"THE EFFECT OF GROWTH MEDIUM ON LEVELS OF FATTY ACIDS IN SOME YEASTS "

Mohamed I.H. Mahmoud, Mehreshan T. El Mokadem and Zeinab H. Khairalla.

Department of Botany, Women's College Ain Shams University, Helipolis, Cairo.

SUMMARY

Saccharomyces cerevisiae and the osmotolerant yeasts S. rouxii and Debaryomyces hansenii were aerobically and anaerobically grown in presence of increasing concentrations of sodium chloride from zero to 15% (w/v). Levels of both saturated and unsaturated fatty acids in the yeast cells were estimated using gas liquid chromatography. Palmitic and stearic acid constituted the major component of saturated fatty acids in the three test organisms. However, palmitoleic, oleic and elaidic were the major components of unsaturated fatty acids.

No linear correlation was found between levels of sodium chloride in the culture medium and levels of either saturated or unsaturated acids in the yeast cells. Levels of total unsaturated fatty acids were higher in aerobically grown cells of S.rouxii and

D. hansenii than those grown anaerobically, although their levels were indifferent in S.cerevisiae. Also, levels of total saturated fatty acids in S. rouxii were lower than their levels in both D. hansenii and S. cerevisiae. However, levels of unsaturated fatty acids in S. rouxii were higher than those of D.hansenii or S. cerevisiae.

INTRODUCTION

The lipid composition of yeasts is very responsive to changes in the chemical and physical properties of the environment. White and Werkman (1948) reported an increase in the lipid content of Saccharomyces cerevisiae following the growth in presence of sodium chloride. Combs and Pisano (1968) found that on increasing the concentration of sodium chloride from zero to 10%, the lipid content as well as levels of the unsaturated palmitoleic fatty acid in Candida albicans increased. Mahmoud et al (1981) found that levels of both total lipids and phospholipids in S. rouxii, Debaryomyces hansenii and S. cerevisiae decreased with the increase of sodium chloride concentration.

The aim of the present study was to show the effect of sodium chloride on the levels of both saturated and unsatuarated fatty acids in the osmotolerant yeasts S. rouxii and D. hansenii in comparison with those of S. cerevisiae.

MATERIALS AND METHODS

Test Organisms

Saccaromyces rouxii was isolated from a sample of dried Egyptian dates, while Debaryomyces hansenii was isolated from a sample of an Egyptian cheese brine commonly called (Mesh). Both organisms were isolated and identified by Mahmoud (1978). S. cerevisiae, however, was isolated from a sample of commercial compressed yeast, a local product of the Egyptian " Starch Products and Yeast Co.".

Growth Medium

The test organisms were grown in 100 ml fractions of a medium containing the desired concentration of sodium chlorids, and dispensed in 250 ml capacity Erlenmeyer flasks. The glucose-yeast extract broth medium of Crabtree and Hindstill (1974) was used with slight modifications. It has the following composition (g. %): 0.5 peptone, 0.3 yeast extract, 1.0 glucose, 0.2 KH_2PO_4 and 0.05 $MgSO_4.7H_2O$ (initial pH 6.5).

Sodium chloride was added as gram per cent (w/v). However, the final readings of the water activity of the growth medium were measured using a Beckman Hygroline recorder, model SMT . Each measurement was taken until the recorder was stable for 30 minutes to one hour.

Inoculated flasks were shaken at 30°C using a rotary shaker. For anaerobic growth, however each flask aseptically received 10 ml of sterile paraffin oil, and incubated (unshaken) at 30°C. The incubation period varied between 2-15 days for reaching the early stationary phase of growth. This depended upon the test organism as well as the concentration of sodium chloride.

Washing of cells:

After the proper incubation period, the yeast cells were harvested and washed three times using distilled water by centrifugation at 2400 r.p.m.

