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FUNGAL PRODUCTION OF EICOSAPENTAENOIC AND ARACHIDONIC ACIDS FROM INDUSTRIAL WASTE STREAMS AND CRUDE SOYBEAN OIL

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Ming-Huei Cheng

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DEDICATION

This thesis is dedicated to my father

SHU-SHIUAN CHEN,

my mother

SHIH CHEN WANG,

and my sister

SHAO-HWA CHEN

for their support, understanding and love throughout this endeavor.

獻給

最親愛的

父親，陳書軒先生

母親，陳王柿女士

和妹妹，陳韶華女士

是 您們的犧牲和愛 完成了這本論文

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ABSTRACT

A series of polyunsaturated fatty acids (PUFAs), including 5,8,11,14,17-*cis*-eicosapentaenoic acid (EPA) and 5,8,11,14-*cis*-arachidonic acid (ARA), have widespread nutritional and pharmaceutical value. This study investigated the potential production of the two economically important fatty acids with a fungal fermentation process. The substrates for the fungal fermentation process were crude soybean oil (SBO), a sucrose waste stream (SWS) and a soymeal waste stream (SMW). Glucose (GLU) was used as a substrate in control groups. The microorganisms used were *Mortierella elongata* NRRL 5513 and *Pythium irregulare* ATCC 10951.

The use of *P. irregulare* ATCC 10951 is preferred since it produced high levels and reasonable ratios of EPA and ARA at various temperatures (12, 18, and 24°C). An advantage of *P. irregulare* was its ability to produce EPA at room temperature, which is desirable for commercial applications. Soybean oil had a unique characteristic of stabilizing pH; the optimal initial pH was 6.0. An emulsifier, Tween 80, allowed much greater dispersion of the SBO in aqueous broth and helped increase EPA and ARA production.

In experiments exploring the combination effects of sugars (1, 2, and 3%) with soybean oil (4%) and Tween 80 (0.2%) at 12, 18, and 24°C, we found that EPA yields of SMW+SBO were significantly higher than those of GLU+SBO and SWS+SBO ($P < 0.05$). The greatest EPA production (1400 mg/L) was obtained at 12°C (1% SMW, 4% SBO). Cultivation of *P. irregulare* at reduced temperatures increased lipid unsaturation.

An orbital shaker (300 rpm) provided better mixing of SBO and Tween 80 than a water-bath shaker did.

The highest ARA level appeared at 18°C-SMW+SBO (2000 mg/L), which was a statistically interactive temperature-media combination ($P<0.05$). Therefore, SMW+SBO other than GLU+SBO or SWS+SBO is suggested when 18°C is used for *P. irregulare*. The ARA/EPA ratio in this study ranged from 0.2 to 4.0, which would be reasonable for food additive or supplement applications, e.g. infant formula.

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CHAPTER I

INTRODUCTION

Nutritional studies have shown the beneficial effects of omega-3 polyunsaturated fatty acids in the diet on coronary heart disease, hypertension, psoriasis, arthritis, other autoimmune disorders and several types of cancer (Table 1)(Bajpai and Bajpai, 1993). Our target fatty acids were the omega-3 fatty acid, 5,8,11,14,17-*cis*-eicosapentaenoic acid (EPA), and the omega-6 fatty acid, 5,8,11,14-*cis*-arachidonic acid (ARA), since both are important intermediates of eicosanoid metabolism, responsible for heart and circulatory, blood clotting, and autoimmune systems in mammals (Braden and Carroll, 1986; Dyerberg, 1986; Dyerberg *et al.*, 1978; Kremer *et al.*, 1985; Bajpai and Bajpai, 1993).

Because of theses health benefits, demand for these compounds is increasing. Van der Wende (1988) reported that the approximate U.S. market value for omega-3 fatty acids was $100 million in 1987 and the projected market was $790 million per year.

Today the major commercial source of EPA is marine fish oil (Yongmanitchai and Ward, 1989). EPA also occurs as a component of cellular lipids in protozoal, algal, bacterial and animal cells (Hartmann *et al.*, 1986; Hulanicka *et al.*, 1964; Seto *et al.*, 1984; Yazawa *et al.*, 1988). EPA has drawn much interest because of its unique biological activities in the prevention and treatment of a number of human diseases and disorders. EPA is an important intermediate in eicosanoid metabolism so that it provides nutritional and pharmacological effects, especially with respect to three areas: the heart

Table 1. List of diseases and afflictions which are potentially reduced by omega-3 PUFAs

Disease class		
Heart and circulatory	Inflammatory	Cancers
Atherosclerosis	Asthma	Breast
Thrombosis	Arthritis	Prostrate
Hypertriglyceridaemia	Migraine headaches	Colon
High blood pressure	Psoriasis	
	Nephritis	
	Diabetes	
	Graft rejection	

Source: Bajpai and Bajpai (1993)

and circulatory system, the inflammatory area and cancers of mammary and colon tissue (Simopoulos, 1989). Other benefits include blood cholesterol reduction, thus reducing the risk of atherosclerosis (Shirasaka and Shimizu, 1995).

