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Short communication

Saccharomyces cerevisiae cell fatty acid composition and release during fermentation without aeration and in absence of exogenous lipids

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Abstract

Medium-chain fatty-acids (MCFA) are among the main aroma compounds of fermented beverages. High concentrations of MCFA have been found in sluggish and stuck fermentations. It has been suggested that they arrest cell growth, as they may be toxic, but the causes of sluggish and stuck fermentations are still unclear. The aim of this work was to see whether the production of MCFA is related to fatty acid synthesis in the absence of exogenous lipids and aeration, and whether their increase can be regarded as a consequence, instead of the cause, of sluggish and stuck fermentations. Two possibilities were considered: (i) MCFA are produced to replace unsaturated fatty acids (UFA) for cell membrane fluidity when the lack of oxygen makes desaturation of saturated fatty acids (SFA) impossible; or (ii) MCFA are produced following the release of medium-chain acyl-CoA from the fatty acid synthetase complex (FAS) due to the accumulation of SFA, and their hydrolysis to recycle CoA-SH. In the first hypothesis, MCFA should be active in cell metabolism and be found in cell structures; in the second, MCFA should be a discard and prevalently found outside the cell. We carried out a *Saccharomyces cerevisiae* fermentation experiment in a synthetic, lipid-free medium without aeration. We measured the fatty acid composition of yeast cells and the amounts of MCFA and their ethyl esters in the medium throughout the fermentation. Cell growth and the oxygen content of the medium were also monitored. We found that MCFA are not immobilized in cell structures, but mainly released into the medium. Cell growth is arrested because fatty acid biosynthesis is prevented by the lack of oxygen. The higher MCFA concentrations found in sluggish and stuck fermentations can be thus regarded as an effect, and not the cause, of this arrest. Some suggestions for the prevention of these events are proposed.

Keywords: Yeasts; Fatty acids; Oxygen; Stuck fermentation; Sluggish fermentation

1. Introduction

Sluggish and stuck fermentations are major industrial problems (Geneix et al., 1983; Lafon-

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Lafourcade, 1984) that cannot be prevented because their causes are not fully understood. High concentrations of medium-chain fatty acids (MCFA) are sometimes found in the medium when yeast growth ceases and in sluggish and stuck fermentations (Taylor and Kirsop, 1977; Geneix et al., 1983; Lafon-Lafourcade, 1984; Lafon-Lafourcade et al., 1984; Viegas et al., 1985, 1989; Edwards et al., 1990; Viegas and Sa-Correia, 1991; Delfini et al., 1993; Ravaglia and Delfini, 1993). As MCFA are toxic, they have been held responsible for the arrest of growth (Lafon-Lafourcade, 1984; Lafon-Lafourcade et al., 1984; Viegas et al., 1985, 1989; Edwards et al., 1990). However, this toxicity has only been demonstrated for exogenous MCFA as carriers of protons that reduce cytoplasmic pH (Viegas and Sa-Correia, 1991; Stevens and Hofmeyer, 1993), and not for MCFA produced inside the cell.

It is not fully clear why yeasts should produce MCFA physiologically. A solution to the problems mentioned above may perhaps be discovered through a fuller understanding of the ways in which such production is enhanced.

MCFAs, mainly hexanoic (C_6), octanoic (C_5) and decanoic acid (C_{10}), are always produced during fermentation of any raw material with anaerobic yeasts, such as brewer's yeasts or the wine strains of *Saccharomyces cerevisiae* (Taylor and Kirsop, 1977; Suomalainen and Lehtonen, 1979; Edwards et al., 1990; Hammond, 1993; Ravaglia and Delfini, 1993). In addition, MCFA and their ethyl esters are among the main aroma compounds of alcoholic beverages (Suomalainen and Lehtonen, 1979; Kodama, 1993). In contrast, aerobic or semi-aerobic yeasts, such as baker's yeasts, produce fewer MCFA, but more unsaturated fatty acids (UFA) (Suomalainen and Lehtonen, 1979). MCFA production thus appears to be directly related to the fermentative metabolism of yeasts, but actually promoted by anaerobic growth.

