <sup>b</sup>
12:1	8.70 $\pm$ 0.14	9.40 $\pm$ 0.25	6.50 $\pm$ 0.45 <sup>a</sup>
14:0	10.02 $\pm$ 0.44	11.68 $\pm$ 0.19 <sup>a</sup>	13.37 $\pm$ 0.33 <sup>b</sup>
14:1	3.56 $\pm$ 0.28	3.40 $\pm$ 0.27	2.86 $\pm$ 5.65 <sup>a</sup>
16:0	45.62 $\pm$ 4.00	47.05 $\pm$ 6.11 <sup>a</sup>	41.46 $\pm$ 5.29 <sup>b</sup>
16:1	4.36 $\pm$ 0.48	4.49 $\pm$ 1.74	3.89 $\pm$ 1.38 <sup>a</sup>
18:0	6.95 $\pm$ 0.76	5.65 $\pm$ 0.39	5.78 $\pm$ 0.62
18:1 n-9	11.06 $\pm$ 0.77	9.56 $\pm$ 1.29 <sup>a</sup>	8.28 $\pm$ 1.73 <sup>b</sup>
18:2 n-6	8.05 $\pm$ 1.31	10.40 $\pm$ 2.25 <sup>a</sup>	12.81 $\pm$ 1.74 <sup>b</sup>
SFA	67.60 $\pm$ 7.04	68.63 $\pm$ 7.43	67.76 $\pm$ 9.52
MUFA	23.95 $\pm$ 3.76	22.40 $\pm$ 1.40	18.29 $\pm$ 4.51 <sup>b</sup>
PUFA	7.85 $\pm$ 3.55	8.32 $\pm$ 6.08	9.86 $\pm$ 5.46 <sup>a</sup>

a:  $p < 0.05$ , b:  $p < 0.01$ , c:  $p < 0.001$

oleic acid (18:1, n-9) amount, a decrease was observed in both molasses media when compared to the control group and this decrease was relatively greater in grape molasses group ( $p < 0.01$ ) (Table 2).

## DISCUSSION

At the end of our study, it was observed that vitamin synthesis was made from *Saccharomyces cerevisiae* FMC-16. It was found that the synthesized vitamin values were  $\delta$ -tocopherol,  $\alpha$ -tocopherol, ergosterol,  $D_2$ ,  $D_3$  and  $K_1$ . The values of these vitamins in changing media were determined by calculations with statistical methods. In a similar study that was carried out by Aksu and Eren (2007), they analyzed the carotenoids synthesis from *Rhodotolura glutinis* yeasts, which was isolated around Ipras refinery in media containing glucose, molasses, sucrose and lactose. At the end of their study, they indicated that appropriate carotenoid synthesis from yeast was greater in molasses and sucrose media and the same effect was not observed in lactose media. They reported that the highest carotenoid concentration was in molasses media with 2 g L<sup>-1</sup>.

It was observed that in fatty acid composition from *Saccharomyces cerevisiae* FMC-16 yeasts; there were 12:0, 12:1, 14:0, 14:1, 16:0, 16:1 n-7, 18:0, 18:1 n-9 and 18:2 n-6 and it was discovered that the fatty acid amounts varied according to changes in the media. We concluded that the main reason in the synthesis of these fatty acids was due to the enzymes that had a role in the synthesis transcribed in *Saccharomyces cerevisiae*. In many studies, it was found that the enzymes, which made fatty acid synthesis from *Saccharomyces cerevisiae* yeast and other yeasts species were affected by various factors in the culture.

In their study, in which they analyzed fatty acid synthesis from yeast, McDonough and Roth (2004) observed that in 2 different development media of *Schizosaccharomyces pombe* (20 and 30°C) 16:0, 16:1 n-7, 18:1, n-9 and 18:2 n-6 synthesis took place. In addition, at the end of the study, they found that 18:1, n-9 synthesis in *Schizosaccharomyces pombe* cells was achievable with the control of stearoyl coA desaturase. The synthesis of 18:1 from 18:0 is realized via introduction of a double bond between the 9th and 10th Cs by Delta 9 desaturase (Steroil Co A desaturase) enzyme. Activities of both the Fatty Acid Synthesize (FAS) and delta 9 desaturase enzymes are affected by different diets (Ntambi, 1999; Rimoldi *et al.*, 2001; Ntambi *et al.*, 2002).