Sources of ARA include animals, e.g. pig liver, adrenal glands, and several microorganisms, e.g. certain protozoa, amoebae, algae and fungi (Ratledge, 1986). ARA appears to be more widely distributed than EPA. ARA is also an important intermediate of eicosanoid metabolism, in which ARA is a precursor of prostaglandins and leukotrienes as well as a large group of 20 carbon (C-20) and 22 carbon (C-22) compounds (Braden and Carroll, 1986; Dyerberg, 1986; Kremer *et al.*, 1985). Research

also indicates that ARA helps brain development; ARA has been extracted from algae and used in infant formula (Salem and Carlson, 1991).

However, marine resources are unstable due to the insufficient fish species, and limited seasons and geographic locations (Yongmanitchai and Ward, 1989). In addition, cholesterol and some objectionable tastes and odors are still difficult to remove from fish oil concentrates and animal sources (Jareonkitmongkol *et al.*, 1993a). Therefore, EPA and ARA produced from fungi could be a better product for use as a food additive, supplement, or feedstock.

The objectives of this research were to investigate the potential for fungal production of EPA and ARA, and to determine the feasibility of using food-processing byproducts and crude soybean oil as substrates for the fungal fermentation process. The fungi examined in this research were *Mortierella elongata* NRRL 5513 and *Pythium irregulare* ATCC 10951.

Two food-processing waste streams and crude soybean oil were tested. Crude soybean oil (SBO), provided by Bunge Foods (Chattanooga, Tennessee), was used because it is a cheap and rich source of linoleic and linolenic acids, which are precursors to both ARA and EPA. Soy protein isolate waste or soymeal waste (SMW) was provided by Protein Technologies, Inc. (St. Louis, Missouri), and a sucrose waste stream (SWS) was provided by M&M Mars (Cleveland, Tennessee). The latter two were used as carbon sources, since they are economically viable, and their use would have a potentially positive environmental impact. Glucose (GLU) was used in control groups. Cultures of microorganisms were *Mortierella elongata* NRRL 5513 and *Pythium*

irregulare ATCC 10951 were used as the bioconversion agents, since many studies have indicated that they are good EPA and ARA producers using simple sugars and linseed oil (Bajpai *et al.*, 1991a,b,c,d,e,f; Braden and Carroll, 1986; Shimizu *et al.*, 1989b; Shirasaka and Shimizu, 1995; Yazawa *et al.*, 1988) (Table 2).

Table 2. Comparison of EPA yield and productivity of selected fungi

Cultures	Treatment	Total lipids in biomass (% w/w)	EPA				Reference
			in lipids (% w/w)	in biomass (mg/g)	yield (mg/L)	productivity (mg/L/d)	
Mortierella alpina 1S-4	3% linseed oil with aging	56	12	67	1900	120	Shimizu *et al.* (1989c)
M. alpina 1S-4 Mut. 48	2% linseed oil	33	20	64	970	97	Jareonkitmongkol *et al.* (1993c)
M. elongata NRRL 5513	2% linseed oil	43	6.8	30	610	61	Bajpai *et al.* (1992)
Saprolegnia Sp. 28YTF-1	1% olive oil	--	--	23	180	25	Shirasaka and Shimizu (1995)
P. irregulare ATCC 10951	1.3% sweet whey permeate	9.9	25	25	--	--	O'Brien *et al.* (1993)
M. alpina 20-17	1% SBO	--	--	--	20	--	Shimizu *et al.* (1989b)

CHAPTER II

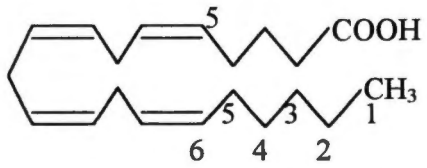
REVIEW OF THE LITERATURE

POLYUNSATURATED FATTY ACIDS (PUFAS)

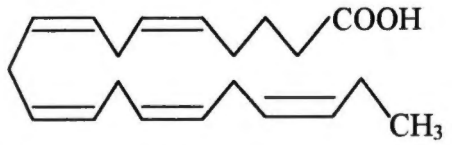
Neutral fats and oils are composed of mono-, di- and tri-esters of glycerol with fatty acids. In the case of polyunsaturated fatty acids, it is convenient to categorize them by the location of the first double bond from the methyl end. Thus, EPA is a C20:5n3 acid, containing 20 carbons, having 5 double bonds, and having the first double bond on the third carbon from the methyl terminal.

Omega-3 fatty acids

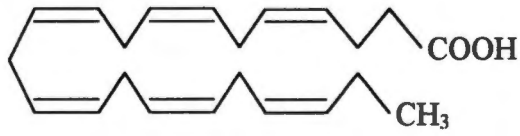
Omega-3 fatty acids like EPA all have the first double bond located on the third carbon atom from the methyl end. The end structure is usually retained even if additional double bonds are introduced into the molecule or if additional carbon atoms are added; thus, α–linolenic acid (C18:3n3) may be changed into eicosatrienoic acid (C20:3n3) while retaining the n-3 structure which confers essential fatty acid character to the molecule (deMan, 1990). Researchers have recently become interested in the health effects of certain n-3 fatty acids, especially EPA (C20:5n3) and docosahexaenoic acid or DHA (C22:6n3). Precise definitions of EPA, DHA, and ARA, along with their chemical structures, are shown in Figure 1.