The fatty acid composition of a cell is partly influenced by the lipid composition of its growth medium, since it can include fatty acids from the medium in its own phospholipids (Taylor et al., 1979; Thurston et al., 1981; Ratledge and Evans, 1989; Quinn et al., 1989; Mauricio et al., 1991; Benchekroun and Bonaly, 1992; Rosi and Bertuccioli, 1992). Fermentation in a low-lipid medium activates fatty acid biosynthesis and results in the production of hexadecanoyl-CoA and octadecanoyl-CoA production (Henry, 1982; Ratledge and Evans, 1989). These compounds can only be converted by Δ^9-desaturase into 9-hexadecenoyl-CoA and *cis*-9-octadecenoyl-CoA in the presence of molecular oxygen (Bloomfield and Bloch, 1960; Jeffcoat and James, 1984; Ratledge and Evans, 1989). In anaerobiosis, long-chain saturated fatty acids (SFA) in the cell increase and inhibit acetyl-CoA carboxylase (ACC), so that fatty acid elongation by fatty acid synthetase complex (FAS) is prevented (Wakil et al., 1983; Schweizer, 1984). In these conditions, FAS releases medium-chain acyl-CoA (Hammond, 1993). The end products of fatty acid synthesis in *Saccharomyces cerevisiae,* in fact, are fatty acyl-CoA esters, whereas mammalian systems yield the corresponding fatty acids (Wakil et al., 1983; Ratledge and Evans, 1989). Increased MCFA production can be explained in two ways:

1. It is activated by the cell to maintain cell membrane fluidity by replacing UFA. In physical terms, the effect of a short chain is similar to that of the double bond of a long chain (Chapman, 1975; Quinn and Chapman, 1980). Higher production of MCFA should be thus devoted to structural formation of the membrane. Their content in the cell should raise with the lack of oxygen and UFA in the medium, and their release into the medium should be proportional to the increase in this content. Moreover, cell growth and vitality should not be strongly affected by the reduced UFA content.
2. It is not the consequence of an active process, but only a secondary effect of the release from FAS of medium-chain acyl-CoA and of their hydrolysis to recycle CoA-SH. MCFA are not bound to cell structures, but are released into the medium. Their concentration does not raise in the cell, but only outside, and in proportion to their ability to diffuse passively through the cytoplasmic membrane. Cell growth ceases because lipid biosynthesis becomes impossible. This could also explain the occurrence of sluggish and stuck fermentations.

To see which hypothesis was correct, we carried out a fermentation in a synthetic lipid-free medium initially saturated with air, in which molecular oxygen decreased spontaneously through its con-

sumption by the growing cells We measured fatty acid content both in the cells and in the medium throughout the fermentation. Cell growth and vitality and the oxygen and MCFA ethyl esters concentrations in the medium were also monitored.

2. Materials and methods

2.1. Yeast strain

A wine strain, S47c, of *Saccharomyces cerevisiae* var. *cerevisiae* with standard oenological characteristics, was selected from the culture collection of Istituto Sperimentale per l'Enologia, Asti (Italy)

2.2. Inoculum

The preculture was obtained in 2% glucose YEPD medium in Erlenmeyer flasks placed on an orbital shaker for 24 h at 28°C. The cells were counted with a haemocytometer and collected by centrifugation at 6 000 rpm for 5′, then washed with sterile bidistillated water and inoculated into the fermentation medium at a concentration of 1×10^5 cells/ml.

2.3. Fermentation medium

The experiments were carried out in 2.5 l Wickerham synthetic medium (Wickerham, 1951) with the following modifications: L(+) tartaric acid 3 g/l; L(+) malic acid 2 g/l, citric acid 2 g/l, D(+) glucose 200 g/l, pH 3.2 obtained by addition of NaOH 2N to recreate vinification conditions. The organic acids also acted as buffers to prevent significant pH fluctuations and maintain a suitable pH value without the addition of substanzial amounts of NaOH.

2.4. Fermentation conditions

Fermentation was carried out for 15 days in an Applikon ADI 1020 fermenter (Applikon Dependable Instruments B.V., Schiedam, NL) under constant temperature (20°C), pH (3.2) and stirring (700 rpm) conditions. The medium was sterilized in an autoclave at 121°C for 20 minutes. Prior to inoculation, it was saturated for 30′ with sterile air. All the oxygen dissolved in the medium was consumed during the first day owing the cell multiplication. Its reduction was monitored with a Clark electrode. At the moment of inoculation, a vitamin complex solution (Wickerham, 1951) sterilized by filtration was added. Cells and medium samples were withdrawn for analysis under sterile nitrogen to maintain anaerobiosis. Cells were counted with a haemocytometer. Dead cells were identified by methylene blue differential staining (Borzani and Vairo, 1958) and expressed as a percentage of the total number of cells.

2.5. Determination of cell fatty acid content

Cell fatty acids were determined according to Cocito and Delfini (1994). Briefly, yeast cells (100–200 mg wet wt) were separated from the medium by centrifugation at 4°C at 6 000 rpm for 5′, washed twice in a 0,9% NaCl solution, resuspended in 10 ml sterile water and transferred to a spherical flask. 1 ml of 1:1 ethanol:water solution of 310 mg/ml nonanoic acid and 1 mg/ml nonadecanoic acid in ethanol were added as internal standard (I.S.). Saponification was carried out by adding 10 ml KOH 2 N and refluxing in a Liebig condenser in ethanol at 80°C for 90′. After cooling to room temperature, the saponified material was washed with 10 ml distilled water, transferred to a separatory funnel, acidified with H_2SO_4 10 N, extracted 3 times with 15 ml $CHCl_3$ and shaken for 2 minutes. The organic phase was transferred to a rotating evaporation flask and concentrated at 30°C to 2 ml; 200 μl were removed, dried under N_2 and silanized with 100 μl pyridine, 100 μl bis(trimethylsilyl)trifluoracetamide and 10 μl trimethylchlorosilane and incubated at 80°C for 60′. This sample was analyzed by gas chromatography (Varian 3600, Varian Associates Inc., Palo Alto, CA, USA) equipped with a flame ionization detector (FID) on a DBI capillary column (J. + W. Scientific, Folsom, CA, USA), 30 m long and 0.25 mm i.d., film thickness 0.25 μm. The operating conditions were: temperature, 40°C to 200°C at 6°C/min, 200°C for 15′, 200°C to 260°C at 6°C/min and 260°C to 290°C at 2°C/min; injector temperature 280°C, detector temperature 300°C; split rate 1:20; carrier gas helium, linear flow rate 1,5 ml/min, pressure 15.7 psig, injection volume 1–2 μl.

2.6. Determination of medium fatty acid and ethyl ester content

Fatty acids and ethyl esters were determined according to Cocito and Delfini (1994). Briefly, 50 ml medium were placed in a separatory funnel. After addition of the 1.S., extraction was carried out by shaking three times with a total of 25 ml of $CHCl_3$. The mixture was centrifuged at 4 500 rpm for 5′ and the organic phase was transferred to a rotating evaporation flask, concentrated at 30°C to about 1 ml, then dehydrated with anhydrous Na_2SO_4. The samples were analysed by gas chromatography (Varian 3600, Varian Associates Inc., Palo Alto, CA. USA) on a Nukol capillary column (Supelco Inc., Bellefonte, PA, USA) 15 m long and 0.53 mm i.d., film thickness 0.50 μm. The operating conditions were: temperature, 60°C to 188°C at 3°C/min; injector temperature 200°C, detector temperature 220°C, split rate 1:25; carrier gas helium 5.2 ml/min; pressure 2.2 psig; injection volume 1–2 μl.

The repeatability and reproducibility of the methods are illustrated in Cocito and Delfini (1994). The average coefficient of variation was 5.63% while the maximum coefficient of variation, checked for C_{14}, was 9.9%.

2.7. Chemicals

All chemical and reagents were from Fluka (Fluka, Bucks, Switzerland); culture media were from Sigma (Sigma chemical Company, St. Louis, MO, USA).

3. Results

The oxygen dissolved in the medium was all consumed in the first day of fermentation (results not shown). Cell UFA as a percentage of the total fatty acids gradually decreased in the first three days (Fig. 1), whereas the SFA percentage increased (Fig. 2). An increase of decanoic (C_{10}) and dodecanoic acid (C_{12}) from about 1–2% to 8–10% of the total fatty acids was also observed, whereas the percentages of hexanoic (C_6) and octanoic acid (C_8) did not change (Fig. 2). From the 4th day on, *cis*-9-octadecenoic acid ($C_{18:1}$) remained constant (Fig. 1), and oc-

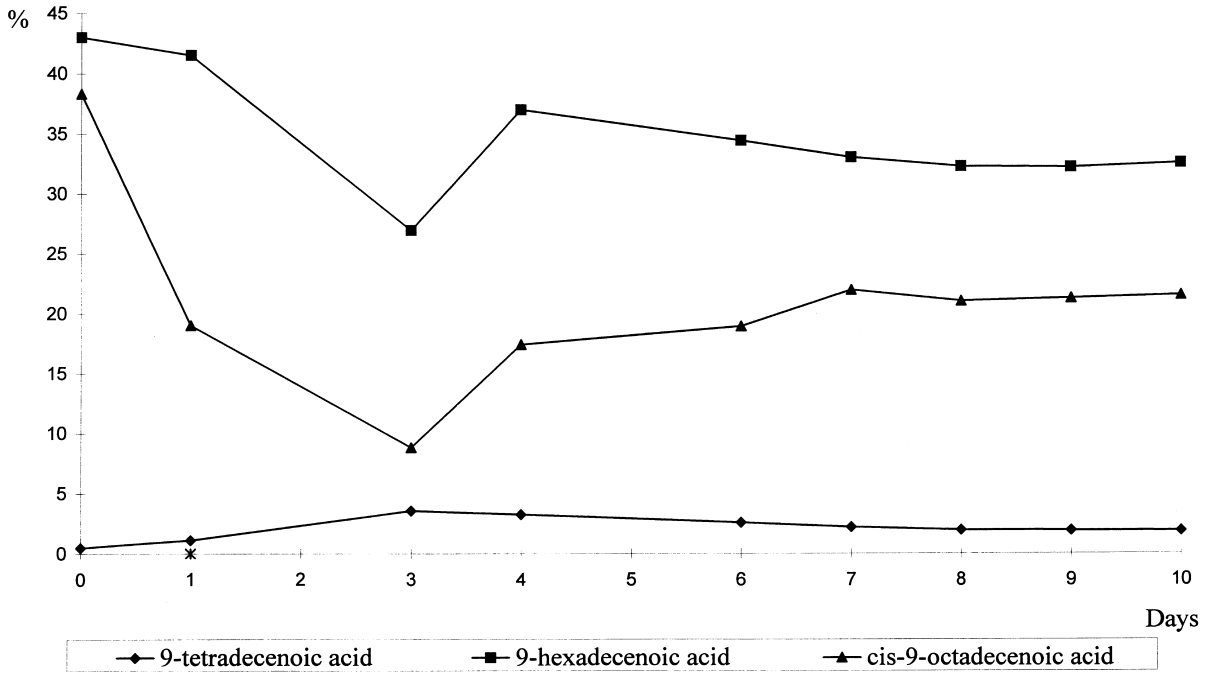

Fig. 1. Cell unsaturated fatty acid percentage of total fatty acids throughout the fermentation without aeration of the Wickerham synthetic medium.

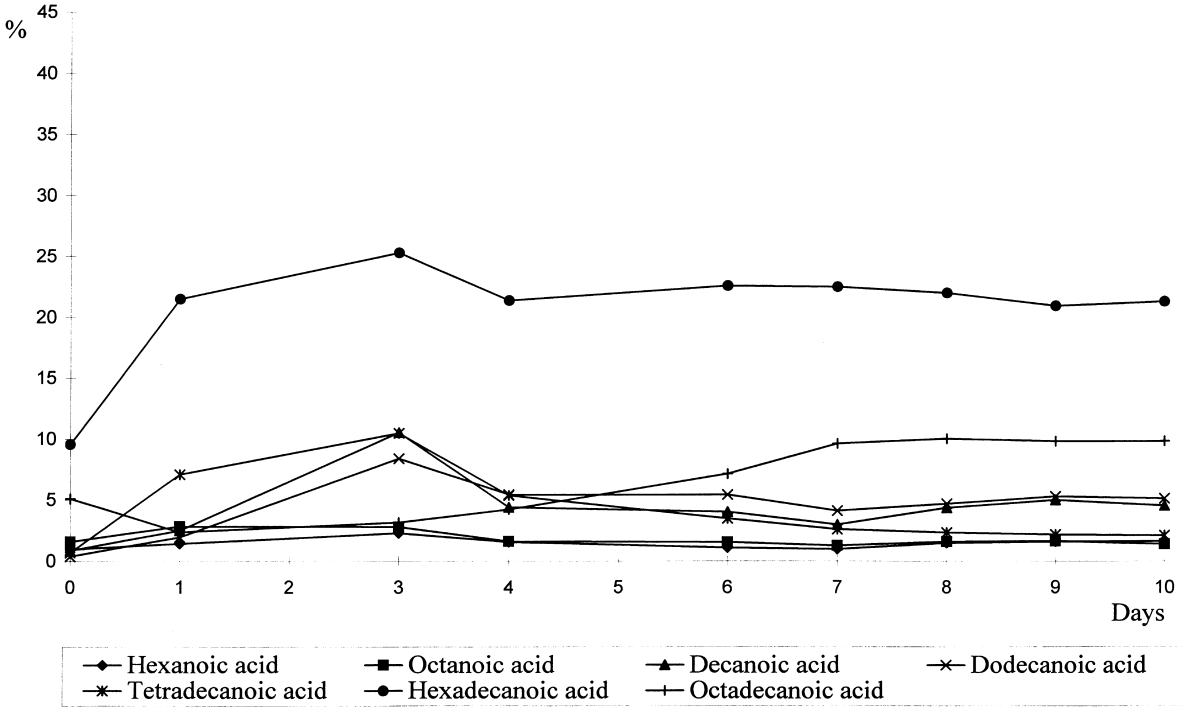

Fig. 2. Cell saturated fatty acid percentage of total fatty acids throughout the fermentation without aeration of the Wickerham synthetic medium.

tadecanoic acid (C_{18}) rose gradually until 7th day (Fig. 2). Between the 3rd and the 4th day, when the dead cell percentage was about 8% (Table 1), the cell fatty acid content changed: SFA decreased and UFA increased (Figs. 1 and 2) and both concentrations then remained constant.

Cell growth (Table 1) leveled all between the 3rd and the 4th day. The dead cell percentage rose slightly 4th day and then became very high between the 10th and the 13th. The maximum cell number was 9.7×10^7, reached on the 7th day, whereas when S47c was cultured in the same medium, but in Erlenmeyer flasks on an orbital shaker, the cell concentration at stationary phase was 2.2×10^8 cells/ml (results not shown).

The C_6 and C_8 concentrations in the medium rose to a maximum on the 4th day and then remained practically constant, whereas C_{10} and C_{12} peaked after six days and then fell to their start values (Fig. 3). C_8 was the most abundant compound. The pattern was the same when the free and esterified forms of MCFA acids were considered. The ethyl esters concentrations, in fact, were very much lower than those of the free forms (Table 2).

4. Discussion

Comparison of the cell and the medium MCFA contents shows that MCFA in a cell are not bound to its structures. Cell C_6 and C_8 values were practically constant (Fig. 2), whereas in the medium these acids

Table 1
Number of cells in suspended phase and dead cell percentage throughout the fermentation

Days	1	2	3	4	6	7	9	10	13
Cells number	4.8×10^6	2.68×10^7	5.92×10^7	6.83×10^7	8.55×10^7	9.7×10^7	9.6×10^7	8.1×10^7	8.2×10^7
Dead cells%	< 1	4.85	6.76	8.49	7.6	8.24	19.79	63.95	95.13

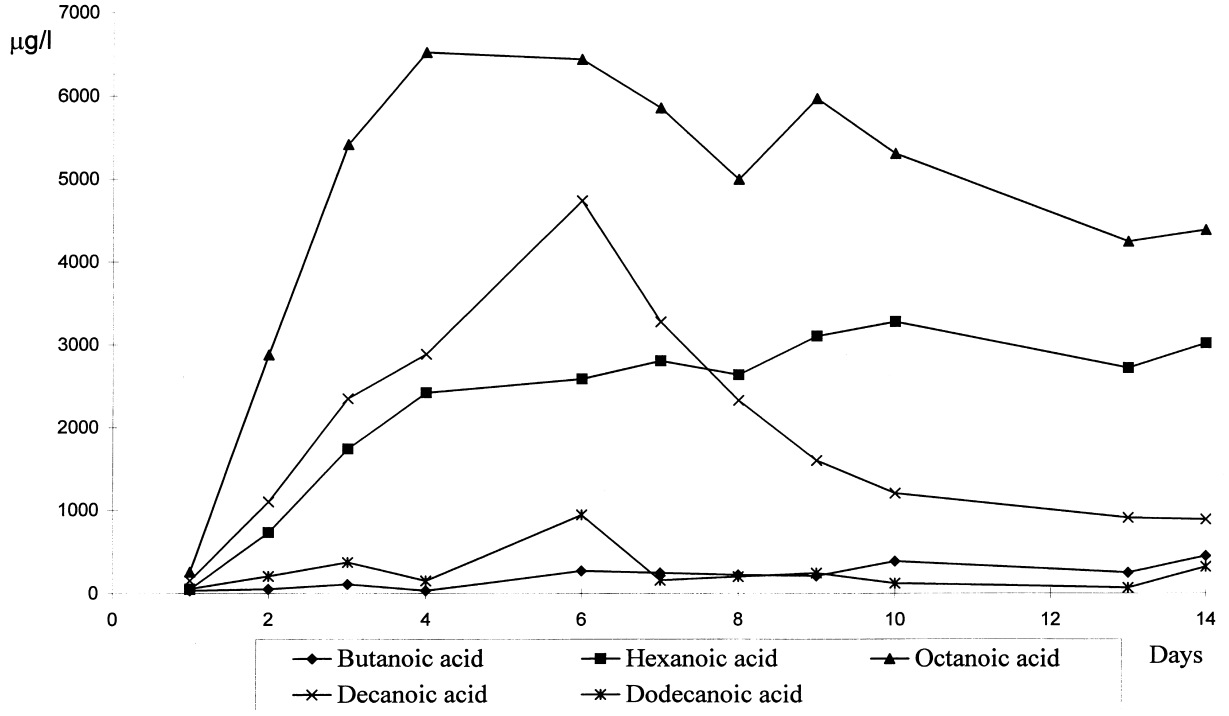

Fig. 3. Concentration of fatty acids (µg/l) released by yeast cells in the Wickerham synthetic medium throughout the fermentation without aeration.

rose to very high concentrations, solely during the growing phase (Fig. 3). C_{10} was relatively abundant both in the cell and in the medium, and C_{12} was almost exclusively present in the cell (Figs. 2 and 3). Since passive diffusion through the cytoplasmic membrane is inversely proportional to fatty acid chain length, the data point to regulation by the membrane of an equilibrium between the inside of a cell and the outside environment. Moreover, the increase in cell MCFA, whether as a concentration or as a percentage of the total fatty acids, was very small. It seems clear from these data that MCFA do not replace UFA in the membrane to maintain its fluidity.

During cell growth, cell UFA decreased and SFA increased as expected because of the lack of oxygen.

Table 2
Concentration of ethylic esters of fatty acids released in the Wickerman synthetic medium throughout the fermentation (µg/l)

Days	Ethyl hexanoate	Ethyl octanoate	Ethyl decanoate	Ethyl didecanoate	Ethyl tetradecanoate	Ethyl hexadecanoate
1	13	4	15	17	40	9
2	9	25	22	4	10	2
3	136	58	0	42	0	15
4	141	45	51	23	19	11
6	228	408	345	189	52	189
7	92	59	86	101	7	35
8	124	193	82	213	14	73
9	242	554	168	295	0	33
10	108	204	197	398	0	56
13	26	67	112	573	27	34
14	66	89	69	34	38	79

When growth levelled off the cell fatty acid composition stabilized and MCFA concentrations in the medium peaked. The functional efficiency of cell membrane, in fact, cannot be maintained when UFA are lacking (Quinn et al., 1989) and growth stops. Fatty acid elongation also stops and the release of MCFA is arrested.

As UFA cannot be synthesized in the absence of oxygen, their small increase and the decrease of SFA observed between the 3rd and the 4th day probably occurred because the living cells used UFA released by lysis of the dead cells. These UFA restarted fatty acid elongation (Hammond, 1993) and resulted in the decrease of C_{10} and C_{12} in the medium after six days (Fig. 3) and the relative increase in cell $C_{18} \leq$ (Fig. 2). At pH 3.2 in the medium, MCFA are mainly undissociated. They can easily pass through cytoplasmic membrane by passive diffusion and be recycled in fatty acid elongation.

Growth ceased on the 7th day without reaching the maximum concentration recorded in the presence of oxygen, showing that growth is arrested by the cessation of lipid synthesis in the absence of oxygen.

It can be concluded that the increase of MCFA in the medium is due to their release from FAS of acyl-CoA and to their hydrolysis to recover CoA-SH from the acyl-bound forms. MCFA are not needed for structural lipid synthesis. Their increase in the medium is thus the consequence, and not the cause, of the events that lead to the arrest of growth, and hence to sluggish and stuck fermentations.

Decrease of MCFA and restarting of sluggish and stuck fermentation by yeast ghosts observed by Lafon-Lafourcade et al. (1984) was attributed to adsorption of MCFA on these yeast ghosts, and thus regarded as evidence of their toxicity. Our results, however, indicate that UFA of the yeast ghosts may restart both cell growth and fatty acid elongation (Ratledge and Evans, 1989; Hammond, 1993). Decreased MCFA concentrations in the medium can therefore be ascribed to their recycling in fatty acid biosynthesis.

Sluggish and stuck fermentations can be prevented in various ways: strong aeration (if consistent with the quality of final product); enrichment of the medium with UFA; inoculation with cells precultured with techniques that promote production of storage lipids, as growth in liquid media with a high C/N ratio (Bardi, 1996).

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References

Bardi, L., 1996. Risultati preliminari sull'importanza della composizione del mezzo nutritivo della precoltura per la crescita e la vitalità di lieviti enologici. Biologia Oggi 10 (3–4), 131–136.

Benchekroun, K., Bonaly, R., 1992. Physiological properties and plasma membrane composition of *Saccharomyces cerevisiae* grown in sequential batch culture and in presence of surfactants. Appl. Microbiol. Biotechnol. 36, 673–678.

Bloomfield, D.K., Bloch, K., 1960. The formation of Δ^9 unsaturated fatty acids. Biol Chem 235, 337–345.

Borzani, W., Vairo, M., 1958. Quantitative adsorption of methylene blue by dead yeast cells. J. Bacteriol. 76, 251–255.

Chapman, D., 1975. Lipid dynamics in cell membranes. In: Weissman, G., Claiborne, R. (Eds.), Cell membranes: biochemistry, cell biology and pathology. Hospital Practice, New York, pp. 13–22.

Cocito, C., Delfini, C., 1994. Simultaneous determination by GC of free and combined fatty acids and sterols in grape musts and in yeasts as silanized compounds. Food Chemistry 50, 297–305.

Delfini, C., Bardi, L., Cocito, C., Conterno, L., Dell'oro, V., Ravaglia, S., 1993. Concentration en oxygène, en acides aminés, en acides gras et en sterols libres du mout et composition aromatique du vin, Actes Symposium International Connaissance aromatique des cépages et qualité des vins. Montpellier, 9–10 février 1993.

Edwards, C.G., Beelman, R.B., Bartley, C.E., McConnel, A.L., 1990. Production of decanoic acid and other volatile compounds and the growth of yeast and malolactic bacteria during vinification. Am J. Enol. Vitic. 41, 48–56.

Geneix, C., Lafon-Lafourcade, S., Ribereau-Gayon, P., 1983. Les causes, la prevention et le traitement des arrets de la fermentation alcoholique. Connaissance Vigne Vin 17, 205–217.

Hammond, J.R.M., 1993. Brewer's yeasts. In: Rose, A.H., Harrison, J.S. (Eds.), The yeasts, Vol. V: Yeast technology. Academic Press Limited, London, 620 pp.

Henry, S.A., 1982. Membrane lipids of yeast: biochemical and genetic study. In: Strathern, J.N., Jones, E.W., Broach, J.R. (Eds.), Molecular biology of the yeast Saccharomyces cerevisiae: Metabolism and gene expression, Cold Spring Harbor Laboratory, pp. 101–158.

Jeffcoat, R., James, A.T., 1984. The regulation of desaturation and elongation of fatty acids in mammals. In: Numa, S. (Ed.), Fatty acid metabolism and its regulation. Elsevier, Amsterdam, pp. 85–112.

Kodama, K., 1993. Sake-Brewing yeasts In: Rose, A.H., Harrison,

J.S. (Eds.), The yeasts, Vol. V: Yeast technology. Academic Press Limited, London, p. 620.

Lafon-Lafourcade, S., 1984. Souches de levures. Bull OIV 637, 185–204.

Lafon-Lafourcade, S., Geneix, C., Ribereau-Gayon, P., 1984. Inhibition of alcoholic fermentation of grape must by fatty acid produced by yeasts and their elimination by yeast ghosts. Appl. Environ. Microbiol. 47, 1246–1249.

Mauricio, J.C., Guijo, S., Ortega, J.M., 1991. Relationship between phospholipid and sterol contents in *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* and their fermentation activity in grape musts. Am. J. Enol. Vitic. 42, 301–308.

Quinn, A.J., Chapman, D., 1980. The dynamics of membrane structure. CRC Crit. Rev. Biochem 8, 1–117.

Quinn, P.J., Joo, F., Vigh, L., 1989. The role of unsaturated lipids in membrane structure and stability. Prog. Biophys. Molec Biol 53, 71–103.

Ratledge, C., Evans, C.T., 1989. Lipids and their metabolism. In: Rose, A.H., Harrison, I.S. (Eds.), The yeasts., Vol. III: Metabolism and Physiology of Yeasts. Academic Press Limited, London, pp. 367–455.

Ravaglia, S., Delfini, C., 1993. Production of MCFA and their ethyl esters by yeast strains isolated from musts and wines. Ital. J. Food. Sci. 1, 21–36.

Rosi, I., Bertuccioli, M., 1992. Influences of lipid addition on fatty acid composition of Saccharomyces cerevisiae and aroma characteristics of experimental wines. J. Inst. Brew. 98, 305–314.

Schweizer, E., 1984. Genetics of fatty acid biosynthesis in yeast. In: Numa, S. (Ed.), Fatty acid metabolism and its regulation. Elsevier, Amsterdam, pp. 59–83.

Stevens, S., Hofmeyer, J.H.S., 1993. Effects of ethanol, octanoic and decanoic acid on fermentation and passive influx of protons through the plasma membrane of *Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol. 38, 656–663.

Suomalainen, H., Lehtonen, M., 1979. The production of aroma compounds by yeast. J Inst Brew 85, 149–156.

Taylor, G.T., Kirsop, B.H., 1977. The origin of the medium-chain length fatty acids present in beer. J. Inst. Brew. 83, 241–243.

Taylor, G.T., Thurston, P.A., Kirsop, B.H., 1979. The influence of lipids derived from malt spent grains on yeast metabolism and fermentation. J. Inst. Brew 85, 219–227.

Thurston, P.A., Taylor, R., Ahvenainen, J., 1981. Effects of linoleic acid supplements on the synthesis by yeast of lipids and acetate esters. J. Inst. Brew. 87, 92–95.

Viegas, C.A., Sa-Correia, I., 1991. Activation of plasma membrane ATPase of *Saccharomyces cerevisiae* by octanoic acid. J. Gen. Microbiol. 137, 645–651.

Viegas, C.A., Sa-Correia, I., Novais, G.M., 1985. Synergistic inhibition of the growth of *Saccharomyces bayanus* by ethanol and octanoic or decanoic acids. Biotechnol. Lett. 7, 611–614.

Viegas, C.A., Rosa, F.M., Sa-Correia, I., Novais, G.M., 1989. Inhibition of yeast growth by octanoic and decanoic acids produced during ethanolic fermentation. Appl. Environ. Microbiol. 55, 21–28.

Wakil, S.J., Stoops, J.K., Joshi, V., 1983. Fatty acid synthesis and its regulation. Ann. Rev. Biochem. 52, 537–579.

Wickerham, L.J., 1951. Technical Bulletin No. 1029, US Department of Agriculture, Washington DC.