Extraction of Lipids

Free lipids were extracted from the yeast cells according to the method presented by Letters (1968) with slight modifications. Yeast cells were disintegrated by mechanical shaking with glass beads, and the broken

cell preperation was extracted with neutral and acid solvents as follows: 20 ml portions of 80% (v/v) aqueous ethanol at 80°C were added to samples of broken cells, each corresponding to 100-130 mg dried yeast cells. The suspension was maintained at 80°C for 15 minutes, then filtered through Whatman No. 44 paper. The extract together with other two ethanol washings were combined, and the antioxidant (BHT) was added at a final concentration of 0,005%. Samples were then stored at - 20°C. The residue samples were shaken with 50 ml of 95% ethanol-ethyl ether (1:1 v/v) for 24 hrs at 30°C. The solvent phase was separated by filtration and added to the ethanol extract. The residue was reextracted twice with chloroform. Bound lipids were extracted according to Letters (1966) by subjecting the residue from ethanol-ethylether and chloroform treatments to digestion in acidic mixture (chloroform-methanol-hydrochloric acid 124:65 : 1 (v/v/v) for 5 hrs. at 50°C . The extract was then neutralized using 2N Na OH. Two extractions were done, the first was with 95% ethanol-ethylether 1 : 7 (v/v), and the second with chloroform. Each extraction rested for 24 hrs. Finally, all extracts were combined and the solvents were removed in vacuum.

Seperation of Neutral Lipids

The solvent partition system described by Galanos and Kapoulas (1962) was adopted.
The lipid extracts dissolved in 15 ml of petroleum ether were transferred to a separatory funnel containing 45 ml of both petroleum ether and 95% aqueous ethanol. After shaking and seperation of layers, the lower layer which contains the phosphlipids was transfered to a second funnel. The neutral lipids in the first funnel were then washed twice by shaking with 12 ml portions of 95% aqeous ethanol to ensure complete separation of phospholipids.

Gas liquid chromatography

Analysis of free fatty acids was done using gas-liquid-chromatography according to Lipsky et al (1959) and Hunter and Rose (1971). All samples of neutral lipids were analyzed using a Perkin-Elmer 910 GLC-type Flame ionization detector after complete estrification of fatty acids. The fetty acid-methylesters were seperated on 10% diethylglycol adipate (DEGA) supported on 35-80 mesh chromosorb w, and packed in a stainless steel column of 6 ft 0.25 inch.

The (DEGA) Column was maintained at 195°C with nitrogen flow rate of 50 ml/min and the detector at 195°C. All samples injected to the column (1.5 ul) were dissolved in 1 ul dimethylether. For statistical analysis of data, the paired-sample t-test was used according to Campbell (1974).

RESULTS AND DISCUSSION

Tables 1, 2 and 3 represent levels of fatty acid esters of the three test organisms, aerobically and anaerobically grown in the presence of increasing concentrations of sodium chloride. In case of aerobically grown cells of Saccheromyces rouxii palmitic acid (16 : 0) and steeric acid (18: 0) were the major components of the saturated fatty acids. In anaerobically grown cells, the fatty acids N-nonoic (9:0), capric (10: 0), undecanoic (11: 0) as well as pentadecanoic (15:0) practically disappeared. While stearic acid disappeared also in presence of sodium chloride, where palmitic acid (16:0) became the major component and its levels were significantly higher than those under aerobic conditions.

In Debaryomyces hansenii , levels of stearic acid in anaerobically grown cells were higher than those grown aerobically, in contrast to palmitic, myristic (14:0) and tridecanoic (13:C), since their levels did not show any statistically significant difference in the aerobically and anaerobically grown cells.

In S. cerevisiae, levels of the fatty acid lauric (12:0) in the aerobically grown cells were higher than in those anaerobically grown, contrary to stearic acid. However, levels of the rest of saturated fatty acids did not show any significant difference in the aerobically and anaerobically grown cells. In both S. rouxii and D. hansenii palmitic acid constituted the major component of saturated fatty acids, while palmitic and stearic acids were dominant in S. cerevisiae.

Palmitoleic (16:1), oleic (18:1), and elaidic (18:2) constituted the unsaturated fatty acids in the three test organisms. Levels of oleic acid in aerobically grown cells of S. rouxii were higher than in the anaerobically grown cells contrary to levels of both palmitoleic and elaidic. Levels of elaidic acid were higher in the aerobically grown cells of D. hansenii then in those anaerobically grown and contrary to the rest of other acids. In S. cerevisiae, however, levels

of palmitoleic and oleic acids did not show any difference in aerobically over anaerobically grown cells. Hunter and Rose (1971) found that levels of palmitoleic acid were higher than oleic in S. cerevisiae when the cells were grown anaerobically at 30°C. In the present study, levels of palmitoleic were higher than oleic in S. cerevisiae (aerobically or anaerobically). However, levels of oleic acid in both S. rouxii and D. hansenii were higher than levels of palmitoleic acid whether the cells were grown aerobically or anaerobically.

As a response to the presence of sodium chloride in the medium, no linear correlation could be found between levels of the fatty acids and salt concentration. However, levels of tridecanoic acid (13:0) in S. cerevisiae aerobically increased and anaerobically decreased in response to salt concentration, although its levels did not show any significant difference in D. hansenii. Levels of palmitic acid showed a decrease in aerobically grown cells of S. rouxii but not under anaerobic conditions. Also, its levels were indifferent in D. hansenii. In S. cerevisiae, however, its levels increased aerobically and decreased anaerbbically with the increase of solute concentration.

Combs and Pisano (1968) found that when Candida albicans was grown in presence of zero to 10 % sodium chloride, levels of palmitoleic acid increased, while levels of oleic acid decreased with the increase of solute concentration. In the present study, levels of palmitoleic acid showed a significant increase in response to sodium chloride under aerobic and anaerobic conditions. Levels of oleic acid in S. rouxii decreased in cells grown under either conditions, although they decreased aerobically and increased anaerobically in S. cerevisiae. However, its levels were indifferent in D. hansenii.

Two general processes for the formation of unsaturated fatty acids have been discussed by Erwin (1973). Introduction of unsaturation into yeast fatty acids appeared to be restricted to oxygen-dependent desaturase systems. Also, he postulated the presence of an alternate anaerobic pathway for the introduction of unsaturation. In the present study, levels of total unsaturated fatty acids were higher in aerobically grown cells of S. rouxii and D. hansenii than in those grown anaerobically. However, their levels were indifferent in aerobically and anaerobically grown cells of S. cerevisiae.

As a response to increasing concentration of sodium chloride, levels of total saturated fatty acids decreased in S. rouxii (aerobically or anaerobically) although they increased in S. cerevisiae grown under both conditions. On the other hand, levels of unsaturated fatty acids increased in S. rouxii (aerobically or anaerobically) but decreased in aerobically grown S. cerevisiae and increased under anaerobic conditions. In D. hansenii, however, levels of both total saturated and unsaturated fatty acids did not show any statistically significant difference in aerobically and anaerobically grown cells as a response to increasing concentration of sodium chloride.

Jollow et al (1968), and Bulder and Reinink (1974), found that when baker's yeast cells were grown anaerobically, the content of unsaturated fatty acids decreased. In the present study, in absence of sodium chloride, levels of total unsaturated fatty acids were higher in aerobically grown cells. However, on increasing the salt concentration, their levels decreased in the aerobically grown cells and increased in the anaerobically cultivated cells.

Demel et al (1967) and Koh (1975) working on the fatty acid composition of an obligate osmophilic mutant of S. rouxii, reported that the changes of the ratios of

saturated palmitic and the unsaturated palmitoleic and oleic acids might cause changes in the cell membrane. The increase in the content of unsaturated fatty acids could also result in increasing the fluidity of the yeas cell membrane. In the present study , increasing the concentration of sodium chloride decreased the levels of palmitic and oleic in aerobically or anaerobically grown cells of S. rouxii. however, levels of palmitoleic and elaidic acids increased in the aerobically cultivated and decreased in the anaerobically grown cells.

Generally, levels of total saturated fatty acids in aerobically and anaerobically grown cells of S. rouxii were lower than their corresponding levels in D. hansenii and S. cerevisiae, where their levels did not show any difference. However, levels of total unsaturated fatty acids in S. rouxii were higher than those of D. hansenii and S. cerevisiae, where their levels were indifferent.

REFERENCES

Bulder, C.J., and M. Reinink (1974)
Unsaturated fatty acid composition of wild type and respiratory deficient yeasts after aerobic and anaerobic growth.
J. Microbiol. Serol., 40, 446.

Campbell, R.C. (1974):
"Statistics for biologists" p. 159.
Cambridge University Press (Publ.)
London.

Combs, T and M. Pisano (1968)
The effect of sodium chloride on the lipid content and fatty acid composition of Candida albicans.
Mycologia 60, 1232.

Crabtree; K., and R. Hindstill (1974)
In "Fundamental Experiments in Microbiology". P. 340. W.B. Saunders (Publ.), Philadelphia, U.S.A.

Demel, R., L. Van Deenen, and B.A Pethica (1967).
Monolayer interactions of phospholipids and cholesterol.
Biochim. Biophys. Acta, 135, 11.

Erwin, J. (1973)
Comparative biochemistry of fatty acids in eukaryotic microorganisms. In " Lipids and biomembranes of eukaryotic microorganisms. Erwin, J. (ed.) p. 41. Academic Press Inc., New York, U.S.A.

Galanos, D., and V. Kapoulas (1952)
Thin layer chromatography of phosphatides and glycolipids.
J. Lipid Res. 3, 134.

Hunter, K., and A. Rose (1971):
Lipid composition of Saccharomyces cerevisiae as influenced by growth temperature.
Biochim. Biophys. Acta, 260, 639.

Jollow, D., G. Kellerman and A. Linnane (1968)
The biogenesis of mitochondria. III-
The lipid composition of aerobically and anaerobically grown Saccharomyces cerevisiae as related to the membrane systems of the cell.
J. Cell Biol. 37, 221.

Koh, T.Y. (1975).
The isolation of obligate osmophilic mutants of the yeast Saccharomyces rouxii.
J. Gen. Microbiol. 88, 184.

Lellers, R. (1966).
Phospholipids of yeasts. II extraction, isolation, and characterization of yeast phospholipids.
Biochim. Biophys. Acta, 116 , 489.

Letters, R. (1968)
Phospholipids of yeasts. P. 303.
in "Aspects of Yeast Metabolism"
Blackwell Press, Oxford. London.

Lipsky, S., R. Landowne and J. Lovelook (1959)
Separation of lipids by gas liquid chromatography.
Anal. Chem. , 31, 852.

Mahmoud, M.I. (1978)
The effect of the water activity of the milieu on rates of glucose uptake by the osmophilic yeasts Saccharomyces rouxii and Debaryomyces hansenii.
Zbl. Bakt. Abt. B.d., 133, 726.

Mahmoud, M.I., M.T. El Mokadem, and Z.H. Khairalla (1981)
The effect of growth medium on the levels of lipids in some yeasts. (Submitted for publication)

White, A.G., and C. Werkman, (1948)
Fat synthesis in yeast.
Arch. Biochem. 17, 478.

Table 1

Levels of Fatty acids in Saccharomyces rouxii (mg/g neutral lipids)

Sodium Chloride%(w/v)		0%	1.7	3.4	6.5	9.8	15.3	0	1.7	3.4	6.5	9.8	15.3
Final water activity		0.998	0.992	0.98	0.96	0.932	0.89	0.998	0.992	0.98	0.96	0.932	0.89
		Aerobic Growth						Anaerobic Growth					
N-Nonoic	9:0	-	3.0	1.5	0.5	traces	3.4	-	-	-	-	-	-
Capric	10:0	2.2	2.2	4.5	1.8	--	-	-	-	0.6	-	-	-
Undecanoic	11:0	-	-	9.0	1.8	1.3	0.8	-	-	-	-	-	-
Lauric	12:0	0.2	0.2	9.0	1.1	1.3	2.1	0.7	0.2	0.5	-	-	-
Myristic	14:0	4.5	4.0	3.0	3.8	4.0	4.7	2.7	-	8.6	-	-	-
Pentodecanoic	15:0	0.3	9.8	1.5	1.1	1.9	2.6	-	-	0.6	-	-	-
Palmitic	16:0	133.9	123.9	124	103.7	87	81.3	183.3	241.8	186.7	187.9	151.8	111.9
Palmitoleic	16:1	105.9	190.4	216.5	212.2	180.1	142.2	45.5	121.7	232.2	257.2	300.3	382.8
Heptodecanoic	17:0	0.8	-	-	-	-	-	-	-	-	-	-	-
Stearic	18:0	21.4	11.1	12.1	13.1	33.0	40.1	74.0	-	-	-	-	-
Oleic	18:1	614.0	530.2	542.1	575.6	564.6	582.7	510.8	489.8	431.3	405.2	396.9	378.4
Elaidic	18:2	107.0	119.5	72.3	82.1	127.1	135.4	169.2	136.5	121.5	112.6	89.7	69.7
Saturated	(Total)	163.3	154.2	164.6	126.9	125.5	135	260.7	242.0	198	187.9	151.8	111.9
Unsaturated	(Total)	826.9	840.1	830.9	869.9	871.8	860.3	725.5	748	785	765	785.1	830.9

Table 2

Levels of Fatty acids in *Debaryomyces hansenii* (mg/g neutral lipids)

Sodium Chloride % (w/v)		0	1.7	3.4	6.5	9.8	15.3	0	1.7	3.4	6.5	9.8	15.3
Final water activity		0.998	0.992	0.98	0.96	0.932	0.89	0.998	0.992	0.98	0.96	0.932	0.89
		Aerobic Growth						Anaerobic Growth					
Capric	10:0	0.6	-	-	0.3	-	-	-	-	0.3	-	-	-
Undecanoic	11:0	0.9	-	-	3.1	4.6	0.8	-	-	1.5	-	2.0	2.5
Lauric	12:0	-	-	-	-	-	-	2.4	0.2	0.3	0.3	3.2	3.0
Tridecenoic	13:0	4.6	11.2	14.7	1.5	traces	traces	1.8	1.8	2.0	1.7	5.9	5.9
Myristic	14:0	1.7	16.4	14.7	6.7	2.2	1.1	1.2	1.5	4.2	5.1	5.5	4.8
Pentadecanoic	15:0	-	-	10.7	4.9	3.3	1.1	-	-	2.7	3.3	3.9	6.7
Palmitic	16:0	215.8	252.6	225.2	95.6	140.4	177.1	267.7	221.8	182.5	184.4	234.6	348.0
Palmitoleic	16:1	46.1	51.8	83.7	98.3	148.9	218.9	36.4	83.9	107.6	87.6	80.9	71.2
Heptadecanoic	17:0	10.1	-	-	-	-	-	-	-	-	-	-	-
Stearic	18:0	10.4	15.5	10.7	17.0	61.7	62.9	109.5	150.1	136.2	140.8	134.4	117.0
Oleic	18:1	520.7	502.4	486.3	463.8	298.3	225.2	481.5	469.0	496	412.4	442.9	349.7
Elaidic	18:2	152.2	100.1	84.9	258.6	230.9	185.9	72.8	31.7	48.3	30.8	22.1	17.3
Saturated	(Total)	244.1	295.7	276	129.1	212.4	243	382.6	372.1	329.7	335.6	389.5	487.9
Unsaturated	(Total)	719	654.3	654.9	820.7	678.1	630	590.7	587.9	651.9	590.8	545.9	438.8

Table 3

Levels of Fatty acids in Saccharomyces cerevisiae (mg/g neutral lipids)

Sodium Chloride % (w/v)		0	1.7	3.4	6.5	9.8	15.3	0	1.7	3.4	6.56	9.8	15.3
Final water activity		0.998	0.992	0.98	0.96	0.932	0.89	0.998	0.992	0.98	0.96	0.932	0.89
		Aerobic Growth						Anaerobic Growth					
Capric	10:0	-	-	-	-	-		-	0.7	0.9	-		
Undecanoic	11:0	2.2	-	-	1.4	-		4.3	7.0	1.8	10.9		
Louric	12:0	2.4	2.9	3.2	8.2	1.4		23.3	30	40	70		
Tridecanoic	13:0	0.3	2.0	2.0	1.4	2.5		5.0	1.6	traces	traces		
Myristic	14:0	1.3	-	-	-	-	GROWTH	26.5	24.6	21.8	61.3	GROWTH	GROWTH
Pentadecanoic	15:0	0.5	-	-	-	-		1.4	3.5	3.0	2.7		
Palmitic	16:0	71.8	133.1	163.9	203.3	214.9		284.8	166.7	178.7	137.8		
Palmitoleic	16:1	278.8	397.6	425.6	444.3	450.1		343.2	405.6	407.3	386.2		
Heptadecanoic	17:0	10.4	18.4	16.3	2.2	traces		-	-	-	-		
Stearic	18:0	110.7	144	130	111.0	110		37.2	44.3	27.6	16.2		
Oleic	18:1	480.0	211.6	215.2	201.7	191.7		273.6	313.0	300.3	298.5		
Elaidic	18:2	1.6	49.4	38.3	24.4	28.5	NO	-	-	-	-	NO	NO
Saturated (Total)		199.6	300.4	315.4	327.5	328.5		381.5	272.3	273.8	298.9		
Unsaturated (Total)		760.4	658.6	679.1	670.4	670.3		616.8	718.6	707.6	684.7		

تأثير بوشنة النمو على مستويات الاحماض الدهنية في بعض قطريات الخميرة

دكتور / محمد ابراهيم حسن محمود ، دكتوره / مهرشامه طه المقدم
والدكتوره / زينب حسن خير الله

قسم النبات — كلية البنات — جامعة عين شمس

ملخص

في هذه الدراسة نبت كل من قطريات الخميرة "سكارومايسزروكسي" و "ديبهاريومايسزهانسنى" و "سكارومايسز سيرفيسى" تحت ظروف هوائية و لا هوائية عند تركيزات متزايدة من كلوريد الصوديوم تتراوح بين صفر و ١٥٪

فوجد ان حمض الباالميتيك و حمض الاستياريك يكونان الحجم الاكبر من كمية الاحماض الدهنية المشبعة في الخمائر تحت الاختبار . أما الاحماض الدهنية بالميتوليك و الايديك فكانت اساس الاحماض الدهنية غير المشبعة في الخلايا .

كما وجد أن مستويات الاحماض الدهنية غير المشبعة في خلايا كل من "سكارومايسز روكسي" و "ديبهاريومايسزهانسنى" النامية هوائيا كانت أعلى من مستوياتها تحت الظروف اللاهوائية ، ولكن لم يلاحظ اى فرق بين مستوياتها في الخلايا النامية و تلك النامية لا هوائيا في خميرة "سكارومايزسيرفيسى" .

كما وجد ايضا ان مستويات الاحماض الدهنية المشبعة في خميرة "سكارومايسزروكسي" منخفضة عن نظائرها في كل من خميرتى "ديبهاريومايسزهانسنى" و "سكارومايسزسيرفيسى" ولكن وجد ان مستويات الاحماض الدهنية غير المشبعة في خميرة "سكارومايسزروكسي" اعلى من نظائرها في كل من خميرتى "ديباروماسزهانسنى" و "سكارومايسزسيرفيسى" .