In a study carried out by Hayashi *et al.* (1978), they indicated that the activity of acyl-coA sentetase enzyme in *Saccharomyces carlsbergensis* changed due to the deficiency of myo-inositol.

In their studies, which they carried out Gentile and Plewa (1975) analyzed fatty acid synthesis of *Saccharomyces cerevisiae* and its temperature-sensitive mutants. They reported that mutant and *Saccharomyces cerevisiae* took place with principal fatty acids of 16:0, 16:1 n-7, 18:0 and 18: n-9 synthesis.

At the end of their studies, which they carried out Sakai and Kajiwara (2003) made an isolation of  $\Delta^9$  desaturase enzyme, which realized the synthesis of 16:1 n-7 and 18:1 n-9 fatty acids from *Basidiomycete lentin*, which is another yeast species.

In their studies, carried out Black and DiRusso (2007) found Acyl-Coa Sentetase (ACS) enzyme existence in *Saccharomyces cerevisiae* yeast and detected affecting points of the enzyme on fatty acid metabolism and regulation.

In his study, which he conducted Martin *et al.* (2006) reported that mono unsaturated fatty acids in *Saccharomyces cerevisiae* and other yeasts were composed of saturated acyl CoA base through  $\Delta^9$  desaturase. Additionally, in the study it was found that desaturase gene in *Saccharomyces cerevisiae*, responded to some various stimulants of OLE1 that contained different carbon sources, metal ions and oxygen levels and regulated gene expression, since membrane fatty acid composition is clearly effected by environmental factors (Benchekroun and Bonaly, 1992; Rosi and Bertuccioli, 1992). In addition, fatty acid composition of the cell may be effected by carbon source in the development media. In another study, which supported this idea it was found that from 18:1, 18:2 n-6 and 16:1, among unsaturated fatty acids in grape, 18:2 n-6 was the most common fatty acid and among saturated fatty acids, 16:0 was the most common one (Torija *et al.*, 2002).

In another study, it was indicated that in many yeasts, the synthesis of poly unsaturated fatty acids such as 18:2 and 18:3 depended on  $\Delta^{12}$  and  $\Delta^{15}$  desaturase enzymes (Weete, 1980).

Romero and Ruiz-Herrera (1987), indicated the activity of Fatty Acid Synthesis (FAS) in *Mucor rouxii*, which is a yeast species. Again, in another study, Karam and Arslanian (1984) determined the activity of fatty acid sentetase enzyme in *Saccharomyces cerevisiae*.

In their study, Meesters and Eggink (1998) made isolation and characterization of  $\Delta^9$  desaturase enyme gene from *Cryptococcus curvatus* CBS570 oily yeast.

In their studies carried out Gurvitz *et al.* (2001), reported that 18:2 n-6 isomer synthesized from *Saccharomyces cerevisiae*, has an important role in human diet. They indicated that these isomers had especially anticarsinogenesis and antiarteriosklerotic effect and also lipoidosis-reducing effects.

Abrodo *et al.* (2004) analyzed the effect of fatty acids on *Saccharomyces cerevisiae* fermentation, which was made with traditional methods and testing and in their studies they reported that 12:0, 14:0, 16:0, 16:1 n-7, 18:0, 18:1 n-7 and 18:2 n-6 synthesis took place similar to what we observed in our study. In addition, they indicated that among the fatty acids, 16:0 has an important value for fermentation technology.

## CONCLUSION

Consequently, the amounts of vitamin and fatty acids have been affected, when it is used to different sugar sources in culture media of *Saccharomyces cerevisiae*. It can be draw a conclusion that the reason for this difference stemmed from the different substances in the contents of mulberry and grape molasses.

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