ARA (5,8,11,14-*cis*-Arachidonic acid, 20:4n6)



EPA (5,8,11,14,17-*cis*-Eicosapentaenoic acid, C20:5n3)



DHA (4,7,10,13,16,19-Docosahexaenoic acid, C22:6n3)

Figure 1. Chemical structures of ARA, EPA and DHA.

EPA (5,8,11,14,17-*cis*-Eicosapentaenoic acid, C20:5n3)

EPA, a rare C-20 PUFA with 5 double bonds, is usually found in marine animals and phytoplankton (Bajpai *et al.*, 1991c; Shirasaka and Shimizu, 1995). Some marine fish require EPA as an essential fatty acid for growth. It is usually one of the major cellular fatty acids in these fish (Shirasaka and Shimizu, 1995). EPA also occurs as a component of cellular lipids in protozoal, algal, bacterial and animal cells (Hartmann *et al.*, 1986; Hulanicka *et al.*, 1964; Seto *et al.*, 1984; Yazawa *et al.*, 1988).

EPA has drawn much interest because of its unique biological activities in the prevention and treatment of a number of human diseases and disorders. EPA is an important intermediate in eicosanoid metabolism so that it provides nutritional or pharmacological effects, especially with respect to three areas: the heart and circulatory system (Daron, 1970; Dyerberg, 1986; Mortensen *et al.*, 1983; Philipson *et al.*, 1985; Urakaze *et al.*, 1986), the inflammatory system (Kremer *et al.*, 1985; Lands, 1986; Robinson *et al.*, 1986; Simopoulos, 1989; Ziboh *et al.*, 1986) and cancers of mammary and colon tissue (Braden and Caroll, 1986; Dyerberg, 1986; Lands, 1986; Maruyama, 1986; Reddy and Kromhout *et al.*, 1985). EPA has also been shown to be effective in preventing or curing thrombosis (Dyerberg, 1986; Dyerberg and Bang, 1978; Kromhout *et al.*, 1985). Other benefits include blood cholesterol reduction, thus reducing the risk of atherosclerosis (Shirasaka and Shimizu, 1995). These benefits have led people to examine potential sources of EPA.

DHA (4,7,10,13,16,19-Docosahexaenoic acid, C22:6n3)

DHA functions in the nervous, visual and reproductive systems (Dratz and Deese, 1986; Neuringer and Connor, 1989; Salem *et al.*, 1986). In mammals and birds DHA is usually associated with phospholipids (Simopoulos, 1989). In human physiology, the benefits of DHA are widely accepted and this has led to extensive nutritional and clinical studies on its effects (Dyerberg, 1986; Braden and Carroll, 1986; Kremer *et al.*, 1985). DHA may be important in the development of brain tissue of babies, because it is one of the most abundant components of the brain's structural lipids, and the lipid fraction of human milk contains thirty times the level of DHA observed in cows' milk lipid (Bajpai

et al., 1991e). Several studies (Anderson, 1970; O'Brian and Sampson, 1965; Poulos *et al.*, 1975) indicated that the cerebral cortex, retina, testes and sperm of mammals, including humans, are also particularly rich in DHA.

There is a deficiency of DHA in the human infant diet as a result of the use of baby formula (Salem and Carlson, 1991). Infant formula reduces DHA content in erythrocyte total lipid, phosphatidylcholine and phosphatidylethanolamine levels to half those found in normal breast-fed infants (Simopoulos, 1989). However, supplementation of pre-term infant formula to provide DHA results in a depression of plasma red blood cell ARA. Therefore, supplements for infant formula should contain both DHA and ARA (Simopolous *et al.*, 1986).

ARA (5,8,11,14-*cis*-Arachidonic acid, C20:4n6)

ARA contains 20 carbon atoms with four double bonds, which are arranged with the last one located five carbon atoms from the methyl end, and therefore, it is referred to as an ω-6 or n-6 fatty acid. ARA is also an important intermediate of eicosanoid metabolism, in which ARA is a precursor of prostaglandins and leukotrienes as well as a large group of 20 carbon (C-20) and 22 carbon (C-22) compounds (Braden and Carroll, 1986; Dyerberg, 1986; Kremer *et al.*, 1985). ARA is usually obtained from pig liver and adrenal glands (Ratledge, 1986). It appears to be more widely distributed than EPA and DHA, having been identified as a product of animals and numerous microorganisms such as certain protozoa, amoebae, algae and fungi. Research also indicates that ARA helps brain development and it has been extracted from algae and used in infant formula (Salem and Carlson, 1991).

MICROBIAL SOURCES OF PUFAS

Several lower fungi of the class Phycomycetes have been reported as promising PUFA producers, particularly strains belonging to the genus *Mortierella*. Other sources of EPA have also been reported, especially from fungi of the orders Mucorales and from several algae. These fungi are rich in γ-linolenic acid (C18:3n6) and the fermentation processes for production of these fatty acid products have been commercialized (Ward, 1989).

Advanced Fungi

Low levels of ARA have been reported in species of some of the more advanced fungal genera, i.e. *Penicillium*, *Aspergillus* and *Fusarium*, when cultured on short-chain fatty acids (Radwan and Soliman, 1988).

Primitive Fungi

With few exceptions, ARA distribution in fungi appears to be mainly in the more primitive species such as those belonging to the classes Hyphochytriomycetes, Chytridiomycetes and Oomycetes of the subdivision Mastigomycotina (Weete *et al.*, 1989), including *Pythium ultimum* (Kerwin and Duddles, 1989). Microbial sources of EPA and other PUFAs have been recently reviewed (Radwan 1991). Several fungi belonging to genera such as *Mortierella*, *Saprolegnia*, and *Pythium* have been reported to produce relatively large amounts of oil with EPA. Therefore, these fungi are also candidates as practical sources of EPA.

Mortierella. Many species of the genus *Mortierella* produce considerable amounts of EPA, ARA, and γ-linolenic acid (C18:3n6) (Sajbidor *et al.*, 1988; Shimizu *et al.*, 1988a; Shimizu *et al.*, 1989a,b,c; Shinmen *et al.*,1989; Totani and Oba, 1987; Weete, 1980), depending on the species and culture. Factors affecting production of ARA and EPA by *Mortierella* strains producing high amounts of these acid components have been investigated (Bajpai *et al.*, 1991a,b,c,d,f, 1992; Shimizu and Yamada, 1990, 1991; Shimizu *et al.* 1988a,b, 1989a,b,c, 1991, 1992). Such oleaginous fungi, which produce PUFAs of both n-6 and n-3 species, could provide an economically viable source of PUFAs if most of the PUFAs occur in the triacylglycerol fraction of lipids which would then be extractable as a plant-like oil rather than a complex array of lipid classes (Kendrick and Ratledge, 1992a,b,c,d, 1996).

Saprolegniales. A survey of the literature (Bajpai *et al.*, 1991e) indicates that some lower fungi within the orders Saprolegniales and Entomorphorales, and especially the strain *Thraustochytrium aureum*, produce significant quantities of DHA (Bajpai *et al.*, 1991e; Haskins *et al.*, 1964). This non-filamentous pigmented marine species has been found to display light-stimulated growth (Ellenbogen *et al.*, 1969). *Saprolegnia* sp. 28YTF-1 (Gellerman and Schlenk, 1979; Shirasaka and Shimizu, 1995), isolated from a fresh-water sample, is also a potent producer of EPA.

Pythium* and *Phytophthora. The Oomycetes are the more primitive fungi, including the pythiaceous species, *Pythium* and *Phytophthora* (O'Brien *et al.*, 1993; Shaw, 1966; Weete and Gandhi, 1992). *Pythium irregulare* is a filamentous fungus of the Oomycetes group of the lower Phycomycetes family of fungi. *P. irregulare* was

identified as a potential organism for EPA production (25% EPA in total lipids, see Table 2) from a specific screening of selected genera of fungi (O'Brien *et al.*, 1993; Stinson *et al.*, 1991; Wessinger *et al.* 1990). Also, *P. ultimum* is potential to produce 20-230 mg ARA/L and 20-220 mg EPA/L (Weete and Gandhi, 1992).

Others (Algae / Microalgae / Bacterium)

Most attention has been focused on marine microalgae such as *Chlorella minutissima* (Seto *et al.* 1984), but there were not high PUFA contents found. Other possible sources of the omega-3 fatty acids include certain algae (Behrens *et al.*, 1989; Erwin, 1973; Yongmanitchai and Ward, 1989), which are actually the dietary origins of such fatty acids in fish. A freshwater alga, *Monodus subterraceus*, a moss, *Leptobryum pyriforme* and *Euglena gracilis* also are sources of EPA, but they have low growth rates and lipid contents (Jareonkitmongkol *et al.*, 1993b). *Entomophthora exitalis*, a strain of obligate aerobic, Gram-negative, motile and short rod-shaped marine bacterium, named SCRC-8132, has been investigated (Kendrick and Ratledge, 1992a; Yazawa *et al.*, 1988), but its oil productivity and the EPA level in the oil or lipid was not satisfactory.

NUTRITIONAL BENEFITS

In recent years, nutritional studies have shown the potential benefits of including long-chain PUFAs in the human diet. These fatty acids exhibit beneficial effects in coronary heart disease, hypertension, psoriasis, arthritis, inflammation, other autoimmune disorders and cancer (Bajpai and Bajpai, 1993; Simopoulos, 1989). Physiological effects

of omega-3 fatty acids have been observed in the areas of heart and circulatory, inflammatory diseases, and cancers (Table 1).

Dietary Balance of n-6 and n-3 PUFAs

The eicosanoids from EPA and ARA are structurally and functionally different and are sometimes even antagonistic in their effects (Bajpai and Bajpai, 1993). Since ARA metabolites are predominantly present in patients with cardiovascular disease, hypertension and autoimmune disorders, it is strongly suggested that a balance between n-3 and n-6 PUFAs is necessary for appropriate platelet function, blood flow, blood viscosity, membrane fluidity, and monocyte function (Simopolous *et al.*, 1991). Nevertheless, the ideal ratio of n-6 and n-3 PUFAs in the diet is not known. The ratio in maternal milk in humans ranges from 1 to 7; however, the present Western diet is low in n-3 PUFAs (ARA/EPA = 10 – 14) (Leaf and Weber, 1987).

NATURALLY SYNTHETIC PATHWAYS VERSUS BIOSYNTHETIC PATHWAYS

In nature, EPA and DHA can be produced slowly from linolenic acid (C18:3) by herbivore animals, marine algae, phytoplankton and some cold-water fish, such as cod, mackerel, tuna, swordfish, sardines, and herring (Ackman, 1982; Simopoulos, 1989). In a mammalian system, on the other hand, it has been demonstrated that dietary α-linolenic acid (C18:3n3) is converted to EPA through the n-3 route (Fulco, 1974; Shimizu *et al.*, 1989b).

Basically, the physiological effects of the omega-3 fatty acids are associated with changes in eicosanoid levels in the circulatory system. ARA (n-6) and EPA serve as precursors of eicosanoid compounds, which are hormone-like substances including prostaglandins (PG), thromboxanes (TX) and leukotrienes (LT) (Figure 2) (Simpopoulos *et al.*, 1986).

Because of the diversity of lipids and fatty acid biosynthetic pathways, lower fungi may find utilization as sources of omega-3 or other PUFAs (O'Brien *et al.*, 1993). PUFAs in microorganisms are commonly synthesized from the starting monounsaturated fatty acid, oleic acid (C18:1n9). There are two principal reactions involved—chain elongation and desaturation. Chain elongation is the same in all pathways where two carbon atoms from the donor (either acetyl CoA or malonyl CoA) are introduced to increase chain length, while desaturation of fatty acids may be varied in two different metabolic directions, omega-3 (n-3) and omega-6 (n-6) routes (Figure 3).

Omega-3 route

The omega-3 route involves desaturation to produce linoleic acid (C18:2n6) and α-linolenic acid (C18:3n3) by the introduction of the first double bond at the 12,13-position of the oleic acid chain to form linoleic acid, and then the second at the 15,16 position to form linolenic acid. Further chain elongation and two more desaturation reactions will yield EPA (C20:5n3). The pathway has been observed in red algae (Korn *et al.*, 1965), some fungi (Shimizu *et al.*, 1988b), and other marine microorganisms (Ben-Amotz *et al.*, 1987; Henderson *et al.*, 1988). Shimizu *et al.* (1989b) also indicated that low temperature may induce EPA production. DHA is produced from EPA by a

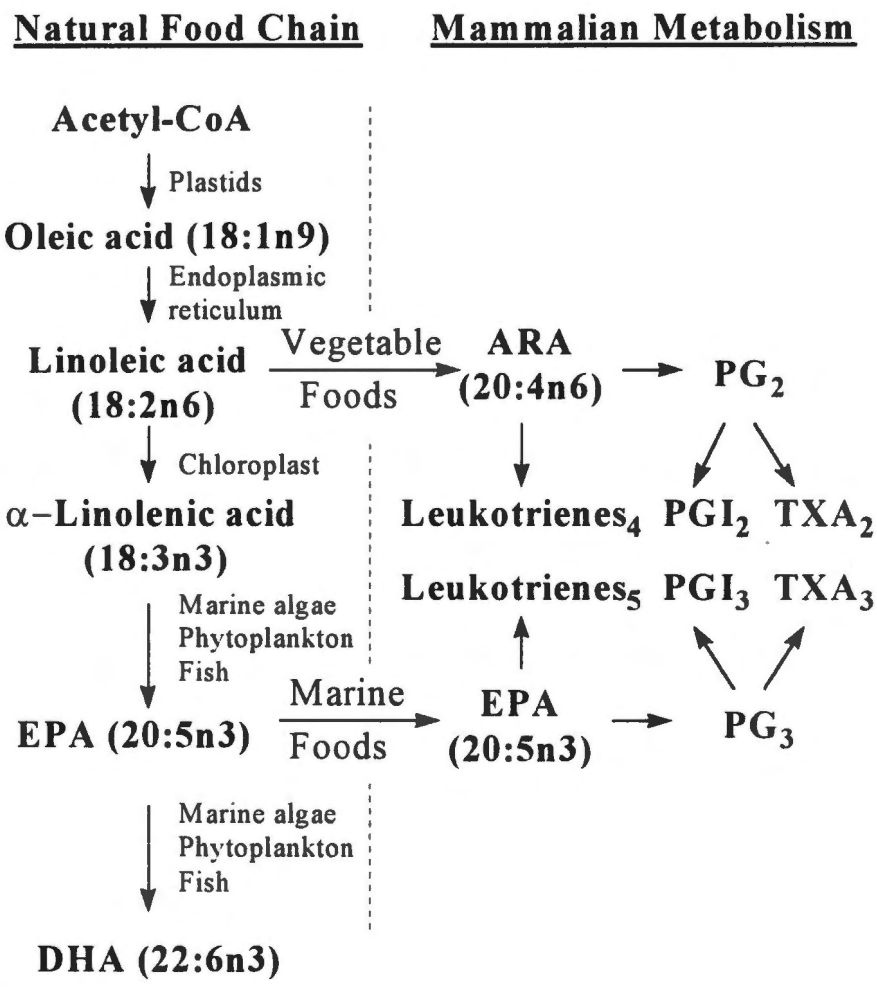


Figure 2. Origin and metabolic pathways of omega-3 and omega-6 fatty acids and biosynthesis of eicosanoids from ARA (20:4n6) and EPA (20:5n3)(Abbreviations: PG_2, prostaglandin 2; PG_3, prostaglandin 3; PGI_2, prostacyclin 2; PGI_3, prostacyclin 3; TXA_2, thromboxane A_2; TXA_3, thromboxane A_3)(Source: Simopoulos *et al.*, 1986)

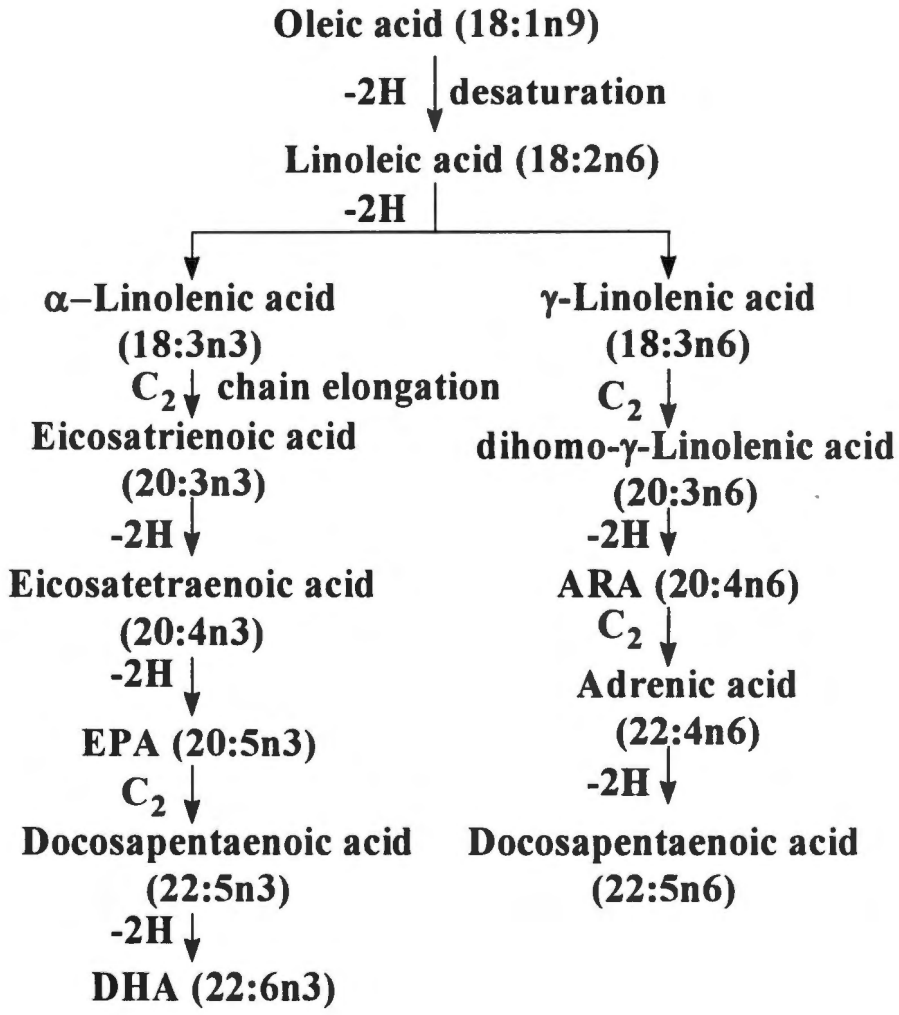


Figure 3. Pathways of PUFAs production in microorganisms (Source: Bajpai and Bajpai, 1993)

desaturation and elongation mechanism and thus it shares a common biosynthetic pathway with omega-3 fatty acid precursors of eicosanoid compounds (Bajpai *et al.*, 1991e; Braden and Carroll, 1986). This is a very promising route for the commercial production of EPA, because there are various kinds of natural oils containing α-linolenic acid that are easily available, and these oils may be expected to be converted to EPA-containing oils on incubation with these fungi (Shimizu *et al.*, 1989b). Jareonkitmongkol *et al.* (1993b) also found a Δ12 desaturase-defective mutant of an ARA-producing fungus, *Mortierella alpina* 1S-4, that could convert α-linolenic acid to EPA.

Omega-6 route

The omega-6 route is also based on the two reactions, but the additional double bonds are generally introduced towards the carboxyl end of the molecule. This pathway produces ARA via either of the following two routes. The first one is desaturation of linoleic acid to γ-linolenic acid, followed by chain elongation (Nichols and Appleby, 1969). Many algae use the second route through which chain elongation of linoleic acid to 11,14-eicosadienoic acid is followed by carboxyl directed desaturation (Nichols and Appleby, 1969; Hulanicka *et al.*, 1964). However, a study of the fungus, *Saprolegnia parasitica* (a fish parasite), showed that the biosynthetic pathway of PUFAs is a combination of the two routes (Gellerman and Schlenk, 1979). Certain oleaginous fungi are able to accumulate up to 85% (w/w) lipid as a storage compound of the biomass (Kendrick and Ratledge, 1992b). They found that the lipid accumulation is triggered by exhaustion of a growth nutrient, other than carbon, thus preventing cell proliferation but

still allowing conversion of substrate into lipid. This finding may have important implications for industrial fermentations.

FACTORS LIMITING OMEGA-3 PUFA PRODUCTION FROM FISH OIL

Today the major commercial sources of EPA and DHA are obtained from marine fish and fish oils. The content of omega-3 fatty acids in fish oils from each source depends on species of fish, seasons, and geographic location of catching sites, which in turn are determined by the availability and make-up of marine organism feed sources (Yongmanitchai and Ward, 1989). Fish oil concentrates are considered unattractive since they contain substantial amounts of undesirable fatty acids and cholesterol (Shimizu *et al.*, 1988a; Wassef, 1977), and are unlikely to meet future demand (Jareonkitmongkol *et al.*, 1993a; Yongmanitchai and Ward, 1989). ARA is presently isolated in low yields from fish and fish oil, and from animal adrenal glands and livers (Ahern, 1984). However, because of the relatively low proportion, e.g., 7-14% DHA (Bajpai *et al.*, 1991e), of omega-3 fatty acids in fish oil and the difficulties encountered in extraction and purification (Aveldan *et al.*, 1983; Haagsma *et al.*, 1982; Nilsson *et al.*, 1988) to remove objectionable tastes and odors, cholesterol, and small amounts of potentially toxic impurities (O'Brien *et al.*, 1993), other sources of ARA, DHA and EPA are being sought among phytoplankton, algae and fungi (Burr, 1989; Shirasaka and Shimizu, 1995; Yongmanitchai and Ward, 1989).

In conclusion, major sources of PUFAs are fish, algae and fungi. However, fish oil has many negative attributes. Some algae produce high amounts of ARA, but are antagonistic to EPA. Therefore, fungi could be the best source of n-3 and n-6 PUFAs.

INDUSTRIAL USES OF EPA AND ARA PRODUCED FROM FUNGI

The approximate U.S. market value for omega-3 fatty acids was $100 million and the projected market was $790 million in 1987 (Van der Wende, 1988). However, major marine resources are insufficient to meet this demand due to the factors mentioned previously. In addition, cholesterol and some objectionable tastes and odors are still difficult to remove from fish oil and animal oils.

For practical purposes, EPA and ARA oils produced from fungi could be used as food additives, supplements or as supplements to animal feeds. They could easily be used as additives to a product such as salad dressing. Also, EPA and ARA-containing fats or oils could be used for further purification and concentration for pharmaceutical applications. However, these applications of fungi for fatty acid production would require design of a process that would include fermentation, mycelium separation from the fermentation broth, and lipid extraction from the fungal mycelium (O'Brien *et al.*, 1993). The focus of this work was the fermentation process. Industrial waste streams (soymeal waste stream and sucrose waste stream) were used as feed sources for the fungi, because they are the most economically viable, and their use would have a potentially positive environmental impact. The sucrose waste stream was composed of water (73%), sucrose (24.4%), glucose (1.7%), and fructose (0.9%). Soymeal waste (soy fiber) derived from

soybean cotyledon was comprised of 73.3% insoluble dietary fiber, 3.1% soluble dietary fiber, and other components (Figure 4) (Lo, 1990). Major carbon sources in soymeal waste were galactose (23.4%), pectin (22.9%), cellulose (13.8%), arabinose (11.9%), xylose (6.9%), and protein (10.8%). Unrefined soybean oil was also used because it is a cheap and rich source of linolenic acid (C18:2n6 > 50%), a major precursor to ARA and EPA production. Comparison of fatty acid compositions of crude soybean oil, refined soybean oil and linseed oil is shown in Table 3. Although linseed oil is one of the best substrates (C18:3n3 > 50%) for EPA production, it is more costly than soybean oil.

TWEEN 80 AS AN EMULSIFIER

In order to promote dispersion of the soybean oil, Tween 80 was used as an emulsifier. An "emulsion" has been defined by Becher (1965) as "a heterogeneous system, consisting of one immiscible liquid intimately dispersed in another one, in the form of droplets with a diameter generally over 0.1 μm." An emulsion system has a minimal stability, which can be enhanced by surface-active agents, also called emulsifiers. Such agents are valuable in the fermentation of lipophilic compounds. Emulsifiers reduce the interfacial tension between air-liquid and liquid-liquid interfaces by a unique ability: the molecules contain two distinct sections, one having polar or hydrophilic character, the other having nonpolar or hydrophobic properties. Most surface active agents reduce the surface tension from about 50 dynes/cm to less than 10 dynes/cm

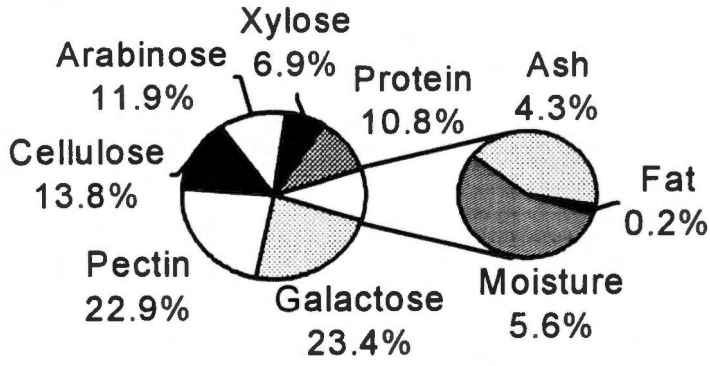


Figure 4. Composition of soymeal waste (soy fiber) (Source: Lo, 1990)

Table 3. Fatty acid composition (%, w/w) of refined and crude soybean oils, and linseed oil

Common name	Symbol	Crude soybean oil	Refined soybean oil	Refined soybean oil [a]	Refined soybean oil [b]	Linseed oil [b]
Palmitic	C16:0	11.6	11.4	10.6	10.0	10.0
Stearic	C18:0	4.5	4.1	4.0	3.7	3.7
Oleic	C18:1n9	21.7	19.9	23.2	23.1	10.7
Linoleic	C18:2n6	57.2	55.1	53.7	53.8	17.5
α -Linolenic	C18:3n3	4.3	8.9	7.6 [c]	8.8 [c]	58.1
Others		0.7	0.6	0.9	0.6	-

[a] Source: Stauffer (1996)
[b] Source: Shimizu *et al.* (1989b)
[c] Total linolenic acid (α– and γ–)

when used in concentrations below 0.2% (deMan, 1990). The HLB (hydrophile-lipophile balance) system, scaled from 0 to 20, is an expression for the relative simultaneous attraction of an emulsifier for water and for oil. Emulsifiers with low HLB tend to form water-in-oil (W/O) emulsions, those with intermediate HLB form oil-in-water (O/W) emulsions, and those with high HLB are solubilizing agents. HLB values are calculated from the saponification number of the ester (S) and the acid value of the fatty acid radical (A_v) as follows (deMan, 1990):

$$HLB = 20\left(1 - \frac{S}{Av}\right)$$

At each end of the scale the compounds would have little emulsifying activity for our application.

Tween 80 (polyoxyethylene sorbitan monooleate)

Tween 80 is a non-ionic liquid detergent with an amber color. It is very soluble in water, and soluble in ethanol, methanol, ethyl acetate, and toluene. Tween 80 belongs to a group of monoglycerides obtained by glycerolysis and molecular distillation under high vacuum. Its 1-monoglyceride content exceeds 70% (responsible for O/W emulsion); pH ranges from 5.5 to 7.2 in 1% solution; and the viscosity ranges from 400 to 620 g/cm-sec (25°C). The chemical structure of Tween 80 is shown in Figure 5. Tween 80 has a solubility at 1 mL in 10 mL of water with a HLB value of 10.0, which is optimal for O/W emulsion (Friberg, 1976). Therefore, it can be used to promote submerged growth of some microorganisms.

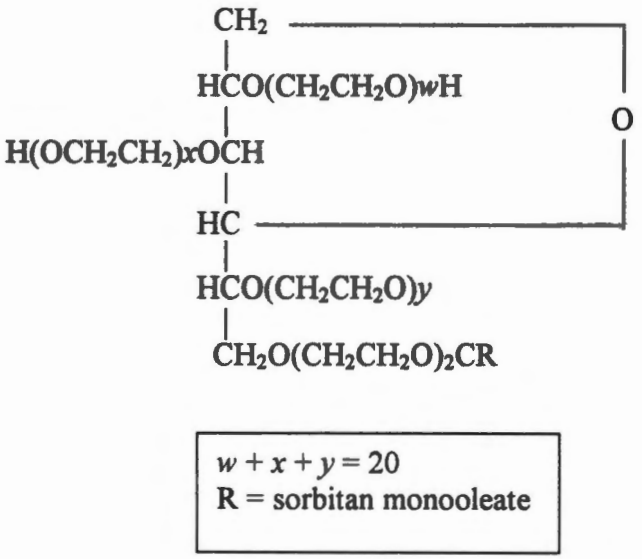


Figure 5. Chemical structure of Tween 80 (Source: Dawson, 1986)

CHAPTER III

MATERIALS AND METHODS

CHEMICALS AND SUBSTRATES

All reagents used in this work were analytical grade and commercially available, including glucose (GLU), yeast extract, potassium phosphate, hexane, isopropanol, sodium sulfate, methyl acetate, sodium methoxide, methanol, Tween 80, FAME 37 standards (authentic fatty acid methyl esters), heptadecanoic acid (C17:0), butylated hydroxytoluene (BHT), acetic acid, and nitrogen, helium, hydrogen and air gases.

Several food byproducts and crude soybean oil were tested. Crude soybean oil (SBO) was obtained in unrefined form from BUNGE Foods, Chattanooga, Tennessee. Sucrose waste stream (SWS), a byproduct from making chocolates, was received from M&M Mars, Cleveland, Tennessee. Defatted soymeal waste (SMW), a byproduct from making protein drinks, was acquired from Protein Technologies, Inc., St. Louis, Missouri.

EXPERIMENTAL DESIGN

Table 4 outlines the four experiments that were performed in this study.

Experiment 1. The experiment was conducted with *M. elongata* and *P. irregulare* in a water-bath shaker at 12 and 24°C in order to find out which fungus was better for EPA and ARA production. