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THE UTILISATION OF USED AND OTHER FATS BY FUNGI

BY

SECHABA BAREETSENG

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Sechaba Bareetseng

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University of the Free State

Bloemfontein

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Promoter: Prof. J.L.F. Kock

Co-promoter: Prof. L. Christov

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CHAPTER 1

INTRODUCTION

1.1 MOTIVATION

When micro-organisms are cultivated on plant and animal fats and oils, little change usually occurs in the fatty acid (FA) profile of the accumulated cellular lipids when compared to the substrate. In this case, lipid synthesis as well as desaturation and elongation reactions are terminated by the presence of substrate FAs (Kendrick and Ratledge, 1996). This will have a negative impact on biotechnological processes aimed at biotransforming plant fats and oils to high value polyunsaturated fatty acids (PUFAs).

Recently we described a substantially improved utilisation of sunflower oil by *Mucor circinelloides f. circinelloides* CBS 108.16 in the presence of sodium acetate which was accompanied by a doubling of the biomass production and an enhancement of the intracellular polyunsaturated γ-linolenic acid (GLA) content as compared to growth conditions with sunflower oil as sole carbon source (Jeffery *et al.*, 1997). A biotechnological process utilising acetate as carbon source has also recently been patented for the production of GLA by *Mucor circinelloides f. circinelloides*. GLA is a high value essential FA produced from plants with the potential of being replaced with single cell oils from fungi (Kock and Botha, 1995). GLA, which is a precursor to the vital cellular lipid hormones (prostaglandins, thromboxanes or leukotrienes) in humans, is prescribed for the treatment of eczema. Consequently, the aim of this study became to screen other fungi capable of utilising used and other low cost fats and oils in the presence of sodium acetate and transform these fats and oils to high value lipids such as GLA. The ultimate aim is to pinpoint those taxa that will have

the best potential of transforming plant fats and oils to high value lipids in the presence of acetate. In addition, the general validity of the enhancing effect of acetate on the overall performance of fungi when grown on fats and oils will be investigated.

1.2 EDIBLE FATS AND OILS

According to Ratledge and Wilkinson (1988a), edible fats and oils are defined as lipids, which are sparingly soluble in water but soluble in organic solvents such as chloroform, alcohols and ethers. These compounds are divided into two groups according to their chemical structures namely (i) those with long chain FAs or their immediate derivatives such as alkanes and alkenes (ii) and those derived from isoprene units, and which are usually known as terpenoid lipids. These compounds are produced in large quantities, on commercial scale by plants for the production of high value PUFAs such as GLA. GLA is at present mainly produced from the Evening Primrose plant, *Oenothera biennis* (Cisowski *et al.,* 1993; Redden *et al.,* 1995).

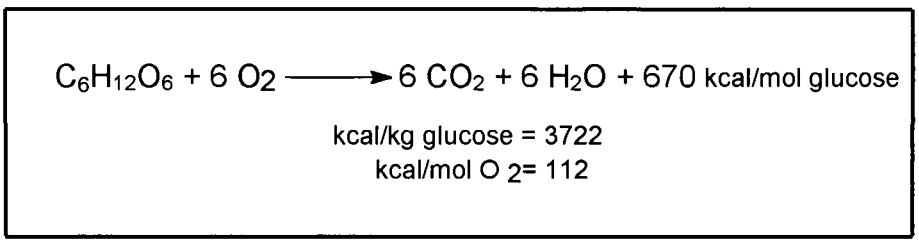
Edible fats and oils are also bulk storage materials produced by plants, animals as well as micro-organisms and contain FA derivatives such as triacylglycerols (TAGs; Fig. 1A), diacylglycerols (DAGs; Fig. 1B), monoacylglycerols (MAGs; Fig 1C), phospholipids (PLs; Fig. 1D) and free fatty acids (FFAs; Fig 1E). These compounds

are classified as fats or oils depending on whether they are liquid or solid at room temperature (Ratledge and Wilkinson, 1988b).

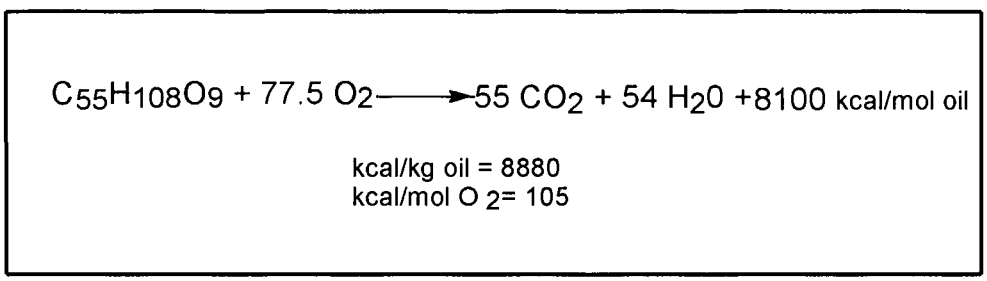
1.2.1 Energy in edible fats and oils

The general reactions for the oxidation of glucose and a typical fat and oil are shown below:

For the oxidation of glucose:



For the oxidation of a typical fat and oil:



From these reactions it is clear that there is substantially more energy produced from fats and oils than from glucose on a weight per weight basis. Consequently, a typical fat and oil contain about 2.4 times the energy of glucose on a weight per weight basis. In addition, fats and oils are also cheaper than glucose. Fats and oils cost about 40% of what sugar cost on an energy basis (Bader *et al.*, 1984). As a

result of this, fats and oils are favoured in biotechnological processes where substrate costs plays an important role in the economics of such a process.

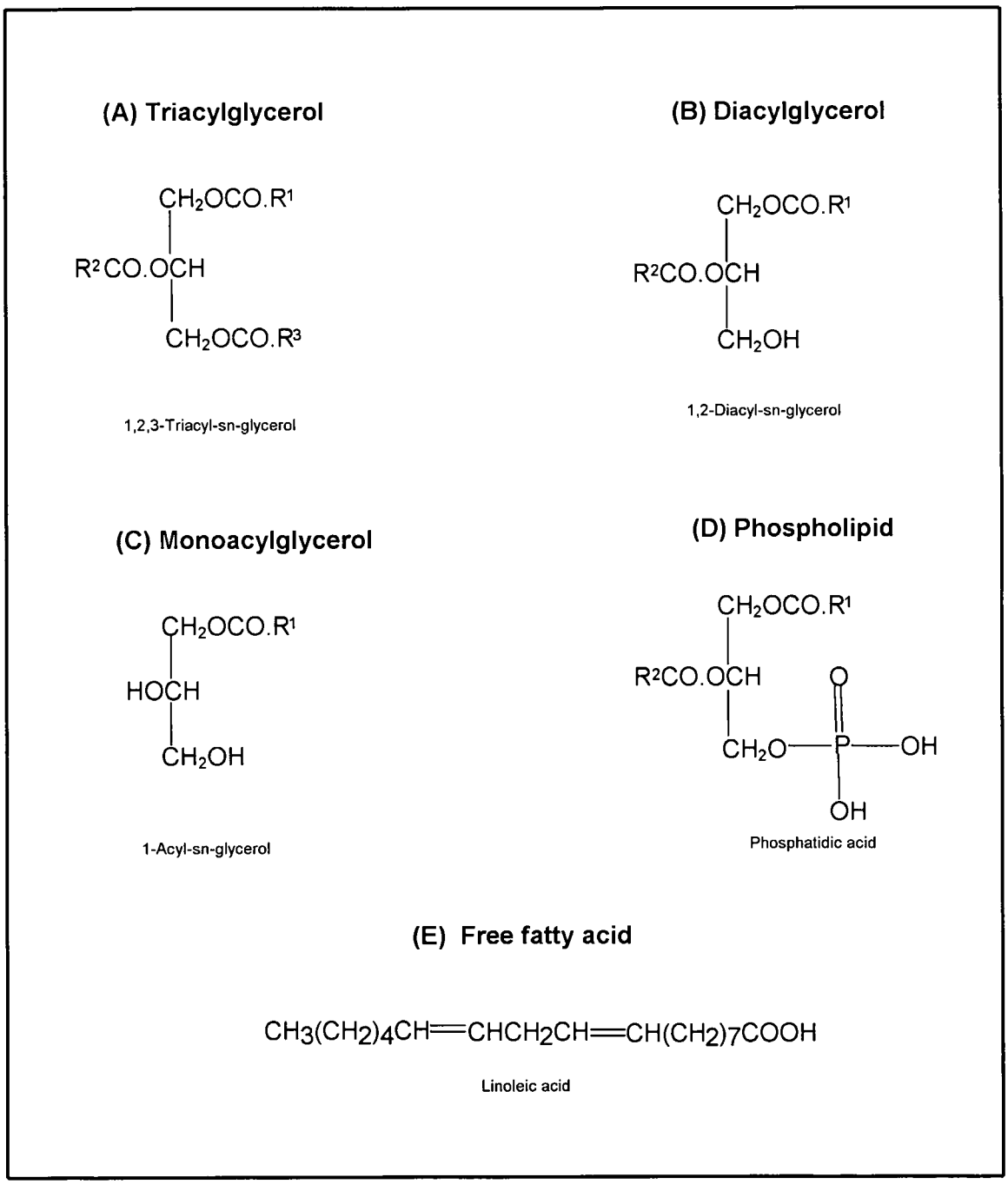


Fig. 1. Structures of FA derivatives. R^1CO-, R^2CO-, R^3CO- represents fatty acyl groups (Ratledge and Wilkinson, 1988b).

1.2.2 Types of fats and oils

Fats and oils are essential nutrients in human and animal diets because they provide the most concentrated form of energy (*ca.* 9 kcal/g). They also provide essential FAs such as linoleic acid (18:2) for the production of lipid hormones such as prostaglandins, serve as carriers for fat-soluble vitamins and provide the feeling of satiety after eating.

Consequently, oil-bearing plants have been cultivated for fat and oil production in the past decades, i.e. rapeseed was cultivated in India 2000 BC, while sesame seed was already known in ancient times. The cultivation of oil-bearing plants for the production of edible fats and oils is increasing considerably even today (Shukla, 1994).

World-wide, edible fats and oils are cultivated for different reasons. For example, 80% of these fats and oils is for human consumption, 6% is for animal feed and 14% is used in the oleochemical industry for the production of mainly soap (Shukla, 1994). Also, about 70% of these fats and oils are derived from oilseed crops (i.e. vegetable oils) and 30% is from animals of which fish oils comprise 2% (Shukla, 1994).

Most of the vegetable fats and oils have large amounts of unsaturated FAs (Table 1). Examples are olive oil which comprises of 71% w/w oleic acid (18:1) and 10%

w/w linoleic acid (18:2) and rapeseed oil comprising of 62% w/w 18:1 and 22% w/w 18:2. Safflower oil on the hand contains only as much as 13% w/w 18:1 and 78% w/w 18:2 and sunflower oil 19% w/w 18:1 and up to 68% w/w 18:2. The animal fats and oils comprise mostly of saturated FAs (no double bond present), e.g. butterfat, beef tallow and lard which comprise of 63% w/w, 46% w/w and 42% w/w saturated FAs respectively (Shukla, 1994). The FAs acyl profiles of these fats and oils are shown in Table 1. From this Table, it is evident that most of the vegetable fats and oils comprise of variable amounts of saturated FAs of which stearic acid (18:0) is present in smaller quantities than palmitic acid (16:0). The most common FAs among both animal and vegetable fats and oils are 16:0 and 18:1.

It is important to mention that the FA compositions summarised in Table 1 may vary according to the strain and climate, e.g. the 18:2 content of corn oil can vary between 35 and 60% and of peanut oil from 20 to 40% (Shukla, 1994).

Another oil of commercial interest is tall oil. This is regarded as non-edible oil. During the kraft pulping of pine wood, the resins in the wood are saponified and dissolved in an alkaline cooking medium to form sodium soaps. At high concentrations, these sodium soaps separate as soap skimmings, which are removed and acidified to yield tall oil (Gunstone *et al.,* 1994).

Tall oil is a mixture of FAs (45%), resin acids (45%) and neutrals (10%). The FAs fraction of tall oil comprises of saturated and unsaturated FAs. The main FAs of tall

oil are 18:1 (approx. 46.0% w/w) and 18:2 (approx. 41.0% w/w). The other FAs are 16:0 (5.0% w/w), 18:0 (3.0% w/w), arachidic acid (20:0) (2.0% w/w) and α18:3 (3.0% w/w) (Gunstone *et al.,* 1994).

The resin acids in tall oil consist of rosins and the neutrals consist of long chain fatty alcohols and sterols. Beta-sitosterol is the major component of the sterols. Tall oil today is produced in large quantities, i.e. about 12 000 tonnes per year in South Africa at R1000/tonne (IOP, personal communication, 1999). Tall oil is used mostly as a varnish and in cosmetic industries.

Table 1. FAs profiles of edible fat and oil sources (%w/w) (Shukla, 1994).

Fat or oil	4:0	6:0	8:0	10:0	12:0	14:0	16:0	18:0	20:0	16:1	18:1	20:1	18:2	18:3	S	M	P
Saturated																	
Beef tallow						3	24	19		4	43		3	1	46	47	4
Butterfat	4	2	1	3	3	11	27	12		2	29		2	1	63	31	3
Cocoa butter							26	35	1		35		3		62	35	3
Coconut oil		1	8	6	47	18	9	3			6		2		92	6	2
Lard						2	26	14		3	44	1	10		42	48	10
Palm kernel oil		1	3	4	48	16	8	3			15		2		83	15	2
Palm oil						1	45	4			40		10		50	40	10
Mono-unsaturated																	
Olive oil							13	3	1	1	71		10	1	17	72	11
Peanut oil							11	2	1		48	2	32		14	50	32
Rapeseed oil							4	2			62		22	10	6	62	32
Polyunsaturated																	
Corn oil							11	2			28		58	1	13	28	59
Cottonseed oil						1	22	3		1	19		54	1	26	20	55
Safflower							7	2			13		78		9	13	78
Soybean							11	4			24		54	7	15	24	61
Sunflower oil							7	5			19		68	1	12	19	69

4:0 = butyric acid; 6:0 = caproic acid; 8:0 = caprylic acid; 10:0 = capric acid; 12:0 = dodecanoic acid; 14:0 = myristic acid; 16:0 = palmitic acid; 16:1 = palmitoleic acid; 18:0 = stearic acid; 18:1 = oleic acid; 18:2 = linoleic acid; α18:3 = alpha-linolenic acid; 20:0 = arachidic acid; 20:1 = eicosenoic acid; S = total saturated fatty acid; M = total mono-unsaturated fatty acid; P = total polyunsaturated fatty acids.

1.2.3 Demand for edible fats and oils

In South Africa about 40 000 tonnes of vegetable fats and oils are sold annually as waste by frying establishments at *ca.* R1000/tonne to the informal sector (Table 2). These waste fats and oils are also re-used by these frying establishments or renderers for animal feed and for the production of low cost oleochemicals (Prof. J.L.F. Kock, personal communication, 1999). In total, 350 000 tonnes of vegetable fats and oils are used per year in the food industry in South Africa (Table 2).

This amount is small when compared to the annual consumption for fats and oils in the world which will increase by 32%, i.e. from 80 million tonnes (MMT) found in 1990 to 105 MMT in the year 2000 (Mielke, 1992).

Table 2. Edible fat and oil consumption and value in South Africa.

Type	Consumption (Tonnes)	Price (R/tonne)
Sunflower oil	155 000	R2596.00
Soya oil	5 000	R2528.00
Groundnut oil	8 000	R2802.00
Cottonseed oil	3 800	R2608.00
*Palmolein and Sunflower oil	138 200	
**Waste fat and oil	40 000	R1000.00
TOTAL	350 000	

*Imported Oil Seed Board, 1996 (Personal Communication).
** Waste fat and oil produced after frying of food.

1.3 FAT AND OIL UTILISATION BY MICRO-ORGANISMS

The exploitation of naturally occurring fats and oils by fungi has been well-studied (Lösel, 1989). It was found that during fungal fat and oil utilisation, extracellular lipases are released for the hydrolysis of TAGs to FFAs and glycerol. This phenomenon was demonstrated by the attack of lipid-rich substrates by lipophilic fungi and the subsequent production of extracellular lipases. Lipases are widely produced in fungi and their production and activity in fungi, when present in sunflower seeds, have been well-studied (Roberts *et al.,* 1987). Lipase activity has been investigated in species such as *Rhizopus*, *Mucor* (Akhtar *et al.,* 1980) and the human and animal pathogen *Geotrichum candidum* (Jensen, 1971). Factors affecting lipase production by *Aspergillus* and *Syncephalastrum* (Chopra and Khuller, 1983) were also investigated. Lipase production by the mushroom species *Agaricus bisporus* and *Agaricus bitorquis* was also demonstrated. Consequently, the fungi can utilise a wide variety of commercially available vegetable fats and oils as carbon sources (Fermor and Wood, 1981).

Several species of thermophilic fungi were isolated which utilise FAs as carbon sources. *Talaromyces emersonii* has shown maximum production of FAs and high lipase activity at incubation temperatures of 40 - 45^{o}C. Investigations, conducted in the degradation of oil-palm products by thermophilic fungi (Ogundero, 1981), showed that 16:0, the major FA in palm oil, is a good carbon source for fungal growth. Lauric acid (12:0) on the other hand was not utilised. It was also shown

that when fungi were grown on stored rapeseed, this resulted in TAG degradation and the formation of FFAs and glycerol. In 1994, it was discovered in our laboratory that *Mucor circinelloides f. circinelloides* CBS 108.16 in the presence of sodium acetate, is able to rapidly emulsify and utilise sunflower oil within seven days of cultivation, while producing more biomass and GLA than when it was grown on only sunflower oil as sole carbon source (Jeffery *et al.*, 1997). This phenomenon was attributed to change in pH (Jeffery *et al.,* 1999). When the pH increased to about seven, the FFAs in the medium formed soaps which in turn affected lipid emulsification and hence lipid utilisation by the fungus.

1.3.1 How are fats and oils utilised by fungi?

When fungi are confronted with fats and oils as carbon sources in a growth medium, the TAGs (Fig.1A) are hydrolysed by fungal lipases to yield DAGs, MAGs, FFAs and glycerol. These hydrolysis products are then taken up by the cell through mainly facilitated and simple diffusion (Finnerty, 1989).

Fats and oils may be supplied to the fungus in the medium as a growth substrate or in the form of fungal stored lipids (TAGs) which can be consumed by the cell during cultivation (Ratledge, 1989). Most fungi produce lipases, especially when challenged with these compounds as substrates in a medium as sole carbon source. In this case, fats and oils are first hydrolysed. This process is catalysed by lipases, also known as long-chain FA ester hydrolases.

Fungal lipases show a broad substrate specificity and are classified into three main types according to their reaction specificity and include non-specific lipases, 1,3-specific lipases and FA specific lipases, as indicated in Fig. 2 (Ratledge, 1989).

At first, non-specific lipases exhibit no specificity to the FA position on the glycerol backbone and as a result catalyse the total hydrolysis of TAGs to DAGs to MAGs and eventually to FFAs and glycerol. Examples of micro-organisms with this kind of lipases are *Candida cylindracae*, *Corynebacterium acnes* and *Staphylococcus aureus*.

The second type, 1,3-regiospecific lipases involve the catalysis reaction at the C-1 and C-3 positions of TAGs to release FFAs, 2,3-diacylglycerols and 2-monoacylglycerols. Examples of fungi exhibiting this type of lipases are *Aspergillus niger*, *Mucor javanicus* and some *Rhizopus* species.

The third type, acyl-group specific lipases, catalyses the removal of a specific FA from a TAG molecule. Example of a micro-organism with this type of lipases is *Geotrichum candidum*.

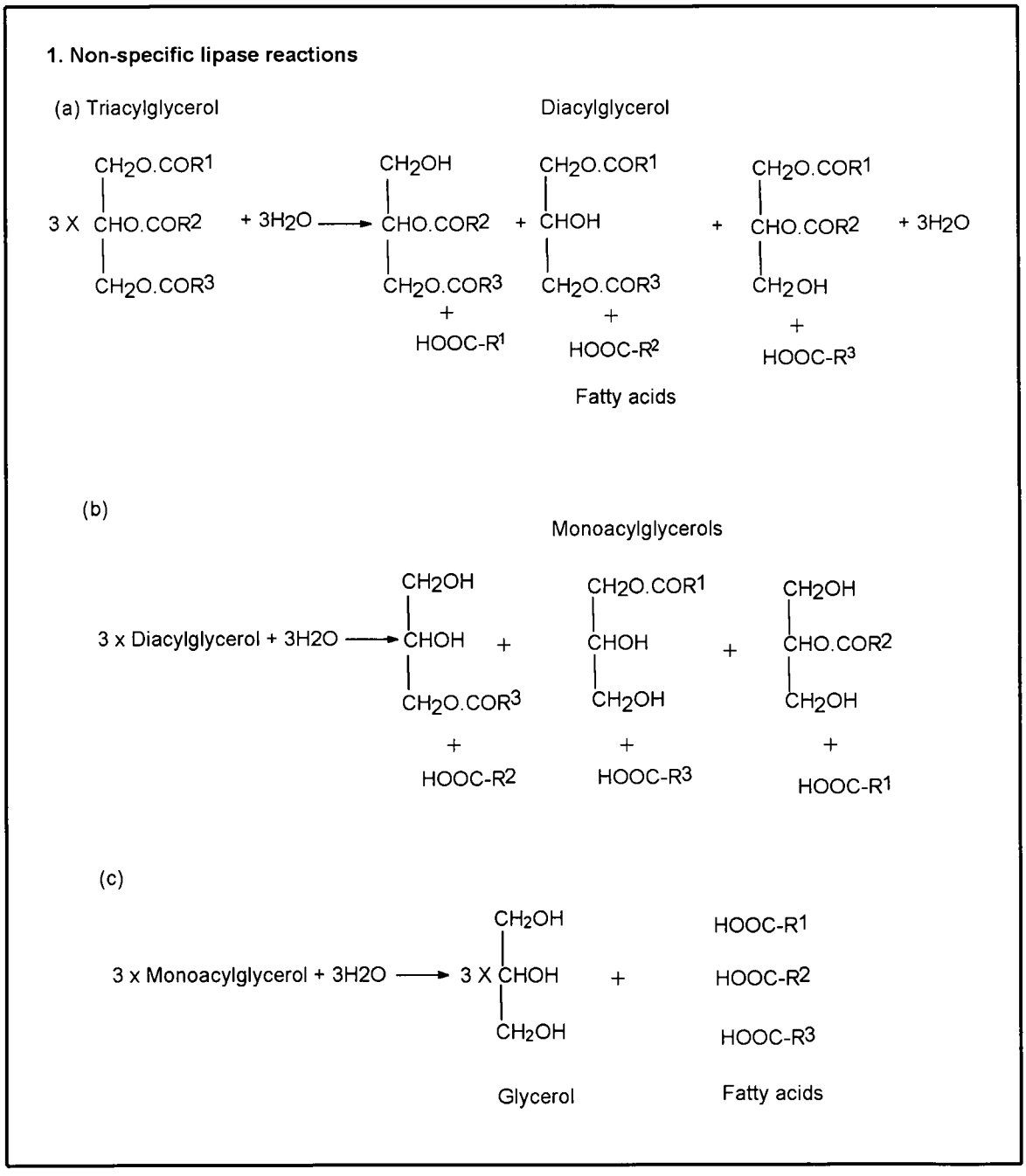


Fig. 2. Fungal lipases (Ratledge, 1989).

Fig. 2. Continued

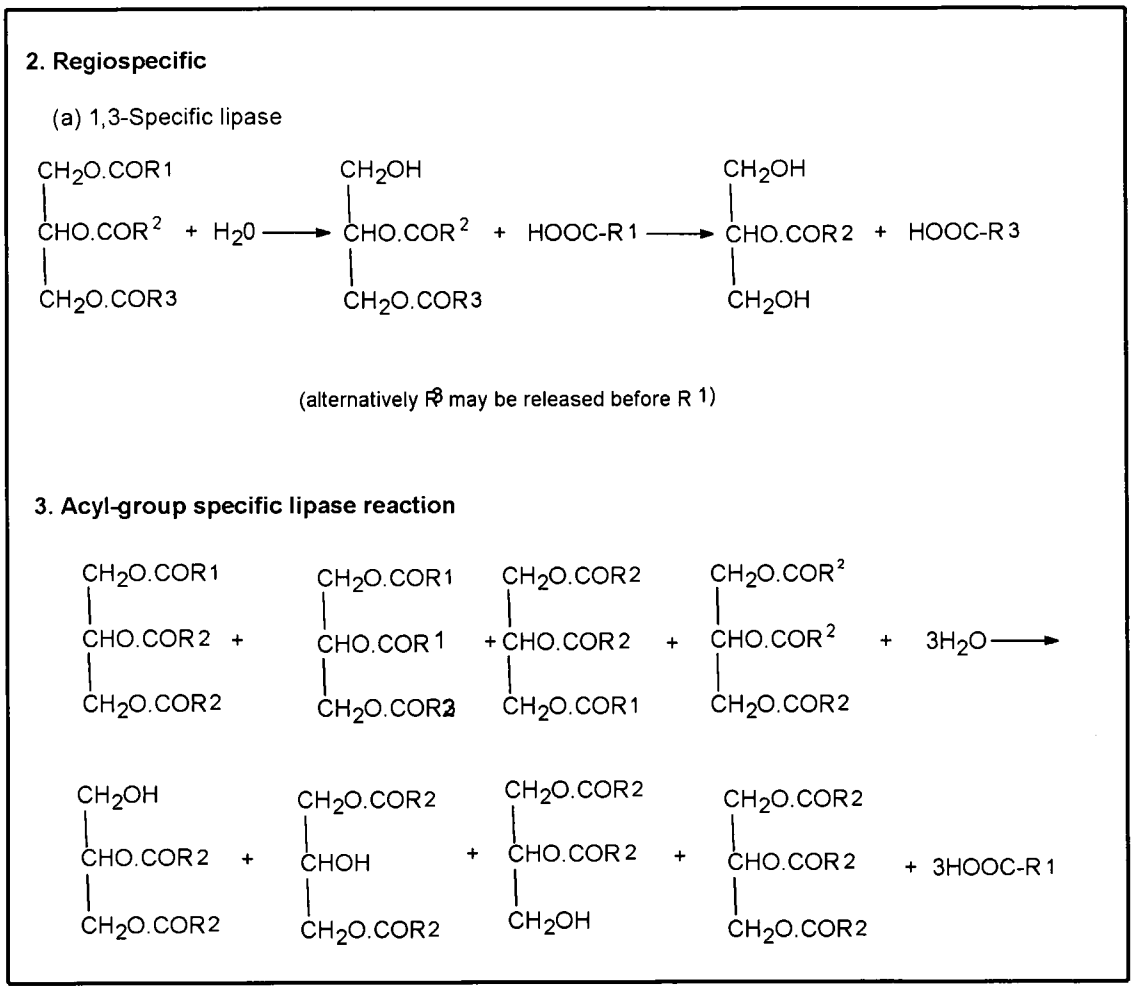


Fig. 2. Fungal lipases (Ratledge, 1989).

1.3.2 Long-chain FAs transport in fungi

The transport of long-chain FAs into fungi has been well investigated (Finnerty, 1989). It was found that long-chain FAs are transported across the cytoplasmic membrane by facilitated and simple diffusion. During transport, the long-chain FAs bind to a protein carrier in the cytoplasmic membrane to facilitate transport across the membrane. Consequently, the long-chain FAs are absorbed and passed through the cytoplasmic membrane to be changed to acyl-CoA esters by acyl-CoA synthetases in the cytoplasm. In this form, the acyl-CoA esters minimise the inhibition of the FFAs on the malic enzyme present in the cytoplasm (Finnerty, 1989). This enzyme is responsible for the fuelling of lipid synthesis and desaturation reactions through NADPH production.

1.3.3 β-Oxidation of fats and oils

Long-chain FAs in the form of acyl-CoA esters in the cytoplasm cannot pass through the mitochondrial membrane. A specific transport system is required. This system works in conjunction with the β-oxidation enzymes needed to initiate the β-oxidation pathway, i.e. epimerase and isomerase. These enzymes are involved in the cyclic 2-carbon shortening of FA acyl-CoA during β-oxidation.

The conversion of long-chain unsaturated FAs to acyl-CoA esters by acyl-CoA synthetase in the cytoplasm represents the activation of FAs (or FFAs) and the first

step in FAs oxidation, which will later take place in the mitochondria (Fig. 3). Acyl-CoA synthetase is a loosely membrane-bound enzyme and is found both inside and outside the mitochondria. Several acyl-CoA synthetases have been described, each specific for FAs of different chain lengths. The fatty acyl-CoA esters pass through the mitochondrial membrane only in combination with the transporter system, carnitine.

After the transport of an acyl moiety through the mitochondrial membrane, 2 carbon atoms are cleaved from the active fatty acyl-CoA ester starting from the β-carbon to form acetyl-CoA. Inside the mitochondrial matrix, two hydrogen atoms are removed from the active fatty acyl-CoA molecule starting from α and β carbon atoms to form Δ^2-trans-enoyl-CoA. This reaction is catalysed by acyl-CoA dehydrogenase. Then the enzyme, Δ^2-enoyl-CoA hydratase catalyses the addition of water to Δ^2-trans-enoyl-CoA to saturate the double bond and eventually convert the latter to 3-hydroxyacyl-CoA. Thereafter, this metabolite undergoes dehydrogenation on the third-carbon atom to form a 3-ketoacyl-CoA molecule. This reaction is catalysed by 3-ketoacyl-CoA dehydrogenase enzyme. Lastly, the enzyme thiolase splits 3-ketoacyl-CoA into acetyl-CoA and Acyl-CoA derivatives. Consequently, the resulting CoA derivatives contain two carbon atoms less than the original acyl-CoA that initially underwent oxidation. Following this, the acyl-CoA molecule is re-cycled back to the oxidative pathway to be completely broken down to acyl-CoA (i.e. C2 units) and further oxidised to CO_2, water and energy. In this way a complete β-oxidation of long-chain FAs is achieved (Mayes, 1990).

β-Oxidation of long-chain unsaturated FAs is similar to that of saturated FAs. The differences between the two pathways reside in the two enzymes namely enoyl-CoA isomerase and 3-hydroxyacyl-CoA epimerase. Mono-unsaturated FAs, i.e. *cis-octadec*-9-enoic acid (18:1) are oxidised to *cis-dodec*-3-enoic acid (12:1) by normal β-oxidation enzymes. The *cis-dodec*-3-enoic acid (12:1) cannot be metabolised by the enzyme acyl-CoA dehydrogenase and is isomerised to *trans*-dodec-2-enoyl-CoA by the enzyme enoyl-CoA isomerase which is a normal substrate for enoyl-CoA hydratase (Finnerty, 1989).

On the other hand, PUFAs, i.e. *cis,cis*-octadec-9,12-dienoic acid (18:2) are β-oxidised to *cis,cis*-dodec-3,6-dienoic acid (12:2). This molecule is isomerised to *trans,cis*-dodec-2,6-dienoic acid by enoyl-CoA isomerase and then β-oxidised to form *cis*-oct-2-enoyl-CoA. This intermediate is in turn converted to D-3-hydroxyoctanoyl-CoA by enoyl-CoA hydratase. The D-3-hydroxyoctanoyl-CoA is not a substrate for the enzyme 3-hydroxyoctanoyl-CoA dehydrogenase because the enzyme only recognises the L-configuration of hydroxylated FAs. However, 3-hydroxyacyl-CoA epimerase converts this substrate to the L-3- hydroxyoctanoyl-CoA allowing the resumption of β-oxidation (Finnerty, 1989).

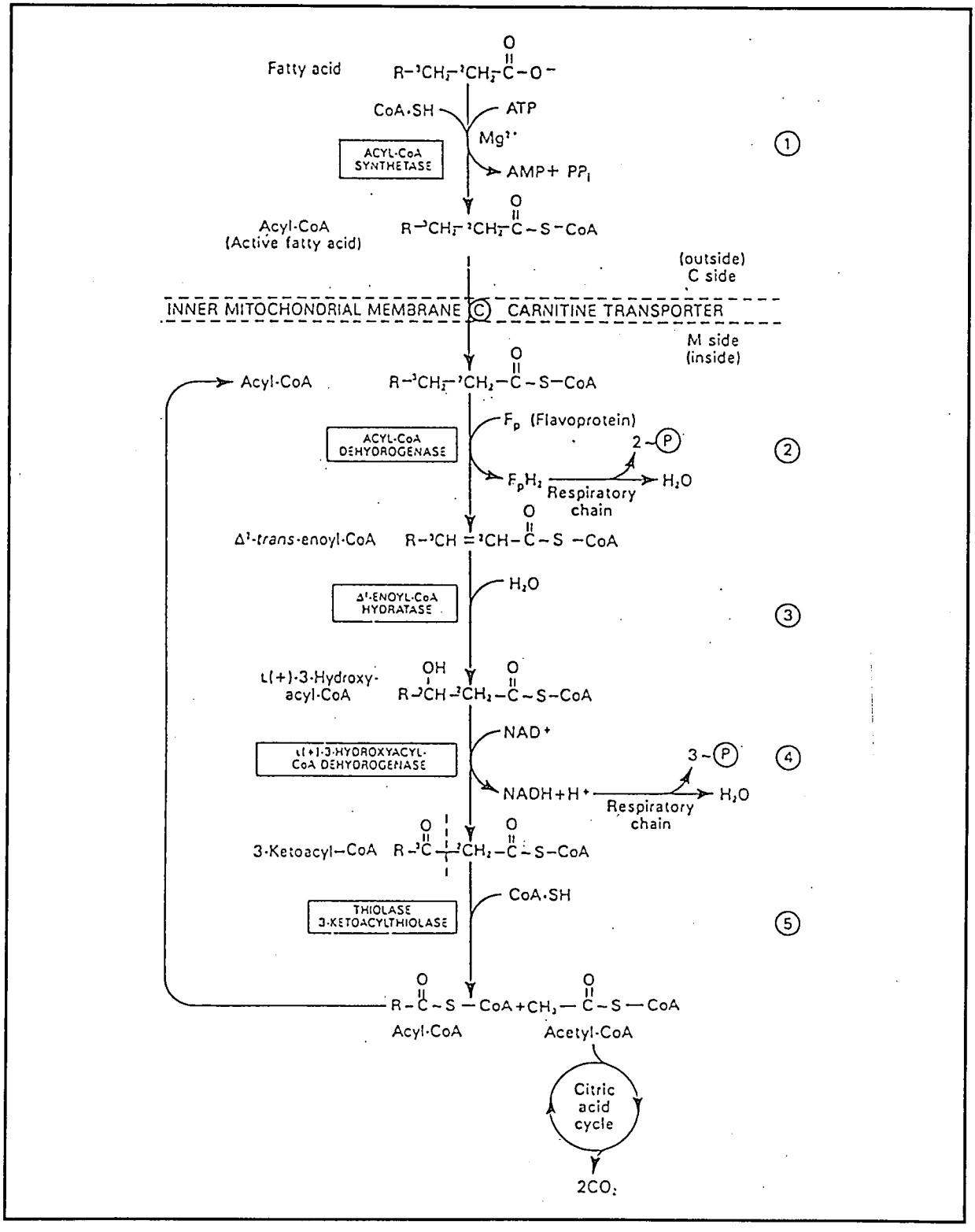


Figure 3. β - Oxidation cycle of long-chain FAs in fungi (Mayes, 1990).

1.3.4 Fungal lipids from fats and oils

Many micro-organisms, i.e. moulds and yeasts are able to grow, accumulate and utilise edible fats and oils as sole carbon sources (Koritala *et al.,* 1987; Ratledge, 1989). Micro-organisms that accumulate more than 20% - 25% of fats and oils within cells are known as oleaginous species (Ratledge, 1991). The micro-organisms that are regarded as oil-bearing include a number of yeast species (Table 3) and moulds (Table 4). The accumulated intracellular lipid occurs in the form of TAGs and can occupy up to *ca.* 85% of the total cell volume. During lipid accumulation, it is important to ensure that the growth medium is nitrogen limited. This causes the cells to accumulate more lipids. The pH of the medium should also be maintained near neutrality for high lipase activity and uptake of the FA anions into the cell (Tan and Gill, 1985a; Tan and Gill, 1985b). However, at a low medium pH, the hydrolysis of the lipid substrate will be poor compared at high pH medium. At high pH the hydrolysis of the TAGs to FFAs increases and hence results in the emulsification of the lipid substrate and increased utilisation by fungi. The FFAs, resulted from the hydrolysis of TAGs, are toxic to the malic enzyme present in the cytoplasm. This in turn results in low NADPH production, which is necessary for lipid synthesis and desaturation (Bell, 1971; Hunkova and Flench, 1977).

In 1989, Ratledge showed that the fat and oil fed to fungi in a growth medium inhibits FA desaturation and elongation. This phenomenon was demonstrated by using the yeast, *Yarrowia lipolytica* cultivated on different fat and oil substrates as

carbon sources. The results of this experiment are shown in Table 5. According to these results, the lipids recovered from the yeast cells were in general similar to the lipids fed at cultivation. However, when this yeast was cultivated on linseed oil as substrate, only 29% 18:3 accumulated inside the cell compared to 54% of this FA present in the original oil substrate (Ratledge, 1989).

In 1996, Kendrick and Ratledge screened four filamentous fungi grown on edible fats and oils and glucose as sole carbon sources (Table 6). They performed this experiment in an attempt to produce higher amounts of PUFAs using micro-organisms. According to the results obtained, the lipid content of the fungi after growth on edible fats and oils was higher than when grown on glucose. Other authors in the literature obtained similar results (Aggelis *et al.*, 1991a; Aggelis *et al.,* 1991b; Ćertick *et al.*, 1997).

Table 7 shows the fatty acyl profiles of the intracellular lipids of fungi grown on edible fats and oils tested in the experiment. All the fungi produced intracellular lipids similar to that of the original oil substrates fed to the fungi. Some major exceptions are 18:1, *Entomophthora exitalis* grown on triolein; 18:1, *Conidiobolus nanodes* and *Mucor circinelloides* grown on sesame oil; 18:1, *Entomophthora exitalis* grown on safflower oil and 18:1, *Conidiobolus nanodes* grown on linseed oil. In contrast to these results, two Japanese groups observed increased PUFA production by the filamentous fungi, *Conidiobolus* spp. and *Mortierella* spp. when grown on edible fats and oils as carbon sources (Kendrick, 1991; Yamada *et al.,*

1992). Aggelis et al (1991a and 1991b) on the other hand, found that when *Mucor circinelloides* CBS 172.27 was grown on sunflower oil, it contained 65% (w/w) cellular lipids and produced 17.4% (w/w) GLA after cultivation.

Furthermore Jeffery et al (1997) reported an increase in GLA production by *Mucor circinelloides f. circinelloides* CBS 108.16 in the cellular lipids when grown on sunflower oil in the presence of sodium acetate.

Table 3. Oleaginous yeast species (Ratledge, 1991).

Yeast species	Maximum cellular lipid content (% w/w)
Candida curvata	58
Candida diddensiae	37
Candida guilliermondi	22
Candida tropicalis	23
Candida sp. 107 (NCYC 911)	42
Cryptococcus albidus	65
Cryptococcus laurentii	32
Cryptococcus neoformans	22
Hansenula ciferri	22
Hansenula saturnus	22
Lipomyces lipofer	64
Lipomyces starkeyi	63
Lipomyces tetrasporus	67
Rhodosporidium toruloides	66
Rhodotorula glutinis	72
Rhodotorula graminis	36
Rhodotorula mucilaginosa	28
Schwanniomyces occidentalis	23
Trichosporon cutaneum	45
Trichosporon pullulans	65
Trigonopsis variabilis	40
Yarrowia lipolytica	36

Table 4. Oleaginous moulds (Ratledge, 1991).

Mucorales	Maximum cellular lipid content (% w/w)
Blakeslea trispora	37
Cunninghamella echinulata	45
Cunninghamella elegans	56
Cunninghamella homothallica	38
Cunninghamella japonica	60
Mortierella isabellina	86
Mortierella pusilla	59
Mortierella vinacea	66
Mucor albo-ater	42
Mucor circinelloides	65
Mucor mucedo	51
Mucor plumbeus	63
Mucor ramanianus	56
Mucor spinosus	47
Rhizopus arrhizus	57
Rhizopus delemar	45
Rhizopus oryzae	57
Zygorhynchus moelleri	40

Table 5. Intracellular fatty acyl composition of *Yarrowia lipolytica* after cultivated on various fats and oils (Ratledge, 1989).

Fat or oil used	Lipid analysed	Relative % (w/w) of fatty acyl groups					
		16:0	**16:1**	**18:0**	**18:1**	**18:2**	**α18:3**
Bonefat	**Fed**	**25**	**3**	**13**	**40**	**13**	**1**
	Recovered	16	6	11	35	32	tr
Corn	**Fed**	**12**	**-**	**2**	**25**	**62**	**0.5**
	Recovered	11	6	2	36	45	-
Linseed	**Fed**	**7**	**-**	**4**	**20**	**15**	**54**
	Recovered	7	3	7	36	18	29
Mixed soapstock	**Fed**	**10**	**tr**	**4**	**41**	**32**	**2**
	Recovered	4	6	2	38	47	2
Olive	**Fed**	**13**	**2**	**3**	**69**	**11**	**1**
	Recovered	12	3	3	70	13	1
Palm	**Fed**	**30**	**7**	**2**	**45**	**11**	**5**
	Recovered	26	7	8	47	10	2
Rapeseed	**Fed**	**7**	**-**	**1**	**56**	**24**	**8**
	Recovered	3	9	1	55	25	7
Soybean	**Fed**	**10**	**-**	**4**	**22**	**56**	**9**
	Recovered	8	-	4	24	58	6

tr. = trace amounts

Table 6. Growth yield and lipid accumulation in filamentous fungi after growth on glucose and on various fats and oils (Kendrick and Ratledge, 1996).

Fungus	Substrate	Growth yield (g dry wt/l)	Cellular lipid content (% w/w dry wt)
Conidiobolus nanodes	Glucose	7.3	26
	Triolein	10.8	44
	Sesame oil	9.1	43
	Safflower oil	3.9	36
	Linseed oil	12.3	42
	Mortierella oil	11.1	40
Entomophthora exitalis	Glucose	6.3	25
	Triolein	6.8	35
	Sesame oil	8.8	36
	Safflower oil	7.1	25
	Linseed oil	5.3	41
	Mortierella oil	6.1	48
Mortierella isabellina	Glucose	8.2	20
	Triolein	8.4	43
	Sesame oil	6.9	46
	Safflower oil	9.7	46
Mucor circinelloides	Glucose	7.1	30
	Triolein	4.8	38
	Sesame oil	4.1	42
	Safflower oil	6.4	35

Table 7. Fatty acyl profiles of total lipid extracted from four filamentous fungi after growth on various fats and oils compared to the fatty acyl profile of the original fat or oil (Kendrick and Ratledge, 1996).

	Relative % (w/w) fatty acyl groups														
Fungus	**14:0**	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**α18:3**	**γ18:3**	**20:0**	**20:1**	**20:2**	**20:3**	**20:4**	**20:5**	**22:6**
Original fat or oil (Triolein)	**2.5**	**5.6**	**6.4**	**1.8**	**68.1**	**11.1**	**1.2**	**1.7**	**0.5**	**0.5**	**0.6**	**-**	**-**	**-**	**-**
Conidiobolus nanodes	1.0	8.9	3.6	1.1	50.6	6.0	tr.	1.0	tr	14.0	2.1	1.4	2.4	tr	1.4
Entomophthora exitalis	2.1	5.1	0.9	1.1	42.2	31.3	1.3	1.9	-	7.5	4.0	tr	0.6	tr	tr
Mortierella isabellina	2.1	5.8	6.2	1.6	68.2	10.9	0.9	0.5	1.4	tr	tr	tr	tr	-	-
Mucor circinelloides	2.4	5.2	5.8	0.6	60.3	15.1	-	7.8	-	-	-	-	-	-	-
Original fat or oil (Sesame)	**tr**	**9.1**	**tr**	**4.9**	**41.5**	**42.8**	**tr**	**0.7**	**tr**	**tr**	**-**	**-**	**-**	**-**	**-**
Conidiobolus nanodes	tr	12.8	0.6	2.1	16.3	47.2	2.0	0.6	tr	1.9	4.7	1.1	5.8	1.1	2.2
Entomophthora exitalis	1.1	9.4	tr	tr	45.7	37.8	0.8	0.3	-	0.6	0.5	tr	1.1	1.5	0.7
Mortierella isabellina	tr	9.9	tr	6.0	39.6	42.3	-	0.9	0.7	tr	-	tr	tr	-	-
Mucor circinelloides	tr	16.2	1.1	0.7	25.4	49.0	-	2.6	-	-	-	-	-	-	-
Original fat or oil (Safflower)	**2.3**	**4.2**	**tr**	**0.7**	**13.3**	**76.8**	**0.8**	**-**	**tr**	**tr**	**tr**	**-**	**-**	**-**	**-**
Conidiobolus nanodes	0.5	11.7	0.7	2.1	29.7	26.6	2.1	tr	tr	6.5	7.2	2.3	5.7	1.4	2.6
Entomophthora exitalis	2.8	14.6	0.5	tr	1.4	72.6	0.8	tr	-	0.5	0.7	tr	2.2	1.0	2.8
Mortierella isabellina	tr	8.1	tr	3.5	18.5	65.1	1.8	1.4	0.5	tr	-	tr	tr	-	-
Mucor circinelloides	tr	16.2	4.9	0.7	14.8	61.9	-	1.1	-	-	-	-	-	-	-
Original fat or oil (Linseed)	**tr**	**9.0**	**0.1**	**3.3**	**20.8**	**14.6**	**51.5**	**tr**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Conidiobolus nanodes	tr	7.9	tr	3.0	14.7	10.6	38.9	3.7	3.7	1.7	tr	0.9	10.9	3.0	0.7
Entomophthora exitalis	1.3	7.6	0.7	2.6	27.1	14.0	40.8	1.2	-	0.8	tr	0.5	1.5	0.5	1.8
Original fat or oil (Mortierella)	**0.5**	**22.8**	**0.9**	**4.1**	**38.0**	**20.3**	**0.7**	**9.8**	**-**	**-**	**-**	**1.1**	**1.8**	**-**	**-**
Conidiobolus nanodes	0.5	17.5	0.6	3.6	29.5	14.2	0.5	7.4	0.5	2.3	1.8	tr	18.8	tr	2.4
Entomophthora exitalis	0.8	13.8	0.9	2.7	39.0	14.0	0.6	6.2	-	tr	2.1	2.3	13.7	tr	2.7

tr. = trace amounts

1.4 EFFECT OF FAs ON THE FUNGAL MALIC ENZYME ACTIVITY

When *Entomophthora exitalis* and *Mucor circinelloides* were grown in the presence of edible fats and oils (Table 8) (Kendrick, 1991) as carbon sources as opposed to growth on glucose, it was found that the malic enzyme activity in the cytoplasm has decreased significantly.

Table 8. Effect of FAs on the fungal malic enzyme activity (Kendrick, 1991).

Fungus	Carbon source	Malic enzyme activity (nmol/min/mg)
Entomophthora exitalis	Glucose	40.4
	Safflower	0
	Sesame oil	0
	Triolein	9.9
Mucor circinelloides	Glucose	54.3
	Safflower	6.9
	Sesame oil	0
	Triolein	16.9

The malic enzyme also known as malate dehydrogenase [(decarboxylating (NADP+)] is a membrane-bound enzyme. It catalyses the reaction: malate + NADP+ $\rightarrow$ pyruvate + CO2 + NADPH (Kendrick and Ratledge, 1992a). The generation of NADPH enhances FA desaturation reactions by desaturase enzymes associated with cytochrome b5 in the phospholipid membranes. When fungi are grown in the

presence of fat or oil as substrate, the malic enzyme activity becomes inhibited resulting in low NADPH. Under low NADPH conditions, lipid synthesis as well as desaturation and elongating reactions are inhibited in fungi. In this case, the fungi may rather accumulate the FAs without any modifications.

1.5 VALUE OF UTILISING EDIBLE FATS AND OILS BY MICRO-ORGANISMS

According to literature, the FA profiles of fungal cellular lipids after growth on fats and oils are usually similar to those of the original oil substrate fed to the fungi (Bati *et al.,* 1984; Tan and Gill, 1985a; Koritala *et al.,* 1987; Kendrick, 1991). Therefore, the possibility of utilising edible fats and oils for the production of high value PUFAs such as GLA, arachidonic acid (20:4), eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6) seems to be limited (Ratledge, 1994). However, exceptions are found in *Mortierella*. These fungi are capable of accumulating 20:5 when cultivated on medium rich in α18:3. *Mortierella alpina* was found to convert 5.1% (w/w) of α18:3 present in linseed oil to 1.35 g/l (41.5 mg/g dry mycelium) 20:5 when incubated at room temperature. It was also shown that when species of *Conidiobolus* are grown on edible fats and oils as carbon sources these fungi are capable of producing high value PUFAs (Kendrick and Ratledge, 1996).

Also, biosurfactants could be produced from edible fats and oils using micro-organisms (Fiechter, 1992). It was reported that antibiotics could be produced using micro-organisms on these substrates (Bader *et al.,* 1984).

1.6 PURPOSE OF RESEARCH

With this as background, the purpose of the research became to determine the possibility of converting used and other fats and oils by fungi in the presence and absence of acetate to high value lipids such as GLA.

In order to do this, selected members (some only distantly related) of the Zygomycota and Dikaryomycota were screened for their ability to utilise sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings in the presence and absence of acetate. These data should expose those taxa having the best potential of transforming edible fats and oils to high value lipids in the presence and absence of acetate.

It is also an aim of this study to investigate the general validity of the findings of Jeffery et al (1997; 1999). These authors discovered that the addition of acetate to a growth medium containing only fats and oils as carbon source enhances fat and oil utilisation, biomass production and GLA production by *Mucor circinelloides f. circinelloides* CBS 108.16.

CHAPTER 2

EXPERIMENTAL

2.1 Fungal strains studied: Twenty fungi were screened for their ability to utilise the different fat and oil substrates in the presence and absence of acetate. These fungi include ten Zygomycotan fungi and ten Dikaryomycotan fungi (yeasts) (Table 1). *Mortierella alpina* MUFS Mo058 and *Lipomyces starkeyi* CBS 1807 T did not show any growth on any of the fats and oils tested in the study. All the CBS strains were obtained from the Centraalbureau voor Schimmelcultures in Baarn, The Netherlands, and the rest of the strains were from the culture collection of the Department of Microbiology and Biochemistry, University of the Free State, Bloemfontein, South Africa.

Table 1. Strains screened for fat and oil utilisation in the presence and absence of acetate.

ZYGOMYCOTA (moulds)		**DIKARYOMYCOTA (Yeasts)**	
Organism	Culture no.	Organism	Culture no.
Absidia	MUFS 200	*Cryptococcus curvatus*	CBS 0570 T
Actinomucor elegans	MUFS SAS218	*Dipodascopsis uninucleata var. uninucleata*	CBS 0190.37 T
Cunninghamella	MUFS Cu001	*Filobasidiella neoformans var. neoformans*	CBS 0132
Gongronella	MUFS Go001	*Galactomyces geotrichum*	CBS 0772.71 T
Mortierella alpina	MUFS Mo058	*Kluyveromyces marxianus var. marxianus*	CBS 1556
Mucor circinelloides f. circinelloides	CBS 108.16	*Lipomyces starkeyi*	CBS 1807 T
Mucor circinelloides f. circinelloides	MUFS SAS045	*Saccharomyces cerevisiae*	CBS 1171 NT
Rhizomucor pusillus	MUFS Rm001	*Schizosaccharomyces pombe var. pombe*	CBS 0356 T
Rhizopus stolonifer	MUFS R008	*Schwanniomyces occidentalis var. occidentalis*	CBS 2863
Thamnostylum	MUFS SAS025	*Yarrowia lipolytica*	CBS 0599

MUFS: Culture collection, University of the Free State, Bloemfontein, South Africa.

CBS: Centraalbureau voor Schimmelcultures, The Netherlands.

2.2 Medium:. The complex medium consisted of the following in (g/l): fat or oil, 30; sodium acetate, 10.0; yeast extract, 0.1; $MgSO_4.7H_2O$, 0.25; K_2HPO_4, 10.0; $CaCl_2.2H_2O$, 0.05; NH_4Cl, 1.28. These ingredients (except sodium acetate and K_2HPO_4) were dissolved in 800ml distilled water containing the following trace elements: $FeSO_4.7H_2O$, 0.035; $MnSO_4.4H_2O$, 0.007; $ZnSO_4.7H_2O$, 0.011; $CuSO_4.5H_2O$, 0.001; $CoCl_2.6H_2O$, 0.002; $Na_2MoO_4.2H_2O$, 0.0013; H_3BO_3, 0.002; KI, 0.00035; $Al_2(SO_4)_3$, 0.0005. The pH was adjusted to 5.8 with 2 N HCl and then autoclaved. 10.0g Sodium acetate as well as 10.0g K_2HPO_4 were prepared separately and dissolved in 100ml tap water containing the above trace elements. The pH was set to 5.8 and then autoclaved. After autoclaving, all the media were cooled to room temperature. Sodium acetate, K_2HPO_4 and the above medium were mixed together aseptically in one container. As control (Fig. 1), the same medium was prepared with the exception of sodium acetate. In this case, 40g/l fat or oil was added as sole carbon source. Fats and oils tested in the study, i.e. sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings were autoclaved separately in the flasks before addition of the medium and inoculation with the organism.

2.3 Growth and harvesting (Fig.1): Twenty fungi tested in this study were transferred from 4 day old YM medium in petridishes (yeast malt-agar medium; incubated at 25°C) into 10ml sterile medium (as described in 2.2) present in 100ml conical flasks. All the experiments were performed at least in duplicate. The fungi were incubated at 30°C for seven days (stationary phase) while shaking at 160r/min. After cultivation, the cells were harvested by filtration (Whatman no. 1

filter) washed extensively with distilled water and chloroform as described by Kendrick and Ratledge (1996), immediately frozen, freeze-dried and weighed. The pH of the medium was determined before harvesting commenced.

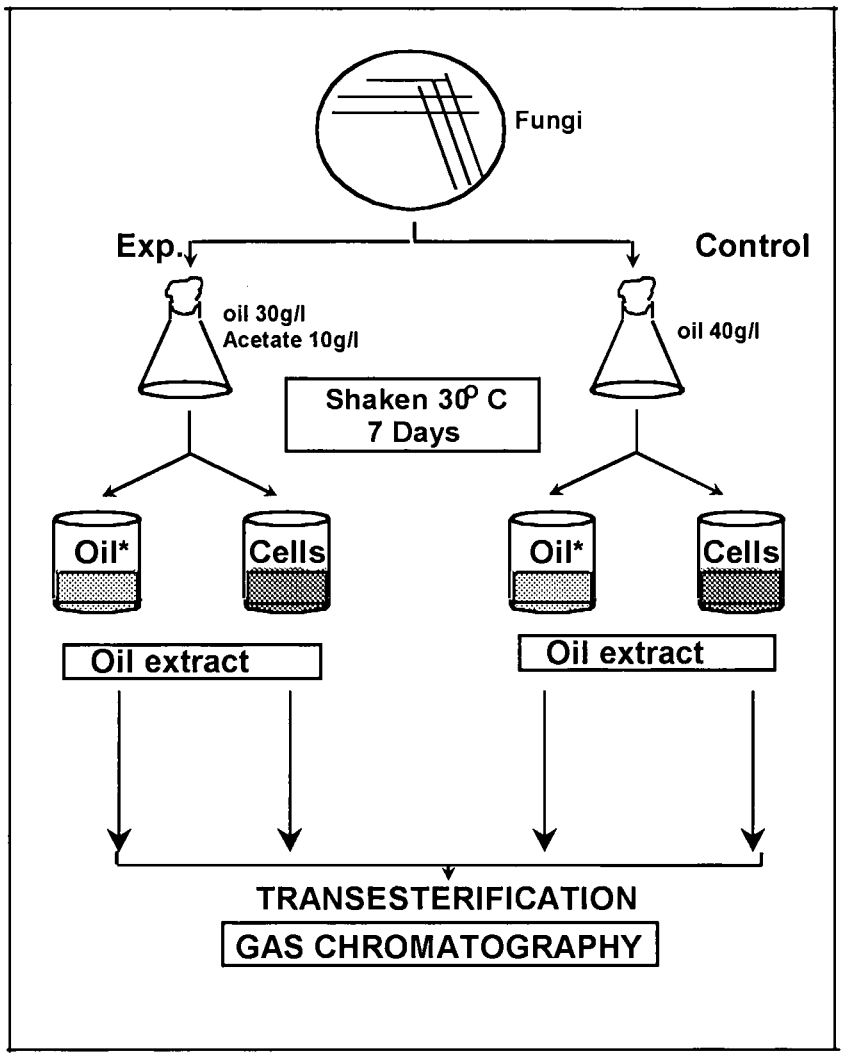


Fig. 1. Experimental procedure. * Oil extracted from supernatant

2.4 Extraction of lipids:

Supernatant: In all experiments the corresponding supernatants were first acidified to pH 4 with 1 N HCl before lipids were extracted with 4 volumes (50ml each) hexane. These were then evaporated to dryness and dried to constant weight over P_2O_5 in a vacuum oven at 55°C and then weighed.

Biomass: Lipids were extracted from the freeze-dried cells as described by Kendrick and Ratledge (1992b). These include extraction with chloroform/methanol (2:1 v/v) according to Folch et al (1957). In short, the biomass was weighed, crushed and homogenised with 2:1 chloroform/methanol mixture (v/v). The homogenate was filtered through a Whatman no. 1 filter paper into preweighed vials and the organic phase evaporated under vacuum. Before the lipids were weighed, samples were dried to constant weight in a vacuum oven over P_2O_5 at 55°C. Samples were stored at -20°C under a blanket of N_2.

2.5 Fatty acid analysis: Intracellular and extracellular lipids were dissolved in chloroform and transesterified by the addition of trimethylsulphonium hydroxide (TMSH) as described by Butte (1983). The fatty acid methyl esters were analysed by gas chromatography (GC) with a flame ionisation detector and supelcowax 10 capillary column (30 m x 0.75 mm). The initial column temperature of 145°C was increased by 3°C/min to 225°C and, following a 10min isothermal period, then increased to 240°C at the same rate. The inlet and detector temperatures were 170

^{o}C and 250^{o}C, respectively. Nitrogen was used as carrier gas at 5ml/min. Peaks were identified by reference to authentic standards.

2.6 Hexane extraction: The intracellular lipids were dissolved in 5ml of hexane and left overnight to extract the neutral lipids (Kim and Norman, 1990). The hexane mixture (hexane and fat or oil) was transferred to pre-weighed vials, dried under nitrogen and weighed.

2.7 Chemicals: All organic chemicals and solvents used were of analytical reagent grade and obtained from major retailers. Fatty acid standards were obtained from Sigma.

2.8 Fats and oils: Sunflower oil and linseed oil were obtained from reputable oil producers in South Africa and were 99% pure (i.e. consist of 99% triglycerides). The used cooking oils were obtained from frying establishments and contained only 80% triglycerides and 20% breakdown products (Dr. D.J. Coetzee, personal communication, 1998). Tall oil (i.e. mixture of fatty acids, resin acids and neutral substances) and soap skimmings were obtained from Sappi mills, Tugela.

CHAPTER 3

RESULTS AND DISCUSSION

In 1997, Jeffery and co-workers discovered that when *Mucor circinelloides f. circinelloides* CBS 108.16 was cultivated on 30g/l sunflower oil and 10g/l sodium acetate, an improved utilisation of the fat or oil, doubling of the biomass production and enhancement of the intracellular polyunsaturated γ-linolenic acid (GLA) content occurred as compared to when this fungus was cultivated on only 40g/l sunflower oil as sole carbon source. Consequently, the aim of this study became to further explore this phenomenon (hypothesis) in selected members of the zygomycotan fungi as well as yeasts when cultivated on various fat and oil substrates in the presence and absence of acetate. The ultimate aim is to identify those taxa that can be further explored for the transformation of edible and tall oils to high value lipids in the presence and absence of acetate.

***Absidia* MUFS 200**

When comparing the growth yields, lipid accumulation, utilisation and the final pH of the growth medium in the presence and absence of acetate, the following results were observed after seven days of growth (Table 1).

Biomass: The fungus produced similar low amounts of biomass (only slight increase) when grown on tall oil (from 4.0g/l to 5.0g/l) and soap skimmings (from 4.0g/l to 4.5g/l) in the absence or presence of acetate. The highest cell yield was obtained when grown on linseed oil in the absence of acetate, i.e. 24.5g/l. When grown on sunflower oil as well as used cooking oil, the addition of acetate to the

growth medium had a significant enhancing effect on biomass production. The biomass increased from 4.0g/l to 14.0g/l on sunflower oil and from 3.0g/l to 13.5g/l on used cooking oil when acetate was added. These results are in accordance with that found by Jeffery et al (1997).

Cellular lipids: The highest amounts of cellular lipids were obtained when the fungus was grown on sunflower oil (52.2% w/w) and on used cooking oil (42.6% w/w) in the presence of acetate. In both cases, the addition of acetate led to a significant increase in lipid content, i.e. from 37.0% w/w to 52.2% w/w on sunflower oil and from 23.4% w/w to 42.6% w/w on used cooking oil. When grown on the other fats and oils, the lipid content remained similar except in the case of tall oil where the lipid content decreased slightly, i.e. from 17.4% w/w to 13.5% w/w when acetate was added. The lipid content of strains of *Absidia* when cultivated on glucose as sole carbon source under static conditions was found to be lower compared to when grown on sunflower and used cooking oils in the presence of acetate (Ratledge, 1989). In this case, *Absidia corymbifera* contained 27% w/w intracellular lipids while *Absidia spinosa* accumulated 28% w/w lipids when cultivated on glucose (Ratledge, 1989).

Residual lipids: Linseed oil was utilised the most after seven days of incubation, i.e. 98.8% and 98.7% in the absence and presence of acetate respectively. In this case, the presence of acetate showed almost no influence on the extend of lipid utilisation. Sunflower and used cooking oils were utilised to a greater extend in the

presence of acetate, i.e. 65.3% and 62.0% respectively. Enhanced utilisation of the latter fats and oils, is probably contributed to the production of biosurfactants (Fiechter, 1992) and to the rise in pH (Jeffery *et al.,* 1999). This phenomenon should be further investigated. Tall oil on the other hand, was poorly utilised in the presence and absence of acetate, i.e. 6.7% and 31.8% respectively. This is probably due to the presence of non-fatty acid compounds such as neutral substances (Gunstone *et al.,* 1994) that resulted in poor growth.

Interesting results were obtained when the fatty acyl profiles of the initial oil substrate and residual lipids present in the media after seven days of incubation were compared (Table 2). In the presence and absence of acetate, a significant decrease in the polyunsaturated fatty acids (PUFAs), i.e. 18:2 and 18:3 occurred in the residual oil fraction of all substrates tested. This probably indicates a preference of this fungus towards the utilisation of PUFAs. In most cases (except tall oil) an enhanced utilisation of 18:2 was observed in the presence of acetate. This is similar to the results of Jeffery et al (1999). All of the α18:3 was utilised in linseed oil, used cooking oil, tall oil and soap skimmings in the presence and absence of acetate while an increase in the saturated FAs, i.e. 16:0 and 18:0 was experienced in most cases when compared to the original oil substrate.

Fatty acyl profiles of the cellular lipids extracted from this fungi after growth on various fats and oils were determined in the presence and absence of acetate and compared to the fatty acyl profiles of the original oil substrate (Table 3).

In most cases, a decrease in PUFAs was experienced in the cellular lipids when compared to the original oils while an increase in saturated cellular FAs occurred. These results are in accordance to the results found by Jeffery et al (1999). From these results it is clear that PUFAs, which were preferentially utilised, are probably rather metabolised for energy and incorporated to a lesser extend into the cellular lipids of this fungus. In the presence of acetate, the high value lipid, i.e. GLA was produced more when sunflower oil was used as substrate while on used cooking oil, a slight decrease on GLA production occurred in the presence of acetate, i.e. from 2.5% w/w to 2.2% w/w.

Table 1. Growth yield, lipid accumulation, utilisation and the final pH by *Absidia* MUFS 200 grown on mixed substrates (oil and acetate) and substrate alone (oil).

Substrate	Growth yield (g dry wt/l)	Cellular lipid content (% w/w dry wt)	Residual lipids (g/l)	pH
Sunflower oil (40g/l)	4.0	37.0	14.6	4.0
Sunflower oil (30g/l) + Sodium acetate (10g/l)	14.0	52.2	10.4	5.7
Linseed oil (40g/l)	24.5	6.9	0.5	4.5
Linseed oil (30g/l) + Sodium acetate (10g/l)	21.5	6.0	0.4	5.5
Used cooking oil (40g/l)	3.0	23.4	20.6	3.5
Used cooking oil (30g/l) + Sodium acetate (10g/l)	13.5	42.6	11.4	5.6
Tall oil (40g/l)	4.0	17.4	27.3	5.7
Tall oil (30g/l) + Sodium acetate (10g/l)	5.0	13.5	28.0	6.2
Soap skimmings (40g/l)	4.0	21.5	13.6	7.3
Soap skimmings (30g/l) + Sodium acetate (10g/l)	4.5	20.0	11.6	6.7

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases.

Table 2. Fatty acyl profiles of the residual lipids of *Absidia* MUFS 200 grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs / Total FAs**
Sunflower oil (40g/l)	15.0	0.0	8.8	26.7	4.8	0.0	0.0	0.09
Sunflower oil (30g/l) + Sodium acetate (10g/l)	17.7	0.0	15.7	31.9	3.3	0.0	0.0	0.05
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	20.3	0.0	11.1	33.9	6.2	0.0	0.0	0.09
Linseed oil (30g/l) + Sodium acetate (10g/l)	22.4	0.0	16.8	28.7	4.0	0.0	0.0	0.06
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	12.4	0.0	5.9	48.3	7.6	0.0	0.0	0.10
Used cooking oil (30g/l) + Sodium acetate (10g/l)	10.1	0.0	8.7	57.1	1.9	0.0	0.0	0.02
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	9.3	0.0	1.6	29.8	15.9	0.0	0.0	0.28
Tall oil (30g/l) + Sodium acetate (10g/l)	8.2	0.0	1.9	30.1	16.3	0.0	0.0	0.29
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	8.4	0.0	1.4	31.1	16.2	0.0	0.0	0.28
Soap skimmings (30g/l) + Sodium acetate (10g/l)	10.0	0.0	2.1	35.2	12.9	0.0	0.0	0.21
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ).

Table 3. Fatty acyl profiles of cellular lipids and GLA production by *Absidia* MUFS 200 grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs / Total FAs**
Sunflower oil (40g/l)	9.7	1.1	3.6	21.4	47.8	1.4	0.0	0.58
Sunflower oil (30g/l) + Sodium acetate (10g/l)	11.1	0.5	9.8	22.6	47.2	1.9	0.0	0.53
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	14.3	0.0	11.1	29.7	4.9	0.0	0.0	0.08
Linseed oil (30g/l) + Sodium acetate (10g/l)	13.9	0.0	11.2	29.3	7.8	0.0	0.0	0.13
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	8.2	0.0	3.2	32.1	24.1	2.5	0.0	0.38
Used cooking oil (30g/l) + Sodium acetate (10g/l)	9.1	1.2	3.0	35.6	37.9	2.2	2.8	0.47
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	6.3	0.0	2.9	20.3	11.1	0.0	0.0	0.27
Tall oil (30g/l) + Sodium acetate (10g/l)	6.4	0.0	3.1	15.9	9.7	0.0	0.0	0.28
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	17.6	0.0	5.3	23.9	0.0	0.0	0.0	0.0
Soap skimmings (30g/l) + Sodium acetate (10g/l)	19.6	0.0	5.4	27.2	0.0	0.0	0.0	0.0
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ).

Actinomucor elegans MUFS SAS218

When comparing the growth yields, lipid accumulation, utilisation and the final pH of the growth medium in the presence and absence of acetate, the following results were obtained after seven days of growth (Table 4).

Biomass: The highest amounts of biomass were obtained when the fungus was cultivated on linseed oil in the presence or absence of acetate, i.e. 30.5g/l. Here, the addition of acetate had no significant effect on biomass production. On tall oil, a small increase in biomass production occurred in the presence of acetate. On soap skimmings on the other hand, the fungus produced similar low amounts of biomass in the presence or absence of acetate. A drastic increase in biomass production was found when the fungus was grown on sunflower and used cooking oils in the presence of acetate. Biomass production increased from 3.5g/l to 18.5g/l on sunflower oil and from 3.0g/l to 17.5g/l on used cooking oil when acetate was added to the growth medium. In both cases, the pH increased to almost neutral in the presence of acetate. These results are in accordance to that found by Jeffery et al (1999).

Cellular lipids: The highest lipid yield was obtained on sunflower oil (43.6% w/w), used cooking oil (60.0% w/w) and tall oil (45.2% w/w) in the presence of acetate. Here, the cellular lipid content increased from 31.4% w/w to 43.6% w/w on sunflower oil, and from 48.0% w/w to 60.0% w/w on used cooking oil. On tall oil, the fungus

produced 45.2% w/w cellular lipids in the presence of acetate and much less (26.0% w/w) in its absence. On the other hand, when the fungus was cultivated on linseed oil in the presence or absence of acetate, similar low amounts of cellular lipids were produced, i.e. 8.7% w/w while a decrease in cellular lipids was experienced on soap skimmings in the presence of acetate, i.e. from 68.0% w/w to 57.0% w/w. According to Botha et al (1995), the lipid content of the same fungus when grown on 10g/l sodium acetate as sole carbon source was found to be much lower, i.e. 6.4% w/w after 72 h of growth.

Residual lipids: Linseed oil was utilised to a similar extend in the presence or absence of acetate. On the other hand, sunflower and used cooking oils were utilised more effectively in the presence of acetate after seven days of growth, i.e. 95.0% and 96.7% respectively. In both cases, the pH increased to almost neutral which may be the cause for enhanced lipid utilisation (Jeffery *et al.,* 1999). When the pH of the medium increases due to the utilisation of acetic acid, the free fatty acids (FFAs) produced as a result of the hydrolysis of triglycerides are transformed to soaps. The latter acts as emulsifier that renders these lipids more soluble in the medium. These fats and oils can then be utilised more efficiently by fungi (Jeffery *et al.,* 1999). A similar trend was experienced for tall oil in the presence of acetate.

Interesting results were obtained when the fatty acyl profiles of the residual lipids present in the medium and the initial oil substrate after seven days of growth were compared (Table 5).

In the presence and absence of acetate, a significance decrease in the PUFAs, i.e 18:2 and 18:3 was experienced in the residual lipid fraction of the medium of all the fat and oil substrates tested when compared to the initial oil substrate. In many cases, the saturated FAs, i.e. 16:0 and 18:0 increased when compared to the original oil substrate. This phenomenon again shows a preference of this fungus towards the utilisation of PUFAs for growth. Interestingly, all of the α18:3 were completely utilised in linseed oil, used cooking oil, tall oil and soap skimmings in the presence and absence of acetate. This is similar to that found in *Absidia* MUFS 200.

Different cellular fatty acyl profiles were found in this fungus after growth on various fat and oil substrates in the presence and absence of acetate when compared to the fatty acyl profiles of the original oil substrate fed (Table 6).

In general a decrease in the cellular PUFAs and an increase in the saturated FAs were experienced in the cellular lipids when compared to the original oil substrates. These results are in accordance to the results found by Jeffery et al (1999) and for *Absidia* MUFS 200. According to these results it is evident that this fungus prefers to utilise the PUFAs probably for energy production. The high value lipid, i.e. GLA was only produced when the fungus was utilising sunflower (1.9% w/w) and used cooking oils (3.2% w/w) in the presence of acetate.

Table 4. Growth yield, lipid accumulation, utilisation and the final pH by *Actinomucor elegans* MUFS SAS218 grown on mixed substrates (oil and acetate) and substrate alone (oil).

Substrate	Growth yield (g dry wt/l)	Cellular lipid content (% w/w dry wt)	Residual lipids (g/l)	pH
Sunflower oil (40g/l)	3.5	31.4	18.7	4.0
Sunflower oil (30g/l) + Sodium acetate (10g/l)	18.5	43.6	1.5	6.7
Linseed oil (40g/l)	30.5	8.7	0.4	4.4
Linseed oil (30g/l) + Sodium acetate (10g/l)	30.5	8.7	0.7	5.3
Used cooking oil (40g/l)	3.0	48.0	29.9	4.9
Used cooking oil (30g/l) + Sodium acetate (10g/l)	17.5	60.0	1.0	6.5
Tall oil (40g/l)	1.0	26.0	24.6	4.2
Tall oil (30g/l) + Sodium acetate (10g/l)	2.5	45.2	15.8	7.3
Soap skimmings (40g/l)	1.0	68.0	9.4	7.3
Soap skimmings (30g/l) + Sodium acetate (10g/l)	1.0	57.0	11.9	7.2

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases.

Table 5. Fatty acyl profiles of the residual lipids of *Actinomucor elegans* MUFS SAS218 grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs / Total FAs**
Sunflower oil (40g/l)	13.8	0.0	9.1	21.4	2.2	0.0	0.0	0.05
Sunflower oil (30g/l) + Sodium acetate (10g/l)	11.3	0.0	31.4	26.4	13.6	0.0	0.0	0.16
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	20.4	0.0	15.3	52.7	4.7	0.0	0.0	0.05
Linseed oil (30g/l) + Sodium acetate (10g/l)	18.3	0.0	26.6	39.0	0.0	0.0	0.0	0.0
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	10.9	0.0	5.3	37.9	0.0	0.0	0.0	0.0
Used cooking oil (30g/l) + Sodium acetate (10g/l)	4.9	0.0	35.5	24.2	0.0	0.0	0.0	0.0
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	6.7	0.0	1.2	29.3	15.2	0.0	0.0	0.29
Tall oil (30g/l) + Sodium acetate (10g/l)	7.5	0.0	2.6	37.2	10.3	0.0	0.0	0.18
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	7.9	0.0	1.6	39.6	11.2	0.0	0.0	0.19
Soap skimmings (30g/l) + Sodium acetate (10g/l)	8.0	0.0	1.5	35.1	12.0	0.0	0.0	0.21
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ).

Table 6. Fatty acyl profiles of cellular lipids and GLA production by *Actinomucor elegans* MUFS SAS218 grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs / Total FAs**
Sunflower oil (40g/l)	12.2	0.0	10.6	25.4	6.4	0.0	0.0	0.12
Sunflower oil (30g/l) + Sodium acetate (10g/l)	10.8	0.9	8.8	32.5	29.8	1.9	0.0	0.37
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	15.2	0.0	12.6	32.5	2.3	0.0	0.0	0.04
Linseed oil (30g/l) + Sodium acetate (10g/l)	15.6	0.8	12.6	31.7	2.3	0.0	0.0	0.04
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	5.7	0.0	5.4	5.8	0.0	0.0	0.0	0.0
Used cooking oil (30g/l) + Sodium acetate (10g/l)	9.2	1.7	4.4	56.7	11.7	3.2	0.2	0.17
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	7.7	0.0	4.3	13.3	5.2	0.0	0.0	0.17
Tall oil (30g/l) + Sodium acetate (10g/l)	7.4	0.0	3.7	24.4	17.6	0.0	0.0	0.33
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	15.2	0.0	5.5	19.2	0.0	0.0	0.0	0.0
Soap skimmings (30g/l) + Sodium acetate (10g/l)	16.3	0.0	6.5	12.8	0.0	0.0	0.0	0.0
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ).

Cunninghamella MUFS Cu001

When comparing the growth yields, lipid accumulation, utilisation and the final pH of the growth medium in the presence and absence of acetate, the following results were obtained after seven days of cultivation (Table 7).

Biomass: The fungus produced similar low amounts of biomass when cultivated on soap skimmings in the presence or absence of acetate, i.e. 2.0g/l. Here, the addition of acetate to the growth medium led to no significant effect on biomass production. On the other hand, a decrease in biomass production was obtained on tall oil in the presence of acetate, i.e. from 8.5g/l to 3.0g/l. When the fungus was grown on sunflower oil and used cooking oil in the presence of acetate, an enhancing effect on biomass production was experienced. In this case, the biomass production increased from 6.0g/l to 18.0g/l on sunflower oil and from 7.5g/l to 26.0g/l on used cooking oil. In both cases, the pH was significantly higher in the presence of acetate reaching neutrality. These results are in accordance to that found by Jeffery et al (1999). The highest amounts of biomass were obtained on linseed oil in the presence and absence of acetate, i.e. 29.0g/l and 30.5g/l respectively. In this case, the addition of acetate had no significant effect on biomass production.

Cellular lipids: The fungus produced the highest amounts of cellular lipids when grown on sunflower oil (46.0% w/w), used cooking oil (43.0% w/w) and soap skimmings (70.3% w/w) in the presence of acetate. Here, the lipid yields increased

from 45.2% w/w to 46.0% w/w for sunflower oil, from 19.6% w/w to 43.0% w/w for used cooking oil and from 47.3% w/w to 70.3% w/w for soap skimmings. When cultivated on linseed oil in the presence of acetate, a smaller rise in cellular lipid was obtained, i.e. from 8.9% w/w to 11.4% w/w. On the other hand, lipid yields decreased from 67.9% w/w to 37.5% w/w when the fungus was utilising tall oil as substrate in the presence of acetate. The lipid content of *Cunninghamella* species when grown on glucose as carbon source, was found to be similar to when grown on various fat and oil substrates in shake flasks (Ratledge, 1989). Here, *Cunninghamella echinulata* produced 45% w/w intracellular lipids while *Cunninghamella japonica* produced 40 to 60% (w/w) cellular lipids when grown on glucose as carbon source. When *Cunninghamella elegans* and *Cunninghamella homothallica* were cultivated on glucose as carbon source under static conditions, they produced 44% w/w and 38% w/w cellular lipids respectively (Ratledge, 1989).

Residual lipids: Sunflower oil and used cooking oil were utilised more in the presence of acetate than in its absence. In this case, the pH increased to neutral in the presence of acetate after seven days of growth, which again may be responsible for this trend (Jeffery *et al.,* 1999). Tall oil and soap skimmings on the other hand were utilised less effectively in the presence of acetate, i.e. 34.0% and 76.7% respectively compared to when grown in the absence of acetate. This is may be due to the presence of non-fatty acid compounds such as neutrals (Gunstone *et al.,* 1994) that again resulted in poor growth of the fungus. About all of linseed oil was utilised in both the presence and absence of acetate.

Interesting results were obtained when the fatty acyl profiles of the initial oil substrate and residual lipids present in the medium after seven days of incubation were compared (Table 8).

In general, a significant decrease in the PUFA content (i.e. 18:2 and 18:3) occurred in the presence and absence of acetate in the residual lipid fraction of the medium. In fact all of these PUFAs were utilised. This indicates a high preference of the fungus towards PUFA utilisation. These results are in accordance to that found by Jeffery et al (1999). Interestingly, again all of the α18:3 were completely utilised in linseed oil, used cooking oil, tall oil and soap skimmings. This phenomenon is in accordance to that found in *Absidia* MUFS 200 and *Actinomucor elegans* MUFS SAS218.

The fatty acyl profiles of the intracellular lipids of this fungus when grown on various fat and oil substrates were determined after seven days of cultivation and compared to the fatty acyl profiles of the original oil substrates (Table 9).

In most cases, a decrease in the PUFAs in the intracellular lipid fraction was experienced while an increase in saturated FAs occurred. According to these results the PUFAs, which were preferably utilised, are probably metabolised for energy production and are not incorporated into the neutral lipid fraction of the cell. When this fungus was cultivated on sunflower oil, used cooking oil and tall oil in the presence of acetate, more GLA was produced as reported by Jeffery et al (1997).

Table 7. Growth yield, lipid accumulation, utilisation and the final pH by *Cunninghamella* MUFS Cu001 grown on mixed substrates (oil and acetate) and substrate alone (oil).

Substrate	Growth yield (g dry wt/l)	Cellular lipid content (% w/w dry wt)	Residual lipids (g/l)	pH
Sunflower oil (40g/l)	6.0	45.2	16.4	2.8
Sunflower oil (30g/l) + Sodium acetate (10g/l)	18.0	46.0	12.1	7.1
Linseed oil (40g/l)	30.5	8.9	0.4	4.2
Linseed oil (30g/l) + Sodium acetate (10g/l)	29.0	11.4	0.6	5.2
Used cooking oil (40g/l)	7.5	19.6	15.3	2.7
Used cooking oil (30g/l) + Sodium acetate (10g/l)	26.0	43.0	1.7	7.1
Tall oil (40g/l)	8.5	67.9	7.8	3.1
Tall oil (30g/l) + Sodium acetate (10g/l)	3.0	37.5	19.8	7.2
Soap skimmings (40g/l)	2.0	47.3	4.8	7.1
Soap skimmings (30g/l) + Sodium acetate (10g/l)	2.0	70.3	7.0	7.7

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases.

Table 8. Fatty acyl profiles of the residual lipids of *Cunninghamella* MUFS Cu001 grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs / Total FAs**
Sunflower oil (40g/l)	12.8	0.0	5.4	12.9	0.0	0.0	0.0	0.0
Sunflower oil (30g/l) + Sodium acetate (10g/l)	3.3	17.5	14.8	9.5	0.0	0.0	0.0	0.0
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	21.2	0.0	10.9	16.8	0.0	0.0	0.0	0.0
Linseed oil (30g/l) + Sodium acetate (10g/l)	18.8	0.0	16.1	8.3	0.0	0.0	0.0	0.0
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	16.7	0.0	8.7	12.7	0.0	0.0	0.0	0.0
Used cooking oil (30g/l) + Sodium acetate (10g/l)	14.9	0.0	17.0	17.1	0.0	0.0	0.0	0.0
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	11.9	0.0	2.5	32.3	0.0	0.0	0.0	0.0
Tall oil (30g/l) + Sodium acetate (10g/l)	14.6	0.0	2.9	38.7	0.0	0.0	0.0	0.0
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	16.4	0.0	3.5	18.9	0.0	0.0	0.0	0.0
Soap skimmings (30g/l) + Sodium acetate (10g/l)	15.6	0.0	3.2	24.5	0.0	0.0	0.0	0.0
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ).

Table 9. Fatty acyl profiles of cellular lipids and GLA production by *Cunninghamella* MUFS Cu001 grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs Total FAs**
Sunflower oil (40g/l)	13.5	1.0	9.0	30.6	14.3	0.0	0.0	0.21
Sunflower oil (30g/l) + Sodium acetate (10g/l)	6.5	0.6	3.9	17.8	62.9	3.7	0.1	0.70
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	17.8	1.0	13.1	36.2	2.0	0.0	0.0	0.03
Linseed oil (30g/l) + Sodium acetate (10g/l)	16.9	0.9	13.7	35.5	7.8	0.0	0.0	0.10
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	7.0	1.0	3.8	41.4	34.2	2.2	3.3	0.43
Used cooking oil (30g/l) + Sodium acetate (10g/l)	6.0	1.0	3.6	40.6	35.6	5.1	2.9	0.46
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	4.3	0.6	1.2	26.2	18.1	1.0	0.9	0.38
Tall oil (30g/l) + Sodium acetate (10g/l)	7.5	0.7	4.7	35.4	21.5	2.0	0.8	0.33
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	16.3	1.3	5.9	26.4	0.0	0.0	0.0	0.0
Soap skimmings (30g/l) + Sodium acetate (10g/l)	15.9	0.0	5.8	29.6	2.7	0.0	0.0	0.05
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ).

***Gongronella* MUFS Go001**

When comparing the growth yields, lipid accumulation, utilisation and the final pH of the growth medium in the presence and absence of acetate, the following results were obtained after seven days of growth (Table 10).

Biomass: The fungus grew on all the lipid substrates and produced biomass in the presence and absence of acetate. In this case, the highest amounts of biomass were produced when the fungus was cultivated on linseed oil in the presence and absence of acetate, i.e. 28.0g/l and 22.0g/l respectively. Here, the addition of acetate had a stimulatory effect on biomass production (Jeffery *et al.,* 1997). Similar results, i.e. acetate enhancement were obtained on sunflower oil and tall oil. On the other hand, when the fungus was cultivated on used cooking oil and soap skimmings in the presence of acetate, a decrease in biomass production was obtained, i.e. from 8.0g/l to 6.0g/l on used cooking oil and from 28g/l to 5.5g/l on soap skimmings. This may be due to toxic substances present in these oils, i.e. oxidised lipids and other neutral compounds.

Cellular lipids: The highest amounts of cellular lipids were obtained when the fungus was cultivated on sunflower oil (62% w/w) and on used cooking oil (46.3% w/w) in the presence of acetate. Interestingly, the fungal cells produced more lipids on all the fat and oil substrates in the presence of acetate. Similar high

concentrations of cellular lipids were reported in this fungus when grown on glucose as sole carbon source (Strauss, 1997).

Residual lipids: Linseed oil and used cooking oil were utilised more in the absence of acetate, i.e. 99.5% and 58.5% respectively compared to 98.7% and 46.7% utilised in the presence of acetate respectively. Sunflower oil and tall oil were utilised more in the presence of acetate, i.e. 89.0% and 57.3% respectively. In both cases, the pH increased to neutrality in the presence of acetate, which most probably caused enhanced lipid utilisation as reported by Jeffery et al (1999). Soap skimmings on the other hand, was utilised to a greater extend in the absence of acetate than in its presence, i.e. 99.8% compared to 55.0% in its presence.

Interesting results were obtained when the fatty acyl profiles of the residual lipids in the medium after seven days of growth and the original oil substrate were compared (Table 11).

Again, a significant decrease in PUFAs was experienced in the residual lipid fraction in the presence and absence of acetate when compared to the initial oil substrate. This phenomenon shows a high preference of the fungus towards PUFA utilisation. The saturated FAs on the other hand, remained in many cases relatively high in the medium after growth when compared to the original oil substrate. Interestingly, α18:3 was completely utilised in all the residual oil fractions (originally containing this compound) tested in the presence and absence of acetate. These results are

similar to that found in *Absidia* MUFS 200, *Actinomucor elegans* MUFS SAS218 and *Cunninghamella* MUFS Cu001.

Different intracellular fatty acyl profiles were found in this fungus in the presence and absence of acetate when grown on various fat and oil substrates and compared to the original oil substrate (Table 12).

Again, a decrease in the cellular PUFAs was experienced in all the lipid substrates tested in the presence and absence of acetate when compared to the original oil substrate used. In many cases, the saturated FAs remained relatively high in the cellular lipid fractions in the presence and absence of acetate. These results are similar to that found in *Absidia* MUFS 200, *Actinomucor elegans* MUFS SAS218, *Cunninghamella* MUFS Cu001 as well as to the results reported by Jeffery et al (1999). About 1.4% w/w GLA was produced by this fungus when grown on used cooking oil in the absence of acetate. In this case, the presence of acetate inhibited GLA production. From these results it seems possible that a Δ^6 desaturase stimulatory compound is present in used cooking (sunflower) oil since no GLA could be detected in pure sunflower oil.

Table 10. Growth yield, lipid accumulation, utilisation and the final pH by *Gongronella* MUFS Go001 grown on mixed substrates (oil and acetate) and substrate alone (oil).

Substrate	Growth yield (g dry wt/l)	Cellular lipid content (% w/w dry wt)	Residual lipids (g/l)	pH
Sunflower oil (40g/l)	6.5	35.3	9.3	2.6
Sunflower oil (30g/l) + Sodium acetate (10g/l)	11.0	62.0	3.3	7.3
Linseed oil (40g/l)	22.0	7.6	0.2	5.2
Linseed oil (30g/l) + Sodium acetate (10 g/l)	28.0	8.2	0.4	5.4
Used cooking oil (40g/l)	8.0	39.4	16.6	2.5
Used cooking oil (30g/l) + Sodium acetate (10g/l)	6.0	46.3	16.0	7.4
Tall oil (40g/l)	5.5	13.5	20.2	4.5
Tall oil (30g/l) + Sodium acetate (10g/l)	7.0	22.1	12.8	7.5
Soap skimmings (40g/l)	28.0	8.6	0.1	7.1
Soap skimmings (30g/l) + Sodium acetate (10g/l)	5.5	19.7	13.5	7.3

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases.

Table 11. Fatty acyl profiles of the residual lipids of *Gongronella* MUFS Go001 grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs Total FAs**
Sunflower oil (40g/l)	8.5	0.0	6.0	24.7	41.7	0.0	0.0	0.52
Sunflower oil (30g/l) + Sodium acetate (10g/l)	18.2	0.0	9.4	47.6	7.9	0.0	0.0	0.10
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	13.9	0.0	47.8	19.0	0.0	0.0	0.0	0.0
Linseed oil (30g/l) + Sodium acetate (10g/l)	19.9	0.0	11.4	55.9	8.2	0.0	0.0	0.09
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	8.4	0.0	3.9	48.6	18.5	0.0	0.0	0.23
Used cooking oil (30g/l) + Sodium acetate (10g/l)	10.2	0.0	5.3	41.6	0.0	0.0	0.0	0.0
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	4.8	0.0	1.3	27.4	13.2	0.0	0.0	0.28
Tall oil (30g/l) + Sodium acetate (10g/l)	5.2	0.0	1.1	28.1	15.6	0.0	0.0	0.31
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	8.0	0.0	1.5	47.0	20.4	0.0	0.0	0.27
Soap skimmings (30g/l) + Sodium acetate (10g/l)	5.4	0.0	1.0	28.2	16.2	0.0	0.0	0.32
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ).

Table 12. Fatty acyl profiles of cellular lipids and GLA production by *Gongronella* MUFS Go001 grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs / Total FAs**
Sunflower oil (40g/l)	7.4	0.0	5.5	21.8	41.5	0.0	0.0	0.54
Sunflower oil (30g/l) + Sodium acetate (10g/l)	10.9	0.0	9.6	30.5	19.0	0.0	0.0	0.27
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	14.8	0.0	12.3	35.7	7.4	0.0	0.0	0.11
Linseed oil (30g/l) + Sodium acetate (10g/l)	15.0	0.0	12.5	35.8	6.1	0.0	0.0	0.09
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	5.8	1.3	3.7	41.2	34.5	1.4	0.0	0.41
Used cooking oil (30g/l) + Sodium acetate (10g/l)	8.3	0.0	5.5	48.2	13.6	0.0	0.0	0.18
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	4.6	0.0	2.2	14.7	8.3	0.0	0.0	0.28
Tall oil (30g/l) + Sodium acetate (10g/l)	5.8	0.0	2.0	22.5	14.3	0.0	0.0	0.32
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	1.5	0.0	1.0	3.5	0.0	0.0	0.0	0.0
Soap skimmings (30g/l) + Sodium acetate (10g/l)	10.6	0.0	3.4	21.9	0.0	0.0	0.0	0.0
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.34**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ).

***Mortierella alpina* MUFS Mo058**

It is important to note that this fungus showed no growth when cultivated on any of the fat and oil substrates in the presence and absence of acetate at 30°C.

***Mucor circinelloides f. circinelloides* CBS 108.16**

When comparing the growth yields, cellular lipid contents, utilisation and the final pH of the growth medium in the presence and absence of acetate, the following results were obtained after seven days of growth (Table 13).

Biomass: A drastic increase in biomass production was obtained on sunflower oil and on used cooking oil in the presence of acetate. In this case, the biomass production increased from 2.5g/l to 25.0g/l on sunflower oil and from 4.0g/l to 20.5g/l on used cooking oil. Here, the addition of acetate again, had a stimulatory effect in biomass production, which is probably due to an increase in pH to neutral as reported by Jeffery et al (1999). Similar results were obtained on linseed oil although the pH did not increase significantly. When cultivated on tall oil and soap skimmings in the presence of acetate, a smaller increase in biomass was observed. However, on tall oil a significant increase in pH was found.

Cellular lipids: Higher amounts of intracellular lipids were obtained on sunflower oil (36.6% w/w), used cooking oil (34.5% w/w), tall oil (43.3% w/w) and on soap skimmings (36.3% w/w) in the presence of acetate. In all cases, the addition of acetate had a stimulatory effect on lipid production, although to a lesser extend when grown on linseed oil and soap skimmings. On the other hand, when the other strains of *Mucor circinelloides* were cultivated on glucose as sole carbon source under static conditions, they produced much higher cellular lipids, i.e. 65.0% w/w

compared to when grown on various fat and oil substrates (Ratledge, 1989). This cannot be explained.

Residual lipids: As expected from the previous results, all the lipid substrates tested were utilised to a greater extend in the presence of acetate after seven days of growth. In all cases (except for linseed oil), the pH increased to neutrality which probably caused enhanced lipid emulsification and utilisation (Jeffery *et al.,* 1999).

Interesting results were obtained when the residual fatty acyl profiles present in the supernatant were compared to the initial oil substrate after seven days of growth (Table 14).

A significant decrease in the PUFAs present in the residual oil fractions was experienced in all the lipid substrates after growth while an increase in saturated FAs occurred in many cases in the presence and absence of acetate. Again, this phenomenon indicates a high preference of the fungus towards PUFA utilisation. The 18:2 on the other hand (except for linseed oil) was significantly more utilised in the presence of acetate. These results are in accordance to that found by Jeffery et al (1999). Again, the α18:3 present in linseed oil, used cooking oil, tall oil and soap skimmings was completely utilised in the presence and absence of acetate.

The fatty acyl profiles of the intracellular lipids of this fungus after growth on various fat and oil substrates were determined after seven days of growth and compared to the fatty acyl profiles of the original oil substrate (Table 15).

In most cases, a decrease in the PUFAs was experienced in the presence and absence of acetate while the saturated FAs remained mostly relatively high. Significant amounts of GLA was produced when the fungus was grown on sunflower oil (5.1% w/w), used cooking oil (4.9% w/w) and tall oil (5.2% w/w) in the presence of acetate. This can most probably be ascribed to the rise in pH as reported by Jeffery et al (1999). On the other hand, when the fungus was cultivated on linseed oil, only small amounts of GLA, i.e. 0.7% w/w were produced in the presence of acetate.

Table 13. Growth yield, lipid accumulation, utilisation and the final pH by *Mucor circinelloides f. circinelloides* CBS 108.16 grown on mixed substrates (oil and acetate) and substrate alone (oil).

Substrate	Growth yield (g dry wt/l)	Cellular lipid content (% w/w dry wt)	Residual lipids (g/l)	pH
Sunflower oil (40g/l)	2.5	17.9	9.1	3.1
Sunflower oil (30g/l) + Sodium acetate (10g/l)	25.0	36.6	1.9	7.3
Linseed oil (40g/l)	26.5	15.8	1.3	4.9
Linseed oil (30g/l) + Sodium acetate (10g/l)	37.0	18.7	0.6	5.5
Used cooking oil (40g/l)	4.0	14.2	18.4	2.8
Used cooking oil (30g/l) + Sodium acetate (10g/l)	20.5	34.5	2.3	7.1
Tall oil (40g/l)	2.0	7.5	27.6	4.1
Tall oil (30g/l) + Sodium acetate (10g/l)	6.0	43.3	9.6	7.1
Soap skimmings (40g/l)	4.0	33.0	7.1	7.4
Soap skimmings (30g/l) + Sodium acetate (10g/l)	7.0	36.3	2.6	8.6

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

Table 14. Fatty acyl profiles of the residual lipids of *Mucor circinelloides f. circinelloides* CBS 108.16 grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs Total FAs**
Sunflower oil (40g/l)	10.6	0.0	11.5	31.5	11.5	0.0	0.0	0.18
Sunflower oil (30g/l) + Sodium acetate (10g/l)	7.5	0.0	24.0	28.1	3.5	0.0	0.0	0.06
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	12.3	0.0	12.7	27.0	0.0	0.0	0.0	0.0
Linseed oil (30g/l) + Sodium acetate (10g/l)	12.3	0.0	12.0	36.2	5.4	0.0	0.0	0.08
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	7.4	0.3	5.6	47.2	26.8	0.0	0.0	0.31
Used cooking oil (30g/l) + Sodium acetate (10g/l)	3.5	0.5	11.9	49.9	8.3	0.0	0.0	0.11
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	4.3	0.0	1.9	25.0	11.9	0.0	0.0	0.28
Tall oil (30g/l) + Sodium acetate (10g/l)	2.3	0.0	2.9	12.9	0.0	0.0	0.0	0.0
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	4.9	0.5	1.3	29.7	17.1	0.0	0.0	0.32
Soap skimmings (30g/l) + Sodium acetate (10g/l)	3.9	0.0	5.2	9.1	0.0	0.0	0.0	0.0
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ). CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

Table 15. Fatty acyl profiles of cellular lipids and GLA production by *Mucor circinelloides f. circinelloides* CBS 108.16 grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs Total FAs**
Sunflower oil (40g/l)	8.8	0.0	4.7	12.8	32.8	4.1	0.0	0.58
Sunflower oil (30g/l) + Sodium acetate (10g/l)	8.3	1.0	3.7	22.4	54.5	5.1	0.0	0.63
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	20.0	1.2	11.8	36.8	5.0	0.0	0.0	0.07
Linseed oil (30g/l) + Sodium acetate (10g/l)	18.9	1.3	11.6	36.3	6.4	0.7	2.6	0.12
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	9.9	2.7	3.0	29.9	30.0	3.3	2.5	0.44
Used cooking oil (30g/l) + Sodium acetate (10g/l)	7.9	1.8	3.5	41.5	33.0	4.9	2.6	0.43
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	6.2	0.0	2.1	10.3	6.1	0.0	0.0	0.25
Tall oil (30g/l) + Sodium acetate (10g/l)	5.5	1.5	3.1	25.1	15.5	5.2	0.0	0.37
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	10.8	2.0	3.0	23.3	0.0	0.0	0.0	0.0
Soap skimmings (30g/l) + Sodium acetate (10g/l)	11.8	0.0	4.1	11.8	0.0	0.0	0.0	0.0
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ). CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

***Mucor circinelloides f. circinelloides* MUFS SAS045**

When comparing the growth yields, lipid accumulation, utilisation and the final pH of the growth medium in the presence and absence of acetate, the following results were obtained after seven days of cultivation (Table 16).

Biomass: In the presence or absence of acetate, the fungus produced similar amounts of biomass, i.e. 6.5g/l when cultivated on soap skimmings. In this case, the addition of acetate had no significant enhancing effect on biomass production. Similar results were also obtained when the fungus was cultivated on linseed oil. When grown on tall oil, a small increase in biomass production was obtained in the presence of acetate, i.e. from 2.0g/l to 4.5g/l. On the other hand, when grown on sunflower and used cooking oils in the presence of acetate, a significant increase in biomass production was obtained, i.e. from 3.0g/l to 16.0g/l and from 2.0g/l to 14.5g/l respectively. In this case, the addition of acetate and an increase in pH to neutrality had a stimulatory effect on biomass production. These results are again similar to that reported by Jeffery et al (1997; 1999) and to that found for *Mucor circinelloides f. circinelloides* CBS 108.16.

Cellular lipids: The fungus produced similar amounts of intracellular lipids when grown on linseed oil in the presence and absence of acetate, i.e. 8.4% w/w and 8.9% w/w respectively. Here, the addition of acetate had again no significant effect on the extend of lipid production. When cultivated on sunflower oil, used cooking oil

and tall oil in the presence of acetate, it produced much more lipids than in its absence. In this case, lipid production increased drastically from 15.7% w/w to 35.7% w/w on sunflower oil, from 23.5% w/w to 28.6% w/w on used cooking oil and significantly from 18.0% w/w to 37.9% w/w on tall oil. When cultivated on soap skimmings in the presence of acetate, a decrease in lipid production was obtained, i.e. from 56.5% w/w to 42.5% w/w. This may be due to the high pH. These results are similar (except for linseed oil) to that found in *Mucor circinelloides f circinelloides* CBS 108.16.

Residual lipids: As expected, sunflower oil, used cooking oil and tall oil were utilised much more in the presence of acetate than in its absence, i.e. 74.0%, 78.7% and 54.7% respectively. In these experiments, the pH increase to neutrality probably played a role in the enhanced lipid utilisation (Jeffery *et al.,* 1999). Soap skimmings on the other hand, was utilised to a greater extend in the absence of acetate than in its presence. Linseed oil was most effectively utilised and to a similar extend in both experiments (oil and oil plus acetate).

Interesting results were obtained when the residual fatty acyl profiles in the supernatant were compared to the initial oil substrate after seven days of growth (Table 17).

From the previous results, a significant decrease in the PUFAs was experienced in the residual oil fractions in the presence and absence of acetate while the saturated

FAs remained in most cases relatively high in the supernatant after growth. Again, this phenomenon shows a high preference of the fungus towards PUFAs utilisation. These results are similar to that found previously. Again, the α18:3 present in linseed oil, used cooking oil, tall oil and soap skimmings were utilised completely in the presence and absence of acetate.

The fatty acyl profiles of the intracellular lipids of this fungus after growth on various fat and oil substrates were determined after seven days of growth and compared to the fatty acyl profiles of the original oil substrate (Table 18).

In the presence and absence of acetate, a general decrease in the PUFAs was experienced while the saturated FAs remained relatively high in the intracellular lipid fractions when compared to the original oil substrates. Small amounts of GLA were produced when cultivated on sunflower oil and linseed oil in the presence of acetate, i.e. 1.6% w/w and 0.3% w/w respectively. Here, the addition of acetate and an increase in pH had a small stimulatory effect on GLA production. It is interesting to note that, when cultivated on used cooking oil and tall oil in the presence of acetate, a decrease in GLA production was obtained, i.e. from 4.8% w/w to 1.8% w/w and from 5.7% w/w to 3.7% w/w respectively.

Table 16. Growth yield, lipid accumulation, utilisation and final pH by *Mucor circinelloides f. circinelloides* MUFS SAS045 grown on mixed substrates (oil and acetate) and substrate alone (oil).

Substrate	Growth yield (g dry wt/l)	Cellular lipid content (% w/w dry wt)	Residual lipids (g/l)	pH
Sunflower oil (40g/l)	3.0	15.7	16.1	4.2
Sunflower oil (30g/l) + Sodium acetate (10g/l)	16.0	35.7	7.8	7.1
Linseed oil (40g/l)	31.5	8.9	0.8	4.9
Linseed oil (30g/l) + Sodium acetate (10g/l)	31.0	8.4	0.5	5.4
Used cooking oil (40g/l)	2.0	23.5	20.9	3.5
Used cooking oil (30g/l) + Sodium acetate (10 g/l)	14.5	28.6	6.4	7.0
Tall oil (40g/l)	2.0	18.0	26.4	4.1
Tall oil (30g/l) + Sodium acetate (10g/l)	4.5	37.9	13.6	7.1
Soap skimmings (40g/l)	6.5	56.5	2.6	6.9
Soap skimmings (30g/l) + Sodium acetate (10g/l)	6.5	42.5	6.0	8.4

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases.

Table 17. Fatty acyl profiles of the residual lipids of *Mucor circinelloides f. circinelloides* MUFS SAS045 grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs Total FAs**
Sunflower oil (40g/l)	19.5	1.1	16.2	13.5	0.0	0.0	0.0	0.0
Sunflower oil (30g/l) + Sodium acetate (10g/l)	17.5	1.4	15.2	11.5	0.9	0.0	0.0	0.02
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	22.3	0.0	17.1	6.8	0.0	0.0	0.0	0.0
Linseed oil (30g/l) + Sodium acetate (10g/l)	17.2	0.2	16.1	10.7	0.0	0.0	0.0	0.0
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	17.7	1.2	9.7	22.6	0.4	0.0	0.0	0.01
Used cooking oil (30g/l) + Sodium acetate (10g/l)	13.6	1.1	16.1	21.2	0.7	0.0	0.0	0.01
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	10.7	0.9	3.5	35.0	0.4	0.0	0.0	0.01
Tall oil (30g/l) + Sodium acetate (10g/l)	5.8	0.7	4.2	32.9	0.5	0.0	0.0	0.01
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	11.8	0.0	6.2	18.3	0.0	0.0	0.0	0.0
Soap skimmings (30g/l) + Sodium acetate (10g/l)	14.3	2.5	4.1	12.4	0.0	0.0	0.0	0.0
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ).

Table 18. Fatty acyl profiles of cellular lipids and GLA production by *Mucor circinelloides f. circinelloides* MUFS SAS045 grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs Total FAs**
Sunflower oil (40g/l)	13.8	2.1	5.8	23.7	24.2	1.0	0.0	0.36
Sunflower oil (30g/l) + Sodium acetate (10g/l)	9.6	0.7	5.8	21.9	49.7	1.6	0.0	0.57
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	18.6	1.0	12.9	38.5	3.8	0.2	0.5	0.06
Linseed oil (30g/l) + Sodium acetate (10g/l)	18.0	0.9	12.9	36.3	10.1	0.3	0.8	0.14
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	10.3	1.9	4.2	33.9	23.4	4.8	1.9	0.37
Used cooking oil (30g/l) + Sodium acetate (10g/l)	10.6	1.6	4.8	45.9	23.7	1.8	1.1	0.30
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	11.9	2.9	3.7	25.2	14.2	5.7	0.0	0.31
Tall oil (30g/l) + Sodium acetate (10g/l)	7.2	1.4	3.8	33.3	25.8	3.7	0.3	0.39
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	10.1	1.1	3.6	36.5	0.9	0.0	0.0	0.02
Soap skimmings (30g/l) + Sodium acetate (10g/l)	15.6	1.0	5.4	34.1	3.0	0.0	0.0	0.05
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ).

Rhizomucor pusillus MUFS Rm001

When comparing the growth yields, lipid accumulation, utilisation and the final pH of the growth medium in the presence and absence of acetate, the following results were obtained after seven days of growth (Table 19).

Biomass: The highest similar amounts of biomass were obtained on linseed oil as carbon source in the presence or absence of acetate, i.e. 29.0g/l. In this case, the effect of acetate on the extend of biomass production was not experienced. On the other hand, the enhancing effect of acetate on biomass production was experienced when cultivated on sunflower oil and on used cooking oil. The biomass production in this case increased significantly from 6.5g/l to 16.5g/l on sunflower oil and from 5.0g/l to 16.0g/l on used cooking oil. In both cases, the pH increased to neutrality due to acetic acid utilisation, which probably played a role in biomass production (Jeffery *et al.,* 1999). When cultivated on tall oil and soap skimmings in the presence and absence of acetate, small amounts of biomass were obtained.

Cellular lipids: The highest lipid yield was obtained on sunflower oil (57.2% w/w), used cooking oil (52.9% w/w) and soap skimmings (46.5% w/w) in the presence of acetate. In both cases, the addition of acetate and the pH increase to neutrality played a significant role on lipid production. It is also important to note that a decrease in lipid content was obtained on tall oil (from 45.0% w/w to 26.0% w/w) and on linseed oil (from 9.2% w/w to 7.4% w/w) in the presence of acetate.

Residual lipids: Again, as expected, sunflower oil and used cooking oil were utilised more in the presence of acetate than in its absence, i.e. 91.0% and 80.3% respectively. This is probably due to the increase in pH followed by emulsification of the lipid (Jeffery *et al.,* 1999). Tall oil and soap skimmings on the other hand, were utilised to a lesser extend in the presence of acetate than in its absence.

Interesting results were obtained when the fatty acyl profiles of the residual oil fractions in the medium were compared to the original oil substrates in the presence and absence of acetate after seven days of growth (Table 20).

From the previous results, a significant decrease in the PUFAs present in the residual lipids was experienced in the presence and absence of acetate. Again, this decrease indicates that the fungus prefers to utilise PUFAs. The saturated FAs on the other hand, remained relatively high in the residual oil fractions in the presence and absence of acetate when compared to the original oil fraction. The α18:3 in linseed oil, used cooking oil, tall oil and soap skimmings on the other hand, was utilised completely in the presence and absence of acetate. These results are similar to that reported previously.

The fatty acyl profiles of the cellular lipid fractions were determined after this fungus was grown on various fat and oil substrates and compared to the original oil substrates in the presence and absence of acetate (Table 21).

In the presence and absence of acetate, a general decrease in the PUFAs was experienced while the saturated FAs remained relatively high in the cellular lipid fractions when compared to the original oil substrate. Only small amounts of GLA were produced when cultivated on sunflower oil (0.2% w/w), linseed oil (0.5% w/w) and used cooking oil (0.2 % w/w) in the presence of acetate.

Table 19. Growth yield, lipid accumulation, utilisation and the final pH by *Rhizomucor pusillus* MUFS Rm001 grown on mixed substrates (oil and acetate) and substrate alone (oil).

Substrate	Growth yield (g dry wt/l)	Cellular lipid content (% w/w dry wt)	Residual lipids (g/l)	pH
Sunflower oil (40g/l)	6.5	29.3	16.7	2.7
Sunflower oil (30g/l) + Sodium acetate (10 g/l)	16.5	57.2	2.7	6.5
Linseed oil (40g/l)	29.0	9.2	0.2	4.7
Linseed oil (30g/l) + Sodium acetate (10g/l)	29.0	7.4	0.4	5.2
Used cooking oil (40g/l)	5.0	41.2	18.2	3.1
Used cooking oil (30g/l) + Sodium acetate (10g/l)	16.0	52.9	5.9	6.8
Tall oil (40g/l)	5.0	45.0	17.0	3.6
Tall oil (30g/l) + Sodium acetate (10g/l)	2.0	26.0	25.0	6.6
Soap skimmings (40g/l)	2.5	34.1	10.7	7.4
Soap skimmings (30g/l) + Sodium acetate (10g/l)	3.5	46.5	10.1	7.2

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases.

Table 20. Fatty acyl profiles of the residual lipids of *Rhizomucor pusillus* MUFS Rm001 grown on sunflower oil, linseed oil, used cooking oil; tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs / Total FAs**
Sunflower oil (40g/l)	14.2	0.0	9.0	19.8	1.5	0.0	0.0	0.03
Sunflower oil (30g/l) + Sodium acetate (10g/l)	17.6	0.0	10.0	34.1	6.5	0.0	0.0	0.1
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	19.9	0.0	11.7	21.9	2.3	0.0	0.0	0.04
Linseed oil (30g/l) + Sodium acetate (10g/l)	22.1	0.0	11.9	29.8	4.9	0.0	0.0	0.07
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	12.3	0.0	5.5	30.5	0.2	0.0	0.0	0.004
Used cooking oil (30g/l) + Sodium acetate (10g/l)	10.8	0.0	6.6	51.4	1.2	0.0	0.0	0.02
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	7.4	0.0	1.7	29.5	11.4	0.0	0.0	0.23
Tall oil (30g/l) + Sodium acetate (10g/l)	9.1	0.0	2.0	36.2	12.8	0.0	0.0	0.21
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	10.7	0.0	2.2	40.8	8.2	0.0	0.0	0.13
Soap skimmings (30g/l) + Sodium acetate (10g/l)	14.5	0.0	2.8	30.8	3.3	0.0	0.0	0.06
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ).

Table 21. Fatty acyl profiles of cellular lipids and GLA production by *Rhizomucor pusillus* MUFS Rm001 grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs / Total FAs**
Sunflower oil (40g/l)	15.3	0.6	9.9	32.5	9.0	0.0	0.0	0.13
Sunflower oil (30g/l) + Sodium acetate (10g/l)	18.0	0.8	13.4	34.0	13.9	0.2	0.0	0.18
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	16.5	0.0	10.7	32.7	2.7	0.0	0.0	0.04
Linseed oil (30g/l) + Sodium acetate (10g/l)	17.6	0.7	12.9	34.1	4.0	0.5	0.7	0.07
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	16.4	0.6	6.0	47.4	3.0	0.0	0.0	0.04
Used cooking oil (30g/l) + Sodium acetate (10g/l)	12.0	0.9	7.0	49.3	12.6	0.2	0.4	0.16
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	6.9	1.0	3.4	29.0	12.6	0.0	0.0	0.24
Tall oil (30g/l) + Sodium acetate (10g/l)	9.1	0.8	6.3	35.4	8.9	0.0	0.0	0.15
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	10.1	0.0	4.1	25.5	7.9	0.0	0.0	0.17
Soap skimmings (30g/l) + Sodium acetate (10g/l)	12.4	0.0	5.7	35.3	4.1	0.0	0.0	0.07
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ).

Rhizopus stolonifer MUFS R008

When comparing the growth yields, lipid content, utilisation and the final pH of the growth medium in the presence and absence of acetate, the following results were obtained after seven days of growth (Table 22).

Biomass: The highest amounts of biomass were obtained on linseed oil in the presence and absence of acetate, i.e. 30.0g/l and 28.0g/l respectively. In this case, the addition of acetate to the growth medium had a small enhancing effect on biomass production. A similar pattern was obtained when cultivated on used cooking oil. A significant enhancing effect of acetate was experienced when this fungus was grown on tall oil. The biomass production in this case, increased significantly from 1.0g/l to 9.5g/l. Again, the pH increase to neutrality in the presence of acetate probably played a role in the enhanced biomass production (Jeffery *et al.,* 1999).

Cellular lipids: The highest amount of cellular lipid production was obtained on sunflower oil and soap skimmings in the absence of acetate, i.e. 65.5% w/w and 80.5% w/w respectively. When cultivated on linseed oil, used cooking oil and tall oil in the presence of acetate, a small increase in the cellular lipid content was experienced. The intracellular lipid content increased slightly from 13.1% w/w to 14.9% w/w on linseed oil, from 31.5% w/w to 35.0% w/w on used cooking oil and from 2.0% w/w to 4.5% w/w on tall oil when acetate was added. As reported by

Ratledge (1989), *Rhizopus arrhizus*, *Rhizopus delemar* and *Rhizopus oryzae*, when cultivated on glucose as sole carbon source under static conditions, produced similar amounts of lipids compared to *Rhizopus stolonifer* MUFS R008 when grown on various fat and oil substrates in this study.

Residual lipids: Sunflower oil, linseed oil and used cooking oil were utilised much more in the absence of acetate than in its presence, i.e 91.3%, 99.0% and 73.5% respectively. In this case, a smaller rise in pH was experienced in the presence of acetate. Tall oil on the other hand, was utilised poorly in the presence and absence of acetate while soap skimmings was utilised more in the presence of acetate than in its absence.

Interesting results were obtained when the residual fatty acyl profiles present in the supernatant were compared to the original oil substrate after seven days of cultivation (Table 23).

As expected, a significant decrease in the PUFAs was experienced after growth when this fungus was cultivated on various fat and oil substrates in the presence and absence of acetate while the saturated FAs remained mostly relatively high. This phenomenon again indicates a high preference of this fungus towards PUFAs utilisation. On the other hand, the α18:3 in linseed oil, used cooking oil, tall oil and soap skimmings was completely utilised in the presence and absence of acetate.

When comparing the fatty acyl profiles of the intracellular lipids extracted from this fungus with the original oil substrates in the presence and absence of acetate, the following results were obtained after seven days of growth (Table 24).

Again, a general decrease in the PUFAs of the intracellular lipid fractions was experienced. The saturated FAs on the other hand, remained relatively high when compared to the original oil substrates. Significant amounts of GLA (except for linseed oil) were produced when this fungus was utilising sunflower oil (2.4% w/w), and used cooking oil (6.5% w/w) in the presence of acetate. Here, the addition of acetate had a significant effect on GLA production (Jeffery *et al.,* 1997).

Table 22. Growth yield, lipid accumulation, utilisation and the final pH by *Rhizopus stolonifer* MUFS R008 grown on mixed substrates (oil and acetate) and substrate alone (oil).

Substrate	Growth yield (g dry wt/l)	Cellular lipid content (% w/w dry wt)	Residual lipids (g/l)	pH
Sunflower oil (40g/l)	14.0	65.5	3.5	3.8
Sunflower oil (30g/l) + Sodium acetate (10g/l)	11.0	27.7	10.5	5.0
Linseed oil (40g/l)	28.0	13.1	0.4	4.9
Linseed oil (30g/l) + Sodium acetate (10g/l)	30.0	14.9	15.4	5.4
Used cooking oil (40g/l)	6.0	31.5	10.6	4.3
Used cooking oil (30g/l) + Sodium acetate (10g/l)	7.5	35.0	15.2	5.6
Tall oil (40g/l)	1.0	2.0	27.4	5.9
Tall oil (30g/l) + Sodium acetate (10g/l)	9.5	4.5	25.5	6.9
Soap skimmings (40g/l)	1.0	80.5	10.6	7.6
Soap skimmings (30g/l) + Sodium acetate (10g/l)	1.0	57.5	7.0	7.4

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases.

Table 23. Fatty acyl profiles of the residual lipids of *Rhizopus stolonifer* MUFS R008 grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs Total FAs**
Sunflower oil (40g/l)	14.9	0.0	7.1	11.3	0.0	0.0	0.0	0.0
Sunflower oil (30g/l) + Sodium acetate (10g/l)	17.4	0.0	16.7	14.9	0.0	0.0	0.0	0.0
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	16.9	0.0	8.5	22.1	0.0	0.0	0.0	0.0
Linseed oil (30g/l) + Sodium acetate (10g/l)	21.3	0.0	17.0	12.7	0.0	0.0	0.0	0.0
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	3.0	19.0	8.3	22.8	0.0	0.0	0.0	0.0
Used cooking oil (30g/l) + Sodium acetate (10g/l)	2.7	15.3	8.9	31.6	0.0	0.0	0.0	0.0
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	13.6	0.0	2.6	36.3	0.0	0.0	0.0	0.0
Tall oil (30g/l) + Sodium acetate (10g/l)	15.2	0.0	6.3	35.5	0.0	0.0	0.0	0.0
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	16.9	0.0	2.8	31.5	0.0	0.0	0.0	0.0
Soap skimmings (30g/l) + Sodium acetate (10g/l)	16.4	0.0	3.6	32.7	0.0	0.0	0.0	0.0
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ).

Table 24. Fatty acyl profiles of cellular lipids and GLA production by *Rhizopus stolonifer* MUFS R008 grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs / Total FAs**
Sunflower oil (40g/l)	15.1	0.8	12.0	30.7	20.0	0.0	0.0	0.25
Sunflower oil (30g/l) + Sodium acetate (10g/l)	14.4	1.2	8.6	28.2	28.2	2.4	0.0	0.37
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	18.8	1.2	13.3	34.9	6.0	0.0	0.0	0.10
Linseed oil (30g/l) + Sodium acetate (10g/l)	18.1	1.0	12.6	35.1	5.7	0.6	1.1	0.08
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	12.5	1.6	7.4	39.9	6.3	0.0	0.0	0.09
Used cooking oil (30g/l) + Sodium acetate (10g/l)	9.7	5.0	2.3	32.3	33.5	6.5	2.4	0.46
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	14.2	2.8	8.0	31.8	7.7	0.0	0.0	0.12
Tall oil (30g/l) + Sodium acetate (10g/l)	11.6	3.2	4.6	29.1	18.7	0.0	0.0	0.28
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	13.7	0.0	5.2	23.1	0.0	0.0	0.0	0.0
Soap skimmings (30g/l) + Sodium acetate (10g/l)	12.8	0.0	4.0	23.2	5.8	0.0	0.0	0.13
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ).

***Thamnostylum* MUFS SAS025**

When comparing the growth yields, lipid accumulation, utilisation and the final pH of the growth medium in the presence and absence of acetate, the following results were obtained after seven days of growth (Table 25).

Biomass: When the fungus was cultivated on sunflower oil and on used cooking oil in the presence of acetate, it produced significant amounts of biomass. In this case, biomass production increased significantly from 2.5g/l to 19.5g/l on sunflower oil and from 3.5g/l to 21.0g/l on used cooking oil. The pH increase to neutrality probably played a role in the enhanced biomass production (Jeffery *et al.,* 1999). Similar trend was observed for *Mucor circinelloides f. circinelloides* CBS 108.16 and *Mucor circinelloides f. circinelloides* MUFS SAS045. When cultivated on linseed oil, tall oil and soap skimmings in the presence of acetate, a small increase in biomass production was obtained.

Cellular lipids: High amounts of cellular lipids were obtained on all the lipid substrates (except for linseed oil) in the presence of acetate. Here, the lipid content of the fungus increased from 42.1% w/w to 58.2% w/w on sunflower oil and from 12.5% w/w to 65.1% w/w on used cooking oil. When cultivated on tall oil and soap skimmings in the presence of acetate, the lipid content increased from 38.7% w/w to 64.5% w/w and from 36.3% w/w to 49.9% w/w respectively. Here, the addition of acetate had a large effect on cellular lipid production. When cultivated on linseed

oil in the presence of acetate, a small increase in lipid content was encountered, i.e. from 8.9% w/w to 10.0% w/w. When this fungus was cultivated on sodium acetate as sole carbon source, it produced only small amounts of cellular lipids, i.e. 6.4% w/w (Botha *et al.,* 1995).

Residual lipids: Sunflower oil and used cooking oil were utilised to a greater extend in the presence of acetate, i.e. 84.3% and 94.0% respectively. In this case, the pH increase to neutrality had probably an effect on lipid emulsification and utilisation (Jeffery *et al.*, 1999). On the other hand, linseed oil was utilised to a similar extend in the absence of acetate and on a mixture of substrates. Tall oil and soap skimmings were utilised much more in the absence of acetate than when acetate was added to the medium.

Interesting results were found in the presence and absence of acetate when the residual fatty acyl profiles and fatty acyl profiles of the original oil substrate were compared. (Table 26).

In the presence and absence of acetate, a general decrease in PUFAs after growth on various fat and oil substrates occurred while the saturated FAs in the medium remained mostly high. This phenomenon again indicates that the fungus prefers utilising PUFAs rather than the saturated FAs. In most cases, the 18:2 was significantly utilised (except for linseed oil) in the presence of acetate. These results are similar to that found by Jeffery et al (1999). Again, α18:3 in linseed oil,

used cooking oil, tall oil and soap skimmings was completely utilised in the presence and absence of acetate. These results are similar to that reported previously.

The fatty acyl profiles of the intracellular lipids in the presence and absence of acetate after growth on various fat and oil substrates were compared to the initial oil substrates after seven days of growth (Table 27).

A general decrease in the PUFAs was experienced in the intracellular lipids in the presence and absence of acetate while the saturated FAs remained high when compared to the original oil substrate. These results are similar to that reported previously. Small amounts of GLA were produced when cultivated on sunflower oil (0.7% w/w), used cooking oil (0.6% w/w) and tall oil (0.9% w/w) in the presence of acetate.

Table 25. Growth yield, lipid accumulation, utilisation and the final pH by *Thamnostylum* MUFS SAS025 grown on mixed substrates (oil and acetate) and substrate alone (oil).

Substrate	Growth yield (g dry wt/l)	Cellular lipid content (% w/w dry wt)	Residual lipids (g/l)	pH
Sunflower oil (40g/l)	2.5	42.1	21.9	3.9
Sunflower oil (30g/l) + Sodium acetate (10g/l)	19.5	58.2	4.7	7.5
Linseed oil (40g/l)	25.0	8.9	0.4	4.8
Linseed oil (30g/l) + Sodium acetate (10g/l)	27.5	10.0	0.3	5.4
Used cooking oil (40g/l)	3.5	12.5	22.6	5.6
Used cooking oil (30g/l) + Sodium acetate (10g/l)	21.0	65.1	1.8	7.4
Tall oil (40g/l)	3.0	38.7	19.7	5.1
Tall oil (30g/l) + Sodium acetate (10g/l)	7.5	64.5	15.5	7.0
Soap skimmings (40g/l)	2.0	36.3	14.6	7.2
Soap skimmings (30g/l) + Sodium acetate (10g/l)	3.0	49.9	12.2	7.6

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases.

Table 26. Fatty acyl profiles of the residual lipids of *Thamnostylum* MUFS SAS025 grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs / Total FAs**
Sunflower oil (40g/l)	11.0	0.0	6.1	35.8	34.2	0.0	0.0	0.39
Sunflower oil (30g/l) + Sodium acetate (10g/l)	11.9	0.0	7.3	28.5	32.4	0.0	0.0	0.40
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	17.2	0.0	9.3	35.8	7.9	0.0	0.0	0.11
Linseed oil (30g/l) + Sodium acetate (10g/l)	12.4	0.0	12.4	43.8	8.2	0.0	0.0	0.11
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	9.8	0.0	4.4	36.3	31.6	0.0	0.0	0.38
Used cooking oil (30g/l) + Sodium acetate (10g/l)	8.6	0.0	8.4	49.5	6.3	0.0	0.0	0.09
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.41**
Tall oil (40g/l)	6.4	0.0	1.6	30.4	13.7	0.0	0.0	0.26
Tall oil (30g/l) + Sodium acetate (10g/l)	5.6	0.0	1.6	27.6	9.2	0.0	0.0	0.21
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	6.4	0.0	1.3	30.5	17.3	0.0	0.0	0.31
Soap skimmings (30g/l) + Sodium acetate (10g/l)	6.6	0.0	1.3	29.8	14.9	0.0	0.0	0.28
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ).

Table 27. Fatty acyl profiles of cellular lipids and GLA production by *Thamnostylum* MUFS SAS025 grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs / Total FAs**
Sunflower oil (40g/l)	10.0	0.0	4.4	19.1	33.8	0.0	0.0	0.50
Sunflower oil (30g/l) + Sodium acetate (10g/l)	10.3	0.7	6.7	26.0	43.8	0.7	0.0	0.50
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	15.6	0.0	10.4	26.1	2.2	0.0	0.0	0.04
Linseed oil (30g/l) + Sodium acetate (10g/l)	14.7	0.0	10.7	26.4	4.4	0.0	0.0	0.08
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	7.8	0.0	3.6	30.7	24.2	0.0	0.0	0.37
Used cooking oil (30g/l) + Sodium acetate (10g/l)	9.7	0.0	3.6	48.4	23.1	0.6	0.3	0.28
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.41**
Tall oil (40g/l)	8.1	0.0	2.6	24.6	9.8	0.0	0.0	0.22
Tall oil (30g/l) + Sodium acetate (10g/l)	9.5	0.9	2.7	34.9	16.5	0.9	0.0	0.27
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	13.5	0.0	3.5	21.3	0.0	0.0	0.0	0.0
Soap skimmings (30g/l) + Sodium acetate (10g/l)	14.0	0.0	4.4	25.0	2.9	0.0	0.0	0.06
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ).

***Cryptococcus curvatus* CBS 0570 T**

When comparing the growth yields, lipid accumulation, utilisation and the final pH of the growth medium in the presence and absence of acetate, the following results were obtained (Table 28).

Biomass: The yeast grew on all the lipid substrates and produced similar low amounts of biomass in the presence and absence of acetate. When grown on linseed oil in the presence of acetate, a notable increase in biomass production was obtained, i.e. from 2.7g/l to 4.8g/l. Similar trends were observed on the other fats and oils except for used cooking oil.

Cellular lipids: A significant increase in cellular lipids was obtained in the presence of acetate when the yeast was cultivated on sunflower oil (from 5.5% w/w to 14.4% w/w), used cooking oil (from 13.9% w/w to 16.7% w/w) and soap skimmings (from 10.7% w/w to 21.9% w/w). When cultivated on linseed oil, a small increase in cellular lipid content was obtained in the presence of acetate, i.e. from 1.3% w/w to 3.5% w/w. On the other hand, when cultivated on tall oil in the presence of acetate, a decrease in cellular lipid occurred, i.e. from 11.1% w/w to 5.4% w/w. When *Cryptococcus albidus* and *Cryptococcus laurentii* were grown on ethanol as sole carbon source, they produced much more cellular lipids, i.e. 65% w/w and 22% w/w respectively (Ratledge, 1989).

Residual lipids: Linseed oil was utilised most efficiently and to a similar extend in the presence and absence of acetate, i.e. 94.3% and 96.0% respectively. Here, the addition of acetate had a small decreasing effect on lipid utilisation. Similar effects were obtained when the yeast was cultivated on the other fats and oils. When the yeast was grown on sunflower oil, used cooking oil and soap skimmings in the presence of acetate, it utilised the fat and oils less effectively. Tall oil was also utilised to a lesser extend in the presence of acetate, i.e. 11.7% compared to 46.3% in its absence. It is interesting to note that the pH was higher in the medium containing sunflower oil and used cooking oil in the presence of acetate. This may be attributed to the utilisation of acetic acid (Jeffery *et al.,* 1999).

Interesting results were found when the fatty acyl profiles of the residual lipids in the supernatant and the original oil substrate were compared after seven days of growth (Table 29).

In the presence and absence of acetate, a decrease in the PUFAs was observed while the saturated FAs remained relatively high in the supernatant when grown on sunflower oil, linseed oil and used cooking oil. Again, this is an indication of the preference of the yeast towards the utilisation of PUFAs as was found previously. With the exception of used cooking oil, the α18:3 present in linseed oil, tall oil and soap skimmings was completely utilised in the presence and absence of acetate. These results are similar to that reported earlier.

Interesting results were found concerning the fatty acyl profiles of the intracellular lipids compared to the original oil substrates in the presence and absence of acetate after seven days of growth (Table 30).

In all cases, a decrease in the intracellular PUFAs was experienced while the saturated FAs remained relatively high. When the yeast was cultivated on various fats and oils in the presence and absence of acetate, no GLA was produced. This was to be expected since members of the Dikaryomycota, i.e. Ascomycotina and Basidiomycotina and affiliated anamorphs are known not to produce GLA (Kock and Botha, 1998).

Table 28. Growth yield, lipid accumulation, utilisation and the final pH by *Cryptococcus curvatus* CBS 0570 T grown on mixed substrates (oil and acetate) and substrate alone (oil).

Substrate	Growth yield (g dry wt/l)	Cellular lipid content (% w/w dry wt)	Residual lipids (g/l)	pH
Sunflower oil (40g/l)	3.8	5.5	21.6	3.4
Sunflower oil (30g/l) + Sodium acetate (10g/l)	4.7	14.4	19.8	7.7
Linseed oil (40g/l)	2.7	1.3	1.6	4.5
Linseed oil (30g/l) + Sodium acetate (10g/l)	4.8	3.5	1.7	5.4
Used cooking oil (40g/l)	5.9	13.9	19.1	3.4
Used cooking oil (30g/l) + Sodium acetate (10g/l)	4.6	16.7	20.0	8.1
Tall oil (40g/l)	3.6	11.1	21.5	5.0
Tall oil (30g/l) + Sodium acetate (10g/l)	4.6	5.4	26.5	5.8
Soap skimmings (40g/l)	2.5	10.7	16.0	7.3
Soap skimmings (30g/l) + Sodium acetate (10g/l)	3.6	21.9	14.1	7.5

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

Table 29. Fatty acyl profiles of the residual lipids of *Cryptococcus curvatus* CBS 0570 T grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs / Total FAs**
Sunflower oil (40g/l)	9.4	0.0	7.4	26.3	37.5	0.0	0.0	0.47
Sunflower oil (30g/l) + Sodium acetate (10g/l)	11.7	0.0	7.6	22.9	33.1	0.0	0.0	0.44
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	17.2	0.0	15.5	34.1	7.5	0.0	0.0	0.10
Linseed oil (30g/l) + Sodium acetate (10g/l)	19.2	0.0	13.2	30.1	5.9	0.0	0.0	0.09
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	6.6	0.0	3.3	39.2	37.4	0.0	3.6	0.46
Used cooking oil (30g/l) + Sodium acetate (10g/l)	7.9	0.0	3.5	39.2	34.8	0.0	3.8	0.43
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	6.2	0.0	2.4	25.7	15.3	0.0	0.0	0.31
Tall oil (30g/l) + Sodium acetate (10g/l)	7.7	0.0	1.7	27.3	15.4	0.0	0.0	0.30
Original oil	**9.0**	**0.0**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	7.0	0.0	2.5	27.6	16.2	0.0	0.0	0.30
Soap skimmings (30g/l) + Sodium acetate (10g/l)	7.0	0.0	1.9	27.8	18.2	0.0	0.0	0.33
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ). CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

Table 30. Fatty acyl profiles of the cellular lipids and GLA production by *Cryptococcus curvatus* CBS 0570 T grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs / Total FAs**
Sunflower oil (40g/l)	11.8	0.0	13.7	27.5	24.3	0.0	0.0	0.31
Sunflower oil (30g/l) + Sodium acetate (10g/l)	12.7	0.0	9.1	20.1	38.5	0.0	0.0	0.48
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	15.2	0.0	8.7	7.2	0.0	0.0	0.0	0.0
Linseed oil (30g/l) + Sodium acetate (10g/l)	16.0	0.0	9.0	13.5	11.2	0.0	0.0	0.23
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	8.8	0.0	5.5	42.9	22.3	0.0	0.0	0.28
Used cooking oil (30g/l) + Sodium acetate (10g/l)	9.3	0.0	5.8	39.2	29.5	0.0	0.0	0.35
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	11.2	0.0	4.4	35.2	23.8	0.0	0.0	0.32
Tall oil (30g/l) + Sodium acetate (10g/l)	21.4	0.0	12.5	19.6	10.4	0.0	0.0	0.16
Original oil	**9.0**	**0.0**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	16.4	0.0	7.1	29.3	15.2	0.0	0.0	0.22
Soap skimmings (30g/l) + Sodium acetate (10g/l)	9.7	0.0	3.9	25.4	13.2	0.0	0.0	0.25
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ). CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

***Dipodascopsis uninucleata var. uninucleata* CBS 0190.37 T**

When comparing the growth yields, lipid accumulation, utilisation and the final pH of the growth medium in the presence and absence of acetate, the following results were obtained after seven days of cultivation (Table 31).

Biomass: In the presence and absence of acetate, the yeast produced similar amounts of biomass when cultivated on linseed oil, tall oil and soap skimmings. When cultivated on sunflower oil and used cooking oil in the presence of acetate, a small increase in biomass production was obtained, i.e. from 2.7g/l to 4.5g/l and from 2.4g/l to 5.1g/l respectively.

Cellular lipids: A significant increase in cellular lipids was obtained when the yeast was grown on tall oil in the presence of acetate, i.e. from 9.0% w/w to 16.3% w/w. On the other hand, when cultivated on the other fats and oils in the presence and absence of acetate, the lipid content remained similar except in the case of soap skimmings where the lipid content decreased significantly, i.e. from 28.8% w/w to 17.9% w/w when acetate was added. A small increase in cellular lipid content was found on used cooking oil in the presence of acetate. It is important to note that after 46 hours of growth, the yeast *Dipodascopsis uninucleata* UOFS Y-128 produced significantly smaller amounts of cellular lipids, i.e. 1.2% w/w when grown on glucose as carbon source (Kock and Ratledge, 1992).

Residual lipids: Sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings were utilised to a lesser extend in the presence of acetate compared to its absence. In all cases (except for linseed oil), the pH increased to above neutrality due to acetic acid utilisation (Jeffery *et al.,* 1999).

Next, the fatty acyl profiles of the residual lipids in the medium (i.e in the presence and absence of acetate) were compared to the fatty acyl profiles of the initial oil substrates (Table 32).

Again, a small decrease in the PUFAs of the residual lipids in the supernatant occurred in most cases when compared to the initial oil substrate. This phenomenon again indicates the preference of the yeast towards PUFA utilisation while the saturated FAs in general (with a few exceptions) remained relatively higher in the supernatant. With the exception of linseed oil and used cooking oil, the α18:3 was completely utilised in the presence and absence of acetate.

The fatty acyl profiles of the intracellular lipids were determined after growth on various fat and oil substrates in the presence and absence of acetate and compared to the fatty acyl profiles of the original oil substrates (Table 33).

Again, a decrease in the PUFAs present in the intracellular lipid fractions occurred in the presence and absence of acetate when compared to the original oil substrate after cultivation. The saturated FAs on the other hand, remained relatively high in

the intracellular lipid fractions when compared to the original oil substrate. As expected (Kock and Botha, 1998), no GLA was produced when cultivated on various fat and oil substrates in the presence and absence of acetate.

Table 31. Growth yield, lipid accumulation, utilisation and the final pH by *Dipodascopsis uninucleata var. uninucleata* CBS 0190.37 T grown on mixed substrates (oil and acetate) and substrate alone (oil).

Substrate	Growth yield (g dry wt/l)	Cellular lipid content (% w/w dry wt)	Residual lipids (g/l)	pH
Sunflower oil (40g/l)	2.7	3.6	28.5	6.1
Sunflower oil (30g/l) + Sodium acetate (10g/l)	4.5	3.6	28.2	8.9
Linseed oil (40g/l)	2.3	6.8	7.9	5.2
Linseed oil (30g/l) + Sodium acetate (10g/l)	3.3	6.7	6.9	5.5
Used cooking oil (40g/l)	2.4	3.2	28.6	6.1
Used cooking oil (30g/l) + Sodium acetate (10g/l)	5.1	4.1	27.7	9.0
Tall oil (40g/l)	4.9	9.0	20.9	5.3
Tall oil (30g/l) + Sodium acetate (10g/l)	4.5	16.3	22.3	7.8
Soap skimmings (40g/l)	2.7	28.8	11.3	7.6
Soap skimmings (30g/l) + Sodium acetate (10g/l)	3.5	17.9	11.3	7.7

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

Table 32. Fatty acyl profiles of the residual lipids of *Dipodascopsis uninucleata var. uninucleata* CBS 0190.37 T grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs / Total FAs**
Sunflower oil (40g/l)	8.6	0.0	5.4	19.8	57.1	0.0	0.0	0.63
Sunflower oil (30g/l) + Sodium acetate (10g/l)	8.9	0.0	4.9	19.9	55.7	0.0	0.0	0.62
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	14.9	0.0	10.4	32.5	10.0	0.0	7.5	0.23
Linseed oil (30g/l) + Sodium acetate (10g/l)	17.1	0.0	9.4	30.0	7.6	0.0	0.0	0.12
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	8.1	0.0	3.8	38.7	34.4	0.0	3.8	0.43
Used cooking oil (30g/l) + Sodium acetate (10g/l)	8.5	0.0	4.1	38.8	32.3	0.0	3.3	0.41
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	4.8	0.0	3.7	19.3	7.4	0.0	0.0	0.21
Tall oil (30g/l) + Sodium acetate (10g/l)	5.7	0.0	2.7	19.9	12.6	0.0	0.0	0.31
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	6.5	0.0	3.3	22.4	14.6	0.0	0.0	0.31
Soap skimmings (30g/l) + Sodium acetate (10g/l)	6.4	0.0	3.3	25.6	17.2	0.0	0.0	0.33
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ). CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

Table 33. Fatty acyl profiles of the cellular lipids and GLA production by *Dipodascopsis uninucleata var. uninucleata* CBS 0190. 37 T grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs / Total FAs**
Sunflower oil (40g/l)	15.2	0.0	4.4	32.7	26.3	0.0	0.0	0.33
Sunflower oil (30g/l) + Sodium acetate (10g/l)	14.1	1.1	4.0	20.7	40.4	0.0	0.0	0.50
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	20.8	2.2	5.0	23.7	14.0	0.0	0.0	0.21
Linseed oil (30g/l) + Sodium acetate (10g/l)	19.2	2.4	4.9	27.2	14.1	0.0	0.0	0.21
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	14.4	2.0	5.0	33.7	20.6	0.0	0.0	0.27
Used cooking oil (30g/l) + Sodium acetate (10g/l)	12.6	1.0	3.5	36.4	30.2	0.0	0.0	0.36
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	7.0	0.5	1.7	37.3	14.1	0.0	0.0	0.23
Tall oil (30g/l) + Sodium acetate (10g/l)	12.9	0.9	2.4	28.1	22.0	0.0	0.0	0.33
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	11.9	0.9	2.2	31.7	19.1	0.0	0.0	0.29
Soap skimmings (30g/l) + Sodium acetate (10g/l)	12.6	1.1	2.6	31.6	19.2	0.0	0.0	0.29
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ). CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

***Filobasidiella neoformans var. neoformans* CBS 0132**

When comparing the growth yields, lipid accumulation, utilisation and the final pH of the growth medium in the presence and absence of acetate, the following results were obtained after seven days of growth (Table 34).

Biomass: A small increase in biomass production was obtained on sunflower oil, linseed oil, used cooking oil and soap skimmings in the presence of acetate. When cultivated on tall oil in the presence of acetate, a decrease in biomass production occurred, i.e. from 7.7g/l to 5.6g/l. This is probably due to the presence of neutral substances that inhibited growth of this yeast on this substrate.

Cellular lipids: A significant increase in cellular lipids was obtained on sunflower oil (from 10.5% w/w to 17.1% w/w), linseed oil (from 7.1% w/w to 12.9% w/w), used cooking oil (from 19.3% w/w to 21.7% w/w) and tall oil (from 18.9% w/w to 44.6% w/w) in the presence of acetate. Here, the addition of acetate had an effect on cellular lipid production. When cultivated on soap skimmings, the yeast produced similar amounts of cellular lipids in the presence or absence of acetate, i.e. 26.9% w/w.

Residual lipids: Linseed oil was utilised more efficiently in the absence of acetate than in its presence, i.e. 97.0%. Sunflower oil, used cooking oil, tall oil and soap skimmings were utilised to a smaller extend in the presence of acetate compared to

when acetate was not added. In most cases, except for soap skimmings, the pH increased due to acetic acid utilisation.

The fatty acyl profiles of the residual lipids in the supernatant were obtained in the presence and absence of acetate and compared to the fatty acyl profiles of the initial oil substrate after growth on various lipid substrates (Table 35).

Again, a decrease in the PUFAs of the residual lipids was experienced in the presence and absence of acetate when compared to the initial oil substrates while the saturated FAs remained relatively high in most lipid substrates. This phenomenon again indicates a preference of the yeast for PUFA utilisation. With the exception of used cooking oil, the α18:3 present in linseed oil, tall oil and soap skimmings was completely utilised in the presence and absence of acetate.

The fatty acyl profiles of the intracellular lipids were also determined after the yeast was cultivated on the various fat and oil substrates in the presence and absence of acetate and compared to the initial oil substrate (Table 36).

A decrease in the PUFAs, which were preferably utilised, occurred in most of the cellular lipids when compared to the original oil substrate. In the presence and absence of acetate, the saturated FAs of the intracellular lipids remained high when compared to the original oil substrates. Since the yeast does not possess the Δ^6 desaturase enzyme, no GLA was produced on any of the fat and oil substrates in

the presence and absence of acetate. These results are similar to that reported earlier for the yeasts.

Table 34. Growth yield, lipid accumulation, utilisation and the final pH by *Filobasidiella neoformans var. neoformans* CBS 0132 grown on mixed substrates (oil and acetate) and substrate alone (oil).

Substrate	Growth yield (g dry wt/l)	Cellular lipid content (% w/w dry wt)	Residual lipids (g/l)	pH
Sunflower oil (40g/l)	2.3	10.5	28.2	6.1
Sunflower oil (30g/l) + Sodium acetate (10g/l)	3.5	17.1	23.2	8.4
Linseed oil (40g/l)	1.4	7.1	1.2	5.1
Linseed oil (30g/l) + Sodium acetate (10g/l)	1.9	12.9	6.8	7.5
Used cooking oil (40g/l)	1.8	19.3	27.8	6.1
Used cooking oil (30g/l) + Sodium acetate (10g/l)	2.8	21.7	25.1	8.5
Tall oil (40g/l)	7.7	18.9	22.2	5.6
Tall oil (30g/l) + Sodium acetate (10g/l)	5.6	44.6	17.3	7.5
Soap skimmings (40g/l)	0.9	26.9	16.0	7.5
Soap skimmings (30g/l) + Sodium acetate (10g/l)	2.4	26.9	16.5	7.5

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

Table 35. Fatty acyl profiles of the residual lipids of *Filobasidiella neoformans var. neoformans* CBS 0132 grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs / Total FAs**
Sunflower oil (40g/l)	10.3	0.0	8.9	16.4	44.2	0.0	0.0	0.55
Sunflower oil (30g/l) + Sodium acetate (10g/l)	9.6	0.0	5.7	19.1	58.1	0.0	0.0	0.63
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	16.3	0.0	16.4	18.4	4.4	0.0	0.0	0.08
Linseed oil (30g/l) + Sodium acetate (10g/l)	18.5	0.0	14.6	31.1	5.4	0.0	0.0	0.08
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	10.2	0.0	5.2	35.0	32.1	0.0	3.5	0.41
Used cooking oil (30g/l) + Sodium acetate (10g/l)	8.9	0.0	4.3	38.1	35.0	0.0	3.8	0.43
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	10.1	0.0	10.6	14.6	5.7	0.0	0.0	0.14
Tall oil (30g/l) + Sodium acetate (10g/l)	8.3	0.0	8.0	19.9	6.3	0.0	0.0	0.15
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	11.3	0.0	11.6	14.0	6.5	0.0	0.0	0.15
Soap skimmings (30g/l) + Sodium acetate (10g/l)	8.2	0.0	5.1	22.7	12.5	0.0	0.0	0.26
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ). CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

Table 36. Fatty acyl profiles of the cellular lipids and GLA production by *Filobasidiella neoformans var. neoformans* CBS 0132 grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs Total FAs**
Sunflower oil (40g/l)	13.1	0.0	5.5	21.1	30.6	0.0	0.0	0.43
Sunflower oil (30g/l) + Sodium acetate (10g/l)	9.4	0.0	5.9	19.4	50.3	0.0	0.0	0.59
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	15.5	0.0	8.2	10.8	0.0	0.0	0.0	0.0
Linseed oil (30g/l) + Sodium acetate (10g/l)	13.1	0.0	6.2	25.1	14.2	0.0	0.0	0.24
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	11.2	0.0	5.2	30.7	20.6	0.0	0.0	0.30
Used cooking oil (30g/l) + Sodium acetate (10g/l)	9.4	0.0	4.4	39.1	31.8	0.0	0.0	0.38
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	9.1	0.0	3.0	32.9	13.4	0.0	0.0	0.23
Tall oil (30g/l) + Sodium acetate (10g/l)	9.5	0.8	1.8	30.1	23.6	0.0	0.0	0.36
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	12.8	0.0	3.9	27.8	14.2	0.0	0.0	0.24
Soap skimmings (30g/l) + Sodium acetate (10g/l)	13.9	0.0	3.5	29.0	16.1	0.0	0.0	0.26
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ). CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

***Galactomyces geotrichum* CBS 0772.71 T**

When comparing the growth yields, cellular lipid content, utilisation and the final pH of the growth medium in the presence and absence of acetate, the following results were obtained after seven days of growth (Table 37).

Biomass: The highest growth yield was obtained on sunflower oil and on used cooking oil in the presence of acetate. In this case, the addition of acetate led to a significant enhancing effect on biomass production, i.e. biomass production increased from 15.0g/l to 20.9g/l on sunflower oil and increased from 10.2g/l to 23.7g/l on used cooking oil. When cultivated on linseed oil (from 6.9g/l to 15.0g/l) and on soap skimmings (from 8.2g/l to 11.5g/l) in the presence of acetate, a significant increase in biomass production was also obtained. On the other hand, the yeast produced similar low amounts of biomass when cultivated on tall oil in the presence and absence of acetate, i.e. 7.4g/l and 7.1g/l respectively.

Cellular lipids: Increased cellular lipids were obtained on linseed oil and on used cooking oil in the presence of acetate. In both cases, the addition of acetate led to a significant enhancing effect on cellular lipid production where it increased from 18.6% w/w to 23.6% w/w on linseed oil and increased from 13.7% w/w to 19.5% w/w on used cooking oil. When cultivated on the other fats and oils, the lipid content decreased in the presence of acetate. However, when *Galactomyces geotrichum*

was cultivated on lipid or non-lipid material, it produced much higher cellular lipids, i.e. about 50% w/w (Ratledge, 1989).

Residual lipids: Linseed oil was utilised most effectively in the absence of acetate, i.e. 96.0% compared to 93.7% in its presence. When grown on sunflower oil and on used cooking oil, it utilised these fats and oils more efficiently in the presence of acetate than in its absence. In both cases, the pH was significantly higher due to acetic acid utilisation. This may probably have played a role in lipid emulsification and hence its utilisation (Jeffery *et al.,* 1999).

The fatty acyl profiles of the residual lipids present in the supernatant were analysed after seven days of growth in the presence and absence of acetate and compared to the fatty acyl profiles of the initial oil substrate (Table 38).

In the presence and absence of acetate, a significant decrease in the PUFAs was observed in all the residual lipid fractions after growth when compared to the original oil substrates while on the other hand, the saturated FAs remained high in most of the lipid substrates. Again, this phenomenon indicates a high preference of the yeast towards PUFA utilisation. The 18:2 present in all the lipid substrates was significantly utilised in the presence of acetate. These results are in accordance to that found by Jeffery et al (1999). The α18:3 on the other hand, was completely utilised in linseed oil, used cooking oil, tall oil and soap skimmings in the presence and absence of acetate.

The fatty acyl profiles of the intracellular lipid fractions were also determined after growth on various lipid substrates in the presence and absence of acetate and compared to the fatty acyl profiles of the original oil substrate (Table 39).

As expected, a decrease in the PUFAs of the intracellular lipid fractions occurred in the presence and absence of acetate when compared to the original oil substrate while the saturated FAs remained relatively high. No GLA was produced in the presence and absence of acetate when cultivated on all the lipid substrates. This is in accordance with literature (Kock and Botha, 1998).

Table 37. Growth yield, lipid accumulation, utilisation and the final pH by *Galactomyces geotrichum* CBS 0772.71 T grown on mixed substrates (oil and acetate) and substrate alone (oil).

Substrate	Growth yield (g dry wt/l)	Cellular lipid content (% w/w dry wt)	Residual lipids (g/l)	pH
Sunflower oil (40g/l)	15.0	27.2	11.9	2.7
Sunflower oil (30g/l) + Sodium acetate (10g/l)	20.9	17.8	2.1	7.0
Linseed oil (40g/l)	6.9	18.6	1.6	2.9
Linseed oil (30g/l) + Sodium acetate (10g/l)	15.0	23.6	1.9	6.9
Used cooking oil (40g/l)	10.2	13.7	16.1	2.8
Used cooking oil (30g/l) + Sodium acetate (10g/l)	23.7	19.5	1.4	7.1
Tall oil (40g/l)	7.1	12.4	19.4	3.9
Tall oil (30g/l) + Sodium acetate (10g/l)	7.4	12.2	16.3	7.4
Soap skimmings (40g/l)	8.2	21.7	3.5	7.3
Soap skimmings (30g/l) + Sodium acetate (10g/l)	11.5	19.8	5.3	9.3

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

Table 38. Fatty acyl profiles of the residual lipids of *Galactomyces geotrichum* CBS 0772.71 T grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs Total FAs**
Sunflower oil (40g/l)	10.2	0.0	10.7	20.4	43.9	0.0	0.0	0.52
Sunflower oil (30g/l) + Sodium acetate (10g/l)	10.0	0.0	12.7	22.7	15.4	0.0	0.0	0.25
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	15.1	0.0	14.1	20.8	12.8	0.0	0.0	0.20
Linseed oil (30g/l) + Sodium acetate (10g/l)	16.1	0.0	16.2	21.4	6.1	0.0	0.0	0.10
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	10.0	0.0	6.7	37.5	26.8	0.0	0.0	0.33
Used cooking oil (30g/l) + Sodium acetate (10g/l)	10.8	0.0	15.3	20.9	7.6	0.0	0.0	0.14
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	4.9	0.0	5.4	16.1	4.4	0.0	0.0	0.14
Tall oil (30g/l) + Sodium acetate (10g/l)	3.8	0.0	5.0	6.7	2.1	0.0	0.0	0.12
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	7.8	0.0	9.5	9.9	2.8	0.0	0.0	0.09
Soap skimmings (30g/l) + Sodium acetate (10g/l)	5.6	0.0	6.6	4.5	2.3	0.0	0.0	0.12
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ). CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

Table 39. Fatty acyl profiles of the cellular lipids and GLA production by *Galactomyces geotrichum* CBS 0772.71 T grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs Total FAs**
Sunflower oil (40g/l)	16.3	0.0	7.5	26.6	14.4	0.0	0.0	0.22
Sunflower oil (30g/l) + Sodium acetate (10g/l)	7.2	0.0	3.5	19.8	62.2	0.0	0.0	0.67
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	19.8	0.0	10.6	18.7	5.4	0.0	0.0	0.10
Linseed oil (30g/l) + Sodium acetate (10g/l)	9.1	0.0	4.2	34.6	26.8	0.0	0.0	0.36
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	11.8	0.0	6.3	33.9	24.1	0.0	0.0	0.32
Used cooking oil (30g/l) + Sodium acetate (10g/l)	6.2	0.0	9.9	12.6	40.5	0.0	0.0	0.59
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	13.3	0.0	5.0	23.5	11.4	0.0	0.0	0.21
Tall oil (30g/l) + Sodium acetate (10g/l)	21.6	0.0	9.9	12.6	8.5	0.0	0.0	0.16
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	12.3	0.0	5.0	26.5	11.7	0.0	0.0	0.21
Soap skimmings (30g/l) + Sodium acetate (10g/l)	18.7	0.0	7.6	13.7	8.8	0.0	0.0	0.18
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ). CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

***Kluyveromyces marxianus var. marxianus* CBS 1556**

When comparing growth yields, lipid accumulation, utilisation and the final pH of the growth medium in the presence and absence of acetate, the following results were obtained after seven days of growth (Table 40).

Biomass: The yeast produced small and almost similar amounts of biomass when cultivated on linseed oil in the presence and absence of acetate, i.e. 0.3g/l and 0.5g/l respectively. When cultivated on sunflower oil, used cooking oil, tall oil and soap skimmings in the presence of acetate, a small increase on biomass production was obtained.

Cellular lipids: The highest cellular lipids were obtained on tall oil (27.6% w/w) and on soap skimmings (36.1% w/w) in the presence of acetate. Here, the addition of acetate led to an increase in cellular lipid contents, i.e. from 24.4% w/w to 27.6% w/w on tall oil and from 28.8% w/w to 36.1% w/w on soap skimmings. When cultivated on sunflower oil and linseed oil in the presence of acetate, a decrease in the cellular lipids was obtained, i.e. from 12.9% w/w to 11.1% w/w on sunflower oil and from 19.5% w/w to 6.9% w/w on linseed oil while a significant increase in cellular lipid was obtained on used cooking oil in the presence of acetate. It was found that *Kluyveromyces polysporus* produced as high as 10.7% w/w of cellular lipid when cultivated on glucose as sole carbon source on an agar plate (Ratledge, 1989).

Residual lipids: Sunflower oil and used cooking oil were utilised more efficiently in the absence of acetate than in its presence while linseed oil was utilised almost to a similar extend in the presence and absence of acetate, i.e. 95.7% and 96.5% respectively. Tall oil was utilised less efficiently in the presence of acetate than in its absence, i.e. 16.7% compared to 39.0% in its absence. Soap skimmings on the other hand, was utilised more in the presence of acetate than in its absence.

Interesting results concerning the fatty acyl profiles present in the supernatant were obtained in the presence and absence of acetate after seven days of cultivation when compared to the fatty acyl profiles of the initial oil substrate (Table 41).

In general, a significant decrease in the PUFAs present in the supernatant was experienced in the presence and absence of acetate when compared to the fatty acyl profiles of the original oil substrate while the saturated FAs remained relatively high. This phenomenon again indicates the high preference of the yeast towards PUFA utilisation. The α18:3 present in linseed oil, used cooking oil, tall oil and soap skimmings was completely utilised in the presence and absence of acetate. These results are similar to that reported earlier.

The fatty acyl profiles of the intracellular lipid fractions after growth on various fat and oil substrates in the presence and absence of acetate were compared to the fatty acyl profiles of the initial oil substrate (Table 42).

In the presence and absence of acetate, a significant decrease in the PUFAs present in the cellular lipid fractions was experienced when compared to the original oil substrate while the saturated FAs remained relatively high. On the other hand, when this yeast was cultivated on various fat and oil substrates in the presence and absence of acetate, no GLA was produced. This is again similar to the results found previously.

Table 40. Growth yield, lipid accumulation, utilisation and the final pH by *Kluyveromyces marxianus var. marxianus* CBS 1556 grown on mixed substrates (oil and acetate) and substrate alone (oil).

Substrate	Growth yield (g dry wt/l)	Cellular lipid content (% w/w dry wt)	Residual lipids (g/l)	pH
Sunflower oil (40g/l)	0.2	12.9	5.5	5.7
Sunflower oil (30g/l) + Sodium acetate (10g/l)	1.3	11.1	8.3	7.5
Linseed oil (40g/l)	0.5	19.5	1.4	4.7
Linseed oil (30g/l) + Sodium acetate (10g/l)	0.3	6.9	1.3	5.4
Used cooking oil (40g/l)	0.4	8.5	11.5	5.2
Used cooking oil (30g/l) + Sodium acetate (10g/l)	1.9	11.3	25.0	8.1
Tall oil (40g/l)	0.9	24.4	24.4	6.0
Tall oil (30g/l) + Sodium acetate (10g/l)	2.0	27.6	25.0	7.3
Soap skimmings (40g/l)	1.2	28.8	14.3	7.5
Soap skimmings (30g/l) + Sodium acetate (10g/l)	1.7	36.1	8.6	8.9

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

Table 41. Fatty acyl profiles of the residual lipids of *Kluyveromyces marxianus var. marxianus* CBS 1556 grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs / Total FAs**
Sunflower oil (40g/l)	14.9	0.0	9.8	32.1	12.1	0.0	0.0	0.18
Sunflower oil (30g/l) + Sodium acetate (10g/l)	16.4	0.0	10.2	35.1	13.1	0.0	0.0	0.18
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	17.0	0.0	12.9	31.4	6.3	0.0	0.0	0.10
Linseed oil (30g/l) + Sodium acetate (10g/l)	17.1	0.0	13.3	38.9	7.5	0.0	0.0	0.10
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	12.8	0.0	5.8	53.0	4.1	0.0	0.0	0.05
Used cooking oil (30g/l) + Sodium acetate (10g/l)	11.1	0.0	4.7	44.8	17.7	0.0	0.0	0.23
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	6.8	0.0	2.3	24.4	14.2	0.0	0.0	0.30
Tall oil (30g/l) + Sodium acetate (10g/l)	6.6	0.0	3.1	21.4	12.6	0.0	0.0	0.29
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	6.6	0.0	2.5	26.2	15.5	0.0	0.0	0.31
Soap skimmings (30g/l) + Sodium acetate (10g/l)	7.2	0.0	3.9	22.8	12.7	0.0	0.0	0.27
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ). CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

Table 42. Fatty acyl profiles of the cellular lipids and GLA production by *Kluyveromyces marxianus var. marxianus* CBS 1556 grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs / Total FAs**
Sunflower oil (40g/l)	15.1	0.0	12.4	20.3	8.5	0.0	0.0	0.15
Sunflower oil (30g/l) + Sodium acetate (10g/l)	17.7	4.8	8.4	20.4	16.7	0.0	0.0	0.25
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	16.9	7.4	13.7	16.6	11.9	0.0	0.0	0.18
Linseed oil (30g/l) + Sodium acetate (10g/l)	17.6	6.3	12.9	12.7	7.8	0.0	0.0	0.14
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	16.4	7.4	14.4	11.8	14.2	0.0	0.0	0.22
Used cooking oil (30g/l) + Sodium acetate (10g/l)	12.3	2.6	5.6	33.0	23.0	0.0	0.0	0.30
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	9.9	2.8	3.5	26.9	20.5	0.0	0.0	0.32
Tall oil (30g/l) + Sodium acetate (10g/l)	8.3	2.4	2.0	28.0	28.4	0.0	0.0	0.41
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	11.1	1.9	3.4	23.8	18.7	0.0	0.0	0.32
Soap skimmings (30g/l) + Sodium acetate (10g/l)	9.9	2.1	4.2	24.2	21.2	0.0	0.0	0.34
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ). CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

***Lipomyces starkeyi* CBS 1807 T**

It is important to note that this yeast did not show any growth on any of the lipid substrates in the presence and absence of acetate after cultivation at 30°C.

Saccharomyces cerevisiae CBS 1171 NT

When comparing the growth yields, cellular lipid content, utilisation and the final pH of the growth medium in the presence and absence of acetate, the following results were obtained after seven days of growth (Table 43).

Biomass: The presence of acetate led to a small increase in biomass production when this yeast was cultivated on sunflower oil, linseed oil, used cooking oil and soap skimmings. In this case, biomass production increased from 1.5g/l to 2.5g/l on sunflower oil and from 2.6g/l to 4.4g/l on linseed oil, while on used cooking oil and soap skimmings, it increased from 0.8g/l to 2.6g/l and from 1.8g/l to 2.2g/l respectively. When cultivated on tall oil in the absence of acetate, an increase in biomass occurred, i.e from 1.0 g/l to 1.6g/l respectively.

Cellular lipids: The highest cellular lipid yield was obtained on soap skimmings in the presence of acetate. In this case, the addition of acetate led to an increase in lipid production, i.e. from 14.3% w/w to 27.0% w/w. When cultivated on the other lipid substrates in the presence of acetate, a small increase in lipid content was obtained except in the case of used cooking oil and tall oil where the cellular lipid content decreased from 19.3% w/w to 13.4% w/w and 17.6% w/w to 13.0% w/w respectively. When *Saccharomyces cerevisiae* CBS 1171 NT was cultivated in a medium containing glucose as sole carbon source, it produced only 6.3% w/w cellular lipids (Morakile, 1998).

Residual lipids: All fats and oils were utilised less efficiently in the presence of acetate. In most cases, the pH was significantly higher (i.e. above neutral) in the presence of acetate.

Next, the results of the fatty acyl profiles of the residual lipids in the supernatant were compared to the fatty acyl profiles of the original oil substrate after seven days of growth (Table 44).

In most cases, a significant decrease in the PUFAs in the supernatant was again experienced in the presence and absence of acetate. This phenomenon indicates a preference of the yeast towards PUFAs utilisation. On the other hand, the saturated FAs remained in many cases relatively high in the supernatant. With the exception of linseed oil and used cooking oil, the α18:3 was completely utilised in the presence and absence of acetate.

When comparing the fatty acyl profiles of the intracellular lipids in the presence and absence of acetate after growth on various fat and oil substrates with the original oil substrate, the following results were obtained (Table 45).

As expected, a general decrease in the PUFAs of the cellular lipid was experienced in the presence and absence of acetate when compared to the fatty acyl profiles of the original oil substrate while the saturated FAs were relatively high in many cases. On the other hand, GLA was not produced when the yeast was cultivated on various

fat and oil substrates in the presence and absence of acetate. These results are similar to that found earlier for the yeasts.

Table 43. Growth yield, lipid accumulation, utilisation and the final pH when *Saccharomyces cerevisiae* CBS 1171 NT is grown on mixed substrates (oil and acetate) and substrate alone (oil).

Substrate	Growth yield (g dry wt/l)	Cellular lipid content (% w/w dry wt)	Residual lipids (g/l)	pH
Sunflower oil (40g/l)	1.5	11.3	25.0	6.1
Sunflower oil (30g/l) + Sodium acetate (10g/l)	2.5	14.2	18.8	7.9
Linseed oil (40g/l)	2.6	6.5	3.4	5.3
Linseed oil (30g/l) + Sodium acetate (10g/l)	4.4	9.0	3.8	5.7
Used cooking oil (40g/l)	0.8	19.3	29.3	6.0
Used cooking oil (30g/l) + Sodium acetate (10g/l)	2.6	13.4	28.4	8.2
Tall oil (40g/l)	1.6	17.6	25.7	5.9
Tall oil (30g/l) + Sodium acetate (10g/l)	1.0	13.0	24.6	7.4
Soap skimmings (40g/l)	1.8	14.3	17.3	7.4
Soap skimmings (30g/l) + Sodium acetate (10g/l)	2.2	27.0	15.3	7.4

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

Table 44. Fatty acyl profiles of the residual lipids of *Saccharomyces cerevisiae* CBS 1171 NT grown on sunflower oil, linseed oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs / Total FAs**
Sunflower oil (40g/l)	10.7	0.0	2.1	22.1	38.5	0.0	0.0	0.52
Sunflower oil (30g/l) + Sodium acetate (10g/l)	13.3	0.0	8.4	23.9	56.4	0.0	0.0	0.55
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	15.4	0.0	10.2	29.7	9.9	0.0	6.4	0.23
Linseed oil (30g/l) + Sodium acetate (10g/l)	17.0	0.0	11.7	33.3	10.1	0.0	6.9	0.22
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	10.0	0.0	4.5	38.7	18.7	0.0	3.4	0.29
Used cooking oil (30g/l) + Sodium acetate (10g/l)	8.7	0.0	4.1	37.2	32.4	0.0	3.3	0.42
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	5.8	0.0	2.1	19.6	12.5	0.0	0.0	0.31
Tall oil (30g/l) + Sodium acetate (10g/l)	6.2	0.0	2.4	22.1	14.0	0.0	0.0	0.31
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	6.3	0.0	2.5	23.1	15.1	0.0	0.0	0.32
Soap skimmings (30g/l) + Sodium acetate (10g/l)	5.5	0.0	2.1	22.1	14.2	0.0	0.0	0.32
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ). CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

Table 45. Fatty acyl profiles of the cellular lipids and GLA production by *Saccharomyces cerevisiae* CBS 1171 NT grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs / Total FAs**
Sunflower oil (40g/l)	13.4	6.4	6.2	23.4	8.7	0.0	0.0	0.15
Sunflower oil (30g/l) + Sodium acetate (10g/l)	13.4	10.7	5.3	21.3	12.8	0.0	0.0	0.20
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	16.6	7.3	7.3	24.6	5.6	0.0	0.0	0.09
Linseed oil (30g/l) + Sodium acetate (10g/l)	15.8	13.7	5.6	26.1	4.9	0.0	0.0	0.07
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	13.2	19.1	5.1	39.0	4.1	0.0	0.0	0.05
Used cooking oil (30g/l) + Sodium acetate (10g/l)	10.3	18.1	3.6	33.4	12.6	0.0	0.0	0.16
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	11.2	5.3	4.1	29.3	11.3	0.0	0.0	0.18
Tall oil (30g/l) + Sodium acetate (10g/l)	15.2	3.6	3.6	24.5	11.3	0.0	0.0	0.19
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	13.3	1.4	2.6	31.4	12.0	0.0	0.0	0.20
Soap skimmings (30g/l) + Sodium acetate (10g/l)	13.6	0.0	3.9	28.5	10.3	0.0	0.0	0.18
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ). CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

***Schizosaccharomyces pombe var. pombe* CBS 0356 T**

When comparing the growth yields, lipid accumulation, utilisation and the final pH of the growth medium in the presence and absence of acetate, the following results were obtained (Table 46).

Biomass: It is important to note that this yeast did not growth on linseed oil in the presence or absence of acetate after cultivation. When cultivated on the other fats and oils in the presence and absence of acetate, it produced significantly smaller amounts of biomass compared to the previous.

Cellular lipids: The highest cellular lipid content was obtained on soap skimmings in the presence of acetate, i.e. 27.6% w/w. Here, the addition of acetate led to an increase in cellular lipid production. When cultivated on the other fats and oils in the presence and absence of acetate, the lipid content remained almost similar. When *Schizosaccharomyces pombe var. pombe* CBS 0356 T was cultivated in a medium containing glucose as carbon source, it produced 10.0% w/w cellular lipid (Jeffery, 1995).

Residual lipids: In general, sunflower oil, used cooking oil and tall oil were utilised to a much lesser extend in the presence of acetate. Also, when cultivated on soap skimmings in the absence of acetate, the yeast utilised the fat or oil more, i.e. 60.3% compared to 47.7% in the presence of acetate. The fatty acyl profiles of the residual

lipids in the supernatant were again analysed and compared to the fatty acyl profiles of the original oil substrate (Table 47).

As expected from the previous results, a general decrease in the PUFA present in the supernatant was experienced in the presence and absence of acetate when compared to the original oil substrate while the saturated FAs especially 16:0 were relatively high when grown on sunflower oil and used cooking oil. This phenomenon again, indicates a preference of the yeast towards PUFA utilisation. The α18:3 was completely utilised (except in used cooking oil) in the presence and absence of acetate. These results are similar to that found for the other yeasts

Next, the fatty acyl profiles of the intracellular lipids were also determined after growth on various fat and oil substrates in the presence and absence of acetate and compared to the fatty acyl profiles of the original oil substrates (Table 48).

Again, as expected a decrease in the cellular PUFAs occurred while the saturated FAs were generally relatively high when compared to the original oil substrate. No GLA was produced after growth on any of the fats and oils in the presence and absence of acetate. Again, these results are similar to that reported earlier.

Table 46. Growth yield, lipid accumulation, utilisation and the final pH when *Schizosaccharomyces pombe var. pombe* CBS 0356 T was grown on mixed substrates (oil and acetate) and substrate alone (oil).

Substrate	Growth yield (g dry wt/l)	Cellular lipid content (% w/w dry wt)	Residual lipids (g/l)	pH
Sunflower oil (40g/l)	0.6	6.0	29.4	5.2
Sunflower oil (30g/l) + Sodium acetate (10g/l)	0.7	6.6	27.6	6.0
Linseed oil (40g/l)	-	-	-	-
Linseed oil (30g/l) + Sodium acetate (10g/l)	-	-	-	-
Used cooking oil (40g/l)	0.9	6.5	29.1	6.2
Used cooking oil (30g/l) + Sodium acetate (10g/l)	0.9	5.2	27.8	6.1
Tall oil (40g/l)	0.8	7.7	27.3	6.5
Tall oil (30g/l) + Sodium acetate (10g/l)	0.6	5.5	26.7	6.5
Soap skimmings (40g/l)	0.8	21.8	15.9	7.6
Soap skimmings (30g/l) + Sodium acetate (10g/l)	2.3	27.6	15.7	7.5

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. - = No growth. CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

Table 47. Fatty acyl profiles of the residual lipids of *Schizosaccharomyces pombe var. pombe* CBS 0356 T grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs Total FAs**
Sunflower oil (40g/l)	15.7	0.0	7.9	34.3	8.3	0.0	0.0	0.13
Sunflower oil (30g/l) + Sodium acetate (10g/l)	12.1	0.0	5.6	28.0	34.3	0.0	0.0	0.43
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Linseed oil (30g/l) + Sodium acetate (10g/l)	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	8.1	0.0	3.4	39.8	35.9	0.0	3.8	0.44
Used cooking oil (30g/l) + Sodium acetate (10g/l)	8.1	0.0	3.4	38.7	36.4	0.0	4.3	0.45
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	6.2	0.0	1.2	27.6	17.1	0.0	0.0	0.33
Tall oil (30g/l) + Sodium acetate (10g/l)	6.5	0.0	1.1	25.6	16.2	0.0	0.0	0.33
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	6.3	0.0	0.9	27.2	17.5	0.0	0.0	0.34
Soap skimmings (30g/l) + Sodium acetate (10g/l)	5.9	0.0	0.9	27.3	17.4	0.0	0.0	0.34
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ). N/D = Not determent. CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

Table 48. Fatty acyl profiles of the cellular lipids and GLA production by *Schizosaccharomyces pombe var. pombe* CBS 0356 T grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs Total FAs**
Sunflower oil (40g/l)	12.1	0.0	5.4	29.0	15.3	0.0	0.0	0.25
Sunflower oil (30g/l) + Sodium acetate (10g/l)	11.0	0.0	4.8	27.3	39.7	0.0	0.0	0.48
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Linseed oil (30g/l) + Sodium acetate (10g/l)	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	10.6	0.0	4.3	38.7	27.5	0.0	0.0	0.34
Used cooking oil (30g/l) + Sodium acetate (10g/l)	9.7	0.0	4.5	39.4	30.5	0.0	0.0	0.36
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	9.4	0.0	10.0	28.5	12.6	0.0	0.0	0.21
Tall oil (30g/l) + Sodium acetate (10g/l)	9.2	0.0	2.4	31.9	14.3	0.0	0.0	0.25
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	11.3	0.0	2.2	30.0	10.3	0.0	0.0	0.19
Soap skimmings (30g/l) + Sodium acetate (10g/l)	10.3	0.0	2.6	32.8	10.8	0.0	0.0	0.19
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ). N/D = Not determent. CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

***Shwanniomyces occidentalis var. occidentalis* CBS 2863**

When comparing the growth yields, lipid accumulation, utilisation and the final pH of the growth medium in the presence and absence of acetate, the following results were obtained after seven days of cultivation (Table 49).

Biomass: A significant increase in biomass production was obtained on tall oil in the presence of acetate, i.e. from 3.9g/l to 19.1g/l. However, when cultivated on the other fats and oils in the presence of acetate, only a small increase in biomass production was obtained except for soap skimmings, where biomass production decreased from 7.7g/l to 4.3g/l.

Cellular lipids: The highest cellular lipid was obtained on sunflower oil and on tall oil in the presence of acetate, i.e. 24.2% w/w and 39.6% w/w respectively. When cultivated on linseed oil, used cooking oil and soap skimmings in the presence of acetate, the lipid content decreased drastically, i.e. from 25.6% w/w to 9.7% w/w on linseed oil, from 29.2% w/w to 10.1% w/w on used cooking oil and from 31.7% w/w to 13.4% w/w on soap skimmings. When cultivated in a medium containing glucose as sole carbon source, it produced 23.0% w/w of cellular lipid (Ratledge, 1989).

Residual lipids: Sunflower oil and tall oil were utilised to a greater extend in the presence of acetate, i.e. 51.0% and 80.7% respectively. In both cases, the pH was significantly higher reaching neutrality due to acetic acid utilisation and which in turn

probably enhanced lipid utilisation (Jeffery *et al.*, 1999). Soap skimmings on the other hand, was utilised almost to a similar extend in the presence and absence of acetate. Used cooking oil was utilised to a lesser extend in the presence of acetate, i.e. 13.0%. In this case, the pH was above neutral in the presence of acetate.

Next, interesting results were obtained when the fatty acyl profiles of the residual lipids in the supernatant and the fatty acyl profiles of the original oil substrate were compared (Table 50).

In general, a significant decrease in the PUFAs present in the supernatant was experienced in the presence and absence of acetate while on the other hand the saturated FAs were higher in many cases (except for tall oil and soap skimmings) when compared to the original oil substrate. This phenomenon again, indicates a preference of the yeast towards PUFA utilisation. It is also important to note that 18:2 was significantly utilised in most cases in the presence of acetate. These results are similar to that found by Jeffery et al (1999). The α18:3 in linseed oil, used cooking oil, tall oil and soap skimmings was utilised completely in the presence and absence of acetate. Again, these results are similar to that found previously.

The fatty acyl profiles of the cellular lipids were obtained after growth on various lipid substrates and compared to the fatty acyl profiles of the original oil substrates (Table 51).

According to the previous results, a decrease in the cellular PUFAs was obtained in the presence and absence of acetate when compared to the original oil substrate while the saturated FAs were relatively higher. No GLA was produced in the presence and absence of acetate. These results are also similar to that previously found.

Table 49. Growth yield, lipid accumulation, utilisation and the final pH when *Schwanniomyces occidentalis var. occidentalis* CBS 2863 was grown on a mixture of substrates (oil and acetate) and substrate alone (oil).

Substrate	Growth yield (g dry wt/l)	Cellular lipid content (% w/w dry wt)	Residual lipids (g/l)	pH
Sunflower oil (40g/l)	1.5	13.1	29.9	6.0
Sunflower oil (30g/l) + Sodium acetate (10g/l)	2.2	24.2	14.7	7.9
Linseed oil (40g/l)	0.8	25.6	1.7	6.0
Linseed oil (30g/l) + Sodium acetate (10g/l)	2.4	9.7	7.3	7.7
Used cooking oil (40g/l)	1.7	29.2	20.2	6.1
Used cooking oil (30g/l) + Sodium acetate (10g/l)	4.5	10.1	26.1	8.5
Tall oil (40g/l)	3.9	31.5	18.9	3.9
Tall oil (30g/l) + Sodium acetate (10g/l)	19.1	39.6	5.8	7.5
Soap skimmings (40g/l)	7.7	31.7	4.2	7.0
Soap skimmings (30g/l) + Sodium acetate (10g/l)	4.3	13.4	4.3	9.5

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

Table 50. Fatty acyl profiles of the residual lipids of *Schwanniomyces occidentalis var. occidentalis* CBS 2863 grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs Total FAs**
Sunflower oil (40g/l)	12.5	0.0	8.6	27.4	33.2	0.0	0.0	0.41
Sunflower oil (30g/l) + Sodium acetate (10g/l)	19.7	0.0	12.3	40.6	0.0	0.0	0.0	0.0
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	18.2	0.0	13.4	46.1	0.0	0.0	0.0	0.0
Linseed oil (30g/l) + Sodium acetate (10g/l)	14.5	0.0	10.4	40.6	0.0	0.0	0.0	0.0
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	9.9	0.0	4.3	46.6	2.4	0.0	0.0	0.04
Used cooking oil (30g/l) + Sodium acetate (10g/l)	9.9	0.0	4.5	44.1	3.1	0.0	0.0	0.05
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	2.9	0.0	1.1	19.6	7.0	0.0	0.0	0.23
Tall oil (30g/l) + Sodium acetate (10g/l)	0.8	0.0	0.5	4.0	1.6	0.0	0.0	0.23
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	2.3	0.0	1.6	11.9	3.3	0.0	0.0	0.17
Soap skimmings (30g/l) + Sodium acetate (10g/l)	1.0	0.0	1.7	8.0	1.0	0.0	0.0	0.10
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ). CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

Table 51. Fatty acyl profiles of the cellular lipids and GLA production by *Schwannimyces occidentalis var. occidentalis* CBS 2863 grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs / Total FAs**
Sunflower oil (40g/l)	22.1	7.6	4.5	38.8	13.3	0.0	0.0	0.15
Sunflower oil (30g/l) + Sodium acetate (10g/l)	17.2	4.5	4.4	37.4	15.6	0.0	0.0	0.20
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	25.1	7.8	4.9	35.5	7.0	0.0	0.0	0.09
Linseed oil (30g/l) + Sodium acetate (10g/l)	21.7	5.2	4.5	37.4	10.5	0.0	0.0	0.13
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	17.5	4.3	4.2	36.8	7.6	0.0	0.0	0.11
Used cooking oil (30g/l) + Sodium acetate (10g/l)	19.1	6.0	3.4	42.1	14.9	0.0	0.0	0.17
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	12.8	0.0	3.0	46.6	19.3	0.0	0.0	0.24
Tall oil (30g/l) + Sodium acetate (10g/l)	6.6	0.0	1.5	21.5	9.2	0.0	0.0	0.24
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	7.7	0.0	2.5	42.9	17.3	0.0	0.0	0.25
Soap skimmings (30g/l) + Sodium acetate (10g/l)	16.8	0.0	4.5	40.7	12.0	0.0	0.0	0.16
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ). CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

***Yarrowia lipolytica* CBS 0599**

When comparing the growth yields, lipid accumulation, utilisation and the final pH of the growth medium in the presence and absence of acetate, the following results were observed after seven days of cultivation (Table 52).

Biomass: In the absence and presence of acetate, the yeast produced variable amounts of biomass when cultivated on the different fats and oils. In these experiments, biomass production decreased from 11.3g/l to 5.1g/l on sunflower oil and from 16.7g/l to 8.1g/l on tall oil when acetate was added. On the other hand, when cultivated on linseed oil, used cooking oil and soap skimmings in the presence of acetate, an increase on biomass production was obtained while the pH increased to neutrality.

Cellular lipid: The highest cellular lipid content was obtained on tall oil in the absence of acetate, i.e. 30.7% w/w. On sunflower oil, linseed oil, used cooking oil and soap skimmings, an increase in cellular lipid content occurred in the presence of acetate. Here, the addition of acetate led to an increase in cellular lipid content. On the other hand, when cultivated on tall oil in the presence of acetate, a decrease in cellular lipid content was obtained, i.e. from 30.7% w/w to 17.9% w/w. When this yeast was cultivated on glucose as sole carbon source, it produced more cellular lipids, i.e. from 32% w/w to 36% w/w (Ratledge, 1989).

Residual lipids: Sunflower oil, linseed oil, used cooking oil and tall oil were utilised much more in the absence of acetate than in its presence. Soap skimmings on the other hand, was utilised to a similar extend both in the presence and absence of acetate, i.e. 82.0%. In the presence of acetate, an increase in pH occurred in most cases.

The fatty acyl profiles of the residual lipids in the supernatant were analysed in the presence and absence of acetate and compared to the fatty acyl profiles of the original oil substrate (Table 53).

In general, a significant decrease in the PUFAs present in the supernatant occurred while in many cases the saturated FAs were relatively higher in the presence and absence of acetate when compared to the original oil substrate. This phenomenon again indicates a preference of the yeast towards PUFA utilisation. On the other hand, the α18:3 in linseed oil, used cooking oil, tall oil and soap skimmings was completely utilised in the presence and absence of acetate.

More interesting results were found when the fatty acyl profiles of the intracellular lipids and the fatty acyl profiles of the original oil substrate were compared in the presence and absence of acetate (Table 54).

From the results, a significant decrease in the PUFAs present in the intracellular lipid fractions occurred while on the other hand the saturated FAs were in many

cases relatively higher after growth. No GLA was produced in the presence and absence of acetate when cultivated on all the lipid substrates. These results are similar to that found for the yeasts previously studied.

Table 52. Growth yield, lipid accumulation, utilisation and the final pH when *Yarrowia lipolytica* CBS 0599 was grown on a mixture of substrates (oil and acetate) and substrate alone (oil).

Substrate	Growth yield (g dry wt/l)	Cellular lipid content (% w/w dry wt)	Residual lipids (g/l)	pH
Sunflower oil (40g/l)	11.3	8.8	0.3	5.3
Sunflower oil (30g/l) + Sodium acetate (10g/l)	5.1	11.9	2.3	7.5
Linseed oil (40g/l)	2.5	7.0	0.2	5.3
Linseed oil (30g/l) + Sodium acetate (10g/l)	5.3	15.4	1.6	7.6
Used cooking oil (40g/l)	4.7	15.0	3.3	3.6
Used cooking oil (30g/l) + Sodium acetate (10g/l)	6.5	19.5	10.0	7.5
Tall oil (40g/l)	16.7	30.7	7.5	4.1
Tall oil (30g/l) + Sodium acetate (10g/l)	8.1	17.9	13.8	7.1
Soap skimmings (40g/l)	9.5	13.5	7.2	6.8
Soap skimmings (30g/l) + Sodium acetate (10g/l)	13.1	16.7	5.4	9.5

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

Table 53. Fatty acyl profiles of the residual lipids of *Yarrowia lipolytica* CBS 0599 grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs / Total FAs**
Sunflower oil (40g/l)	9.0	0.0	11.8	6.7	6.5	0.0	0.0	0.19
Sunflower oil (30g/l) + Sodium acetate (10g/l)	16.9	0.0	11.9	27.0	6.2	0.0	0.0	0.10
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	13.9	0.0	17.2	7.7	3.2	0.0	0.0	0.08
Linseed oil (30g/l) + Sodium acetate (10g/l)	20.7	0.0	13.0	29.2	5.1	0.0	0.0	0.08
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	13.1	0.0	8.5	34.4	1.6	0.0	0.0	0.03
Used cooking oil (30g/l) + Sodium acetate (10g/l)	13.0	0.0	6.0	47.8	4.4	0.0	0.0	0.06
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	5.6	0.0	4.6	11.1	5.0	0.0	0.0	0.19
Tall oil (30g/l) + Sodium acetate (10g/l)	3.9	0.0	2.7	7.3	3.5	0.0	0.0	0.20
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	5.7	0.0	5.4	9.4	3.7	0.0	0.0	0.15
Soap skimmings (30g/l) + Sodium acetate (10g/l)	4.5	0.0	4.0	7.3	4.0	0.0	0.0	0.20
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ). CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

Table 54. Fatty acyl profiles of the cellular lipids and GLA production by *Yarrowia lipolytica* CBS 0599 grown on sunflower oil, linseed oil, used cooking oil, tall oil and soap skimmings and on a mixture of substrates (oil and acetate).

	Relative % fatty acyl groups							
Substrate	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**PUFAs / Total FAs**
Sunflower oil (40g/l)	11.6	0.0	6.9	20.6	0.0	0.0	0.0	0.0
Sunflower oil (30g/l) + Sodium acetate (10g/l)	13.4	5.4	6.6	29.2	7.9	0.0	0.0	0.13
Original oil	**5.9**	**0.0**	**5.6**	**19.6**	**62.5**	**0.0**	**0.0**	**0.67**
Linseed oil (40g/l)	11.1	0.0	18.4	7.4	0.2	0.0	0.0	0.005
Linseed oil (30g/l) + Sodium acetate (10g/l)	18.2	0.0	9.7	31.2	7.8	0.0	0.0	0.12
Original oil	**5.7**	**0.0**	**4.8**	**16.7**	**16.5**	**0.0**	**47.7**	**0.70**
Used cooking oil (40g/l)	13.0	0.0	6.7	31.2	0.0	0.0	0.0	0.0
Used cooking oil (30g/l) + Sodium acetate (10g/l)	12.9	3.9	5.6	42.0	6.0	0.0	0.0	0.09
Original oil	**5.6**	**0.3**	**3.7**	**40.0**	**38.5**	**0.0**	**4.7**	**0.47**
Tall oil (40g/l)	5.8	1.5	1.5	19.5	9.9	0.0	0.0	0.26
Tall oil (30g/l) + Sodium acetate (10g/l)	7.2	4.7	1.6	34.6	17.0	0.0	0.0	0.26
Original oil	**9.0**	**0.7**	**2.3**	**50.1**	**30.0**	**0.0**	**0.5**	**0.33**
Soap skimmings (40g/l)	6.3	2.1	1.7	22.2	11.3	0.0	0.0	0.26
Soap skimmings (30g/l) + Sodium acetate (10g/l)	6.4	5.0	1.7	20.9	11.7	0.0	0.0	0.26
Original oil	**8.9**	**0.0**	**2.1**	**48.1**	**31.5**	**0.0**	**2.6**	**0.37**

Values represent means of at least two repetitions. Standard deviation was <10% of the mean in all cases. 16:0 = Palmitic acid, 16:1 = Palmitoleic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, α18:3 = Alpha-linolenic acid, γ18:3 = Gamma-linolenic acid. PUFAs = 18:2 + 18:3 (α,γ). CBS = Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

CHAPTER 4

CONCLUSIONS

On the basis of the results obtained in this study, the following main conclusions are drawn (Tables 1 – 54; Chapter 3):

1. Similar results were obtained in this study for *Mucor circinelloides f. circinelloides* CBS 108.16 as compared to Jeffery et al (1997; 1999) and the *hypothesis when cultivated on sunflower oil in the presence and absence of acetate. A similar trend was also found when this fungus was grown on the other fat and oil substrates, i.e. used cooking oil and tall oil. It is important to note that the enhancing effect of acetate was not generally observed in the other fungi and on some other fats and oils tested.
2. Most fungi, including the yeasts tested in this study, could grow on fats and oils provided in the presence or absence of acetate. Exceptions to the rule were *Mortierella alpina* MUFS Mo058 and *Lipomyces starkeyi* CBS 1807 T that were unable to grow. *Schizosaccharomyces pombe var. pombe* CBS 0356 T could also not grow on linseed oil.

*Hypothesis = When acetate is added to a growth medium containing fats and oils as sole carbon source, an increase in the following is experienced when fungi are cultivated on this medium: growth yield, cellular lipid, residual lipids utilisation, pH, PUFA utilisation (present in the residual lipid), cellular PUFA content and GLA production.

3. The presence of acetate had in many cases a stimulatory effect on growth. The enhancing effect of acetate was found especially in *Mucor circinelloides f. circinelloides* CBS 108.16 and *Thamnostylum* MUFS SAS025 as well as the yeast *Galactomyces geotrichum* CBS 0772.71 T when cultivated on all the fat and oil substrates tested. In some cases, the addition of acetate had an inhibitory effect on growth.
4. With a few exceptions, most mucoralean fungi, when cultivated on various fats and oils became oleaginous (i.e. contain ≥ 20% lipids) in the presence or absence of acetate. However, when these fungi were cultivated on linseed oil in the presence or absence of acetate they were unable to accumulate more than 20% lipids according to biomass. Less yeasts became oleaginous when cultivated on various fat and oil substrates when compared to the mucoralean fungi tested. It is interesting to note that the non-oleaginous yeasts i.e. *Kluyveromyces*, *Saccharomyces*, *Schizosaccharomyces*, *Schwanniomyces* and *Yarrowia* became oleaginous when cultivated on various fats and oils in the presence of acetate (Kock and Botha, 1998).
5. In most mucoralean fungi and yeasts, the presence of acetate had an effect on cellular lipid content. The enhancing effect of acetate addition on cellular lipid content was experienced especially on *Gongronella*, *Mucor circinelloides f. circinelloides* CBS 108.16 and *Thamnostylum* when cultivated on various fats and oils tested in this study. On the other hand, the addition of acetate had a negative effect on cellular lipid accumulation in some mucoralean fungi and yeasts when cultivated on various fats and oils.

6. In general, both the mucoralean fungi and yeasts tested in this study, utilised different fats and oils [(i.e. containing saturated fatty acids (FAs) and polyunsaturated fatty acids (PUFAs)] as carbon sources in the presence or absence of acetate. *Mortierella alpina* MUFS Mo058 and *Lipomyces starkeyi* CBS 1807 T could not utilise any of these fats and oils. *Schizosaccharomyces pombe var. pombe* CBS 0356 T on the other hand, could not utilise linseed oil, but was capable of utilising the other fats and oils in the presence and absence of acetate.
7. The presence of acetate had a positive effect on fat and oil utilisation in many mucoralean fungi and only in some yeasts.
8. In most cases, a pH increase was observed probably due to acetic acid utilisation during cultivation (Jeffery *et al.,* 1999). It is interesting to note that no pH increase was observed when some mucoralean fungi and yeasts were cultivated on soap skimmings in the presence of acetate. This may be attributed to poor growth on this lipid substrate.
9. In the presence or absence of acetate, most mucoralean fungi and yeasts showed a high preference towards the utilisation of PUFAs present in the residual oil fractions. The addition of acetate had both a positive and negative effect on the degree of preference towards PUFAs in the residual lipids.
10. In most cases, (in both mucoralean fungi and yeasts), lower amounts of PUFAs in the cellular lipid fractions in the presence or absence of acetate were found when compared to the PUFA content of the original oil substrate tested. This

is probably due to the utilisation of these FAs through β-oxidation for the production of energy (Kendrick and Ratledge, 1996).

11. No general pattern was observed regarding the effect of acetate addition on cellular PUFA content. However, in *Mucor circinelloides f. circinelloides* MUFS SAS045, *Dipodascopsis*, *Filobasidiella* and *Yarrowia*, acetate addition improved the cellular PUFA content when cultivated on all the various fats and oils.
12. Most mucoralean fungi when cultivated on various fats and oils (except soap skimmings) could produce GLA in the presence or absence of acetate. All yeasts in this study could not produce GLA when cultivated on any lipid substrate in the presence or absence of acetate. This is probably due to the lack of a Δ^6 desaturase enzyme in yeasts (Kock and Botha, 1998).
13. The addition of acetate improved GLA production in most mucoralean fungi when cultivated on sunflower oil and used cooking oil. These fungi, capable of producing GLA in this study, should now be explored in the transformation of edible fats and oils to high value lipids containing GLA.
14. When for example *Schwanniomyces* and *Yarrowia* were cultivated on tall oil and soap skimmings (Table 51, p. 141; Table 54, p. 147) various FAs of different chain lengths were produced. This resulted in the recording of only small percentage of 16:0, 16:1, 18:0, 18:1, 18:2 and α18:3 when compared to the original substrate. Similar results were found in some other fungi when cultivated on different fats and oils in the presence or absence of acetate.

Table 1. Mucoralean fungi that fit *hypothesis when cultivated on different fats and oils in the presence of acetate.

Organism	Fats and oils	(a) Growth yield	(b) Cell. lipids	(c) Residual lipids	(d) pH	(e) PUFA utilisation	(f) Cell. PUFAs	(g) GLA (g/l)
1. *Absidia*	Sunflower oil	✔	✔	✔	✔	✔	✘	✔
	Linseed oil	✘	✘	✘	✔	✔	✔	✘
	Used cooking oil	✔	✔	✔	✔	✔	✔	✔
	Tall oil	✔	✘	✘	✔	✘	✘	✘
	Soap skimmings	✔	✘	✘	✘	✔	✘	✘
2. *Actinomucor*	Sunflower oil	✔	✔	✔	✔	✘	✔	✔
	Linseed oil	✘	✘	✘	✔	✔	✘	✘
	Used cooking oil	✔	✔	✔	✔	✘	✔	✔
	Tall oil	✔	✔	✔	✔	✔	✔	✘
	Soap skimmings	✘	✘	✘	✘	✘	✘	✘
3. *Cunninghamella*	Sunflower oil	✔	✔	✔	✔	✘	✔	✔
	Linseed oil	✘	✔	✘	✔	✘	✔	✘
	Used cooking oil	✔	✔	✔	✔	✘	✔	✔
	Tall oil	✘	✘	✘	✔	✘	✔	✘
	Soap skimmings	✘	✔	✘	✔	✘	✔	✘
4. *Gongronella*	Sunflower oil	✔	✔	✔	✔	✔	✘	✘
	Linseed oil	✔	✔	✘	✔	✘	✘	✘
	Used cooking oil	✘	✔	✘	✔	✔	✘	✘
	Tall oil	✔	✔	✔	✔	✘	✔	✘
	Soap skimmings	✘	✔	✘	✔	✔	✘	✘
5. *Mucor circinelloides*	Sunflower oil	✔	✔	✔	✔	✔	✔	✔
	Linseed oil	✔	✔	✔	✔	✘	✔	✔
	Used cooking oil	✔	✔	✔	✔	✔	✔	✔
	Tall oil	✔	✔	✔	✔	✔	✔	✔
	Soap skimmings	✔	✔	✔	✔	✔	✘	✘
6. *Mucor circinelloides*	Sunflower oil	✔	✔	✔	✔	✘	✔	✔
	Linseed oil	✘	✘	✔	✔	✘	✔	✔
	Used cooking oil	✔	✔	✔	✔	✘	✔	✔
	Tall oil	✔	✔	✔	✔	✘	✔	✔
	Soap skimmings	✘	✘	✘	✔	✘	✔	✘
7. *Rhizomucor*	Sunflower oil	✔	✔	✔	✔	✘	✔	✔
	Linseed oil	✘	✘	✘	✔	✘	✔	✔
	Used cooking oil	✔	✔	✔	✔	✘	✔	✔
	Tall oil	✘	✘	✘	✔	✘	✘	✘
	Soap skimmings	✔	✔	✘	✘	✔	✘	✘
8. *Rhizopus*	Sunflower oil	✘	✘	✘	✔	✘	✔	✔
	Linseed oil	✔	✔	✘	✔	✘	✘	✔
	Used cooking oil	✔	✔	✘	✔	✘	✔	✔
	Tall oil	✔	✔	✘	✔	✘	✔	✘
	Soap skimmings	✘	✘	✔	✘	✘	✔	✘
9. *Thamnostylum*	Sunflower oil	✔	✔	✔	✔	✔	✔	✔
	Linseed oil	✔	✔	✘	✔	✘	✔	✘
	Used cooking oil	✔	✔	✔	✔	✔	✘	✔
	Tall oil	✔	✔	✘	✔	✔	✔	✔
	Soap skimmings	✔	✔	✘	✔	✔	✔	✘

1 = *Absidia* MUFS 200; **2** = *Actinomucor elegans* MUFS SAS218; **3** = *Cunninghamella* MUFS Cu001; **4** = *Gongronella* MUFS Go001; **5** = *Mucor circinelloides f. circinelloides* CBS 108.16; **6** = *Mucor circinelloides f. circinelloides* MUFS SAS045; **7** = *Rhizomucor pusillus* MUFS Rm001; **8** = *Rhizopus stolonifer* MUFS R008; **9** = *Thamnostylum* MUFS SAS025. **(a)** = increase in growth yield; **(b)** = increase in cellular lipids **(c)** = increase in residual lipid utilisation; **(d)** = increase in pH; **(e)** = increase in PUFA utilisation (present in residual lipids); **(f)** = increase in cellular lipid PUFAs; **(g)** = increase in cellular GLA production. *Hypothesis = When acetate is added to a growth medium containing fats and oils as sole carbon source, an increase in the following is experienced when fungi are cultivated on this medium: growth yield, cellular lipid, residual lipid utilisation, pH, PUFA utilisation (present in the residual lipid), cellular PUFA content and GLA production.

Table 2. Dikaryomycotan yeasts that fit *hypothesis when cultivated on different fats and oils in the presence of acetate.

Organism	Fats and oils	(a) Growth yield	(b) Cell. lipids	(c) Residual lipids	(d) pH	(e) PUFA utilisation	(f) Cell. PUFAs	(g) GLA (g/l)
10. *Cryptococcus*	Sunflower oil	✔	✔	✘	✔	✔	✔	–
	Linseed oil	✔	✔	✘	✔	✔	✔	–
	Used cooking oil	✘	✔	✘	✔	✔	✔	–
	Tall oil	✔	✘	✘	✔	✘	✘	–
	Soap skimmings	✔	✔	✘	✔	✘	✘	–
11. *Dipodascopsis*	Sunflower oil	✔	✘	✘	✔	✔	✔	–
	Linseed oil	✔	✘	✘	✔	✔	✔	–
	Used cooking oil	✔	✔	✘	✔	✔	✔	–
	Tall oil	✘	✔	✘	✔	✘	✔	–
	Soap skimmings	✔	✘	✘	✔	✘	✔	–
12. *Filobasidiella*	Sunflower oil	✔	✔	✘	✔	✘	✔	–
	Linseed oil	✔	✔	✘	✔	✘	✔	–
	Used cooking oil	✔	✔	✘	✔	✘	✔	–
	Tall oil	✘	✔	✘	✔	✘	✔	–
	Soap skimmings	✔	✘	✘	✘	✘	✔	–
13. *Galactomyces*	Sunflower oil	✔	✘	✔	✔	✔	✔	–
	Linseed oil	✔	✔	✘	✔	✔	✔	–
	Used cooking oil	✔	✔	✔	✔	✔	✔	–
	Tall oil	✔	✘	✘	✔	✔	✘	–
	Soap skimmings	✔	✘	✘	✔	✔	✘	–
14. *Kluyveromyces*	Sunflower oil	✔	✘	✘	✔	✘	✔	–
	Linseed oil	✘	✘	✘	✔	✘	✘	–
	Used cooking oil	✔	✔	✘	✔	✘	✔	–
	Tall oil	✔	✔	✘	✔	✔	✔	–
	Soap skimmings	✔	✔	✔	✔	✔	✔	–
15. *Saccharomyces*	Sunflower oil	✔	✔	✘	✔	✘	✔	–
	Linseed oil	✔	✔	✘	✔	✘	✘	–
	Used cooking oil	✔	✘	✘	✔	✘	✔	–
	Tall oil	✘	✘	✘	✔	✘	✘	–
	Soap skimmings	✔	✔	✘	✘	✔	✘	–
16. *Schizosaccharomyces*	Sunflower oil	✔	✔	✘	✔	✘	✔	–
	Linseed oil	–	–	–	–	–	–	–
	Used cooking oil	✘	✘	✘	✘	✘	✔	–
	Tall oil	✘	✘	✘	✘	✔	✔	–
	Soap skimmings	✔	✔	✘	✘	✔	✔	–
17 *Schwanniomyces*	Sunflower oil	✔	✔	✔	✔	✔	✔	–
	Linseed oil	✔	✘	✘	✔	✘	✔	–
	Used cooking oil	✔	✘	✘	✔	✘	✔	–
	Tall oil	✔	✔	✔	✔	✔	✘	–
	Soap skimmings	✘	✘	✘	✔	✔	✘	–
18 *Yarrowia*	Sunflower oil	✘	✔	✘	✔	✔	✔	–
	Linseed oil	✔	✔	✘	✔	✘	✔	–
	Used cooking oil	✔	✔	✘	✔	✘	✔	–
	Tall oil	✘	✘	✘	✔	✔	✔	–
	Soap skimmings	✔	✔	✘	✔	✘	✔	–

10 = *Cryptococcus curvatus* CBS 0570 T; **11** = *Dipodascopsis uninucleata var. uninucleata* CBS 0190.37 T; **12** = *Filobasidiella neoformans var. neoformans* CBS 0132; **13** = *Galactomyces geotrichum* CBS 0772.71 T; **14** = *Kluyveromyces marxianus var. marxianus* CBS 1556; = **15** = *Saccharomyces cerevisiae* CBS 1171 NT; **16** = *Schizosaccharomyces pombe var. pombe* CBS 0356 T; **17** = *Schwanniomyces occidentalis var. occidentalis* CBS 2863; **18** = *Yarrowia lipolytica* CBS 0599. **(a)** = increase in growth yield; **(b)** = increase in cellular lipids **(c)** = increase in residual lipid utilisation; **(d)** = increase in pH; **(e)** = increase in PUFA utilisation (present in residual lipids); **(f)** = increase in cellular lipid PUFAs; **(g)** = increase in cellular GLA production. – = no growth. *Hypothesis = When acetate is added to a growth medium containing fats and oils as sole carbon source, an increase in the following is experienced when fungi are cultivated on this medium: growth yield, cellular lipid, residual lipid utilisation, pH, PUFA utilisation (present in the residual lipids), cellular PUFA content and GLA production.

Table 3. The production of GLA by fungi when cultivated on different fats and oils in the presence and absence of acetate.

Mucoralean fungi	**Fats and oils**	**-Acetate (g/l)**	**+Acetate (g/l)**
Absidia MUFS 200	Sunflower oil	0.02	0.10
	Linseed oil	0.00	0.00
	Used cooking oil	0.02	0.10
	Tall oil	0.00	0.00
	Soap skimmings	0.00	0.00
Actinomucor MUFS SAS218	Sunflower oil	0.00	0.20
	Linseed oil	0.00	0.00
	Used cooking oil	0.00	0.30
	Tall oil	0.00	0.00
	Soap skimmings	0.00	0.00
Cunninghamella MUFS Cu001	Sunflower oil	0.00	0.30
	Linseed oil	0.00	0.00
	Used cooking oil	0.03	0.60
	Tall oil	0.06	0.02
	Soap skimmings	0.00	0.00
Gongronella MUFS Go001	Sunflower oil	0.00	0.00
	Linseed oil	0.00	0.00
	Used cooking oil	0.04	0.00
	Tall oil	0.00	0.00
	Soap skimmings	0.00	0.00
Mucor circinelloides f. circinelloides CBS 108. 16	Sunflower oil	0.02	0.50
	Linseed oil	0.00	0.05
	Used cooking oil	0.02	0.30
	Tall oil	0.00	0.10
	Soap skimmings	0.00	0.00
Mucor circinelloides f. circinelloides MUFS SAS045	Sunflower oil	0.00	0.10
	Linseed oil	0.00	0.01
	Used cooking oil	0.02	0.10
	Tall oil	0.02	0.10
	Soap skimmings	0.00	0.00
Rhizomucor pusillus MUFS Rm001	Sunflower oil	0.00	0.02
	Linseed oil	0.00	0.01
	Used cooking oil	0.00	0.02
	Tall oil	0.00	0.00
	Soap skimmings	0.00	0.00
Rhizopus stolonifer MUFS R008	Sunflower oil	0.00	0.10
	Linseed oil	0.00	0.03
	Used cooking oil	0.00	0.20
	Tall oil	0.00	0.00
	Soap skimmings	0.00	0.00
Thamnostylum MUFS SAS025	Sunflower oil	0.00	0.10
	Linseed oil	0.00	0.00
	Used cooking oil	0.00	0.10
	Tall oil	0.00	0.04
	Soap skimmings	0.00	0.00
Yeasts	NO GLA		

- = absence of acetate

+ = presence of acetate

CHAPTER 5

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SUMMARY

In 1997, Jeffery and co-workers discovered that when *Mucor circinelloides f. circinelloides* CBS 108.16 was cultivated on 30g/l sunflower oil and 10g/l sodium acetate, an improved utilisation of the oil, doubling of the biomass production and enhancement of the intracellular polyunsaturated γ-linolenic acid (GLA) content occurred as compared to when this fungus was cultivated on only 40g/l sunflower oil as sole carbon source. Consequently, the aim of this study became to further explore this phenomenon (hypothesis) in selected members of the zygomycotan fungi as well as yeasts when cultivated on various fat and oil substrates in the presence and absence of acetate. The ultimate aim was to identify those taxa that can be further explored for the transformation of edible and tall oils to high value lipids in the presence and absence of acetate. In this study, similar trends were observed in *Mucor circinelloides f. circinelloides* CBS 108.16 as that found by Jeffery *et al* when cultivated on sunflower oil in the presence and absence of acetate. A similar pattern was also found when this fungus was grown on used cooking oil and tall oil. The enhancing effect of acetate was not generally observed in the other fungi and on some other fats and oils tested. Most fungi, including the yeasts, could grow on fats and oils provided in the presence or absence of acetate. Exceptions to the rule were *Mortierella alpina* MUFS Mo058 and *Lipomyces starkeyi* CBS 1807 T that were unable to grow. *Schizosaccharomyces pombe var. pombe* CBS 0356 T could also not grow on linseed oil. The presence of acetate had in many cases a stimulatory effect on growth. In some cases, the addition of acetate had an inhibitory effect on growth. With a few exceptions, most mucoralean fungi, when cultivated on various fats and oils, became oleaginous (i.e. contain ≥ 20% lipids) in

the presence or absence of acetate. However, when these fungi were cultivated on linseed oil in the presence or absence of acetate they were unable to accumulate more than 20% lipids according to biomass. Less yeasts became oleaginous when cultivated on various fat and oil substrates when compared to the mucoralean fungi tested. The non-oleaginous yeasts i.e. *Kluyveromyces, Saccharomyces*, *Schizosaccharomyces*, *Schwanniomyces* and *Yarrowia* became oleaginous when cultivated on various fats and oils in the presence of acetate. In most mucoralean fungi and yeasts, the presence of acetate had an effect on cellular lipid content. The enhancing effect of acetate addition on cellular lipid content was experienced especially on *Gongronella*, *Mucor circinelloides f. circinelloides* CBS 108.16 and *Thamnostylum* when cultivated on various fats and oils. On the other hand, the addition of acetate had a negative effect on cellular lipid accumulation in some mucoralean fungi and yeasts. In general, both the mucoralean fungi and yeasts utilised different fats and oils [(i.e. containing saturated fatty acids (FAs) and polyunsaturated fatty acids (PUFAs)] as carbon sources in the presence or absence of acetate. *Mortierella* and *Lipomyces* could not utilise any of these fats and oils. The presence of acetate had a positive effect on fat and oil utilisation by most mucoralean fungi and some yeasts. In most cases, a pH increase was observed probably due to acetic acid utilisation during cultivation. In the presence or absence of acetate, most mucoralean fungi and yeasts showed a high preference towards the utilisation of PUFAs present in the residual oil fractions. The addition of acetate had both a positive and negative effect on the degree of preference towards PUFAs in the residual lipids. In most cases (in both mucoralean fungi and yeasts) lower

amounts of PUFAs in the cellular lipid fractions in the presence or absence of acetate were found when compared to the oil substrate fed. This is probably due to the utilisation of these FAs through β-oxidation for the production of energy. No general pattern was observed regarding the effect of acetate addition on cellular PUFA content. Many mucoralean fungi when cultivated on various fats and oils (except soap skimmings) could produce GLA in the presence or absence of acetate. All the yeasts in this study could not produce GLA when cultivated on any lipid substrate in the presence or absence of acetate. This is probably due to the lack of a Δ^6 desaturase enzyme. The addition of acetate improved GLA production in most mucoralean fungi when cultivated on sunflower oil and used cooking oil. Those fungi capable of producing GLA in this study should now be explored in the transformation of edible fats and oils to high value lipids containing GLA.

OPSOMMING

In 1997 het Jeffery en mede-werkers ontdek dat, wanneer *Mucor circinelloides f. circinelloides* CBS 108.16 op 30g/l sonneblomolie en 10g/l natriumasetaat gegroei word, die olieverbruik verbeter, biomassa produksie verdubbel en die intrasellulêre γ-linoleïensuur (GLS) inhoud verhoog, in vergelyking met wanneer die fungus slegs op 40g/l sonneblomolie as enigste koolstofbron gegroei is. Gevolglik is die doel van hierdie studie om die verskynsel (hipotese) verder te ondersoek in geselekteerde lede van die Zygomycota en giste, gegroei op verskeie vet- en oliesubstrate in die teenwoordigheid en afwesigheid van asetaat. Die uiteindelike doel is om die taksa te identifiseer wat verder ondersoek kan word vir die transformasie van eetbare en tallolies na hoëwaarde lipiede in die teenwoordigheid en afwesigheid van asetaat. In hierdie studie is eenderse tendense waargeneem in *Mucor circinelloides f. circinelloides* CBS 108.16 as die gevind deur Jeffery *et al* wanneer die fungus op sonneblomolie in die teenwoordigheid en afwesigheid van asetaat gegroei is. 'n Soortgelyke patroon is ook gevind wanneer die fungus op gebruikte kookolie en tallolie gegroei word. Die verhoogde effek van asetaat is nie oor die algemeen opgemerk in die ander fungi en met sekere getoetsde vette en olies nie. Uitsonderings op die reël was *Mortierella alpina* MUFS Mo058 en *Lipomyces starkeyi* CBS 1807 T wat nie kon groei nie. *Schizosaccharomyces pombe var. pombe* CBS 0356 T kon ook nie op lynsaadolie groei nie. Die teenwoordigheid van asetaat het in baie gevalle 'n stimulerende effek op groei gehad. In sommige gevalle het die byvoeging van asetaat 'n inhiberende effek op groei gehad. Behalwe vir 'n paar uitsonderings, het die meeste mukoraliese fungi, gegroei op verskillende vette en olies en oleogeen (d.i. bevat ≥ 20% lipiede) geraak in die

teenwoordigheid of afwesigheid van asetaat. Wanneer die fungi egter op lynsaadolie in die teenwoordigheid of afwesigheid van asetaat gegroei is, was hulle nie instaat om meer as 20% lipiede in hul biomassa te vergader nie. Minder giste het oleogeen geword wanneer hulle op verskillende vet en oliesubstrate gegroei is. Die nie-oleogene giste d.i. *Kluyveromyces*, *Saccharomyces*, *Schizosaccharomyces*, *Schwanniomyces* en *Yarrowia* het oleogeen geraak wanneer hulle op verskeie vette en olies in die teenwoordigheid van asetaat gegroei is. By meeste mukoraliese fungi en giste het die teenwoordigheid van asetaat 'n invloed op die sellulêre lipiedinhoud gehad. Die verhogende effek van asetaat byvoeging op die sellulêre lipiedinhoud is veral ondervind by *Gongronella*, *Mucor circinelloides f. circinelloides* CBS 108.16 en *Thamnostylum* wanneer hulle op verskeie vette en olies gegroei is. Aan die anderkant het die byvoeging van asetaat 'n negatiewe effek op die sellulêre lipiedophoping in sommige mukoraliese fungi en giste gehad. Oor die algemeen het die mukoraliese fungi en giste verskillende vette en olies benut [d.i. met verskillende versadigde vetsure (VSe) en poli-onversadigde vetsure (POVS)] as koolstofbronne in die teenwoordigheid of afwesigheid van asetaat. *Mortierella* and *Lipomyces* kon nie enige van die vette en olies benut nie. Die teenwoordigheid van asetaat het 'n positiewe effek op vet en olieverbruik van meeste mukoraliese fungi en sommige giste gehad. In meeste gevalle is 'n toename in pH waargeneem, heelwaarskynlik as gevolg van asynsuurverbruik gedurende groei. In die teenwoordigheid of afwesigheid van asetaat, het meeste mukoraliese fungi en giste 'n voorkeur getoon vir die verbruik van POVS teenwoordig in die residuele oliefraksies. Die byvoeging van asetaat het beide 'n positiewe en negatiewe effek op die graad van voorkeur vir

POVS in die residuele lipiede gehad. In die meeste gevalle (by beide mukoraliese fungi en giste) is laer hoeveelhede POVS in die sellulêre lipiedfraksies gevind as in die olie van die getoetsde substraat. Dis heelwaarskynlik as gevolg van die gebruik van die VSe deur β-oksidasie vir die produksie van energie. Geen algemene patroon is opgemerk aangaande die effek van asetaat byvoeging op sellulêre POVS inhoud nie. Meeste mukoraliese fungi, wanneer hulle op verskillende vette en olies gegroei is (behalwe seepskimmings) kon GLS produseer in die teenwoordigheid of afwesigheid van asetaat. Al die giste in die studie kon nie GLS produseer wanneer hulle op enige lipiedsubstraat gegroei is nie. Dis heelwaarskynlik as gevolg van die afwesigheid van 'n Δ^6 desaturase ensiem. Die byvoeging van asetaat verbeter GLS produksie in meeste mukoraliese fungi wanneer hulle gegroei word op sonneblomolie en gebruikte kookolie. Die fungi wat GLS kon produseer in hierdie studie, behoort nou ondersoek te word vir die transformasie van eetbare vette en olies na hoë waarde GLS-bevattende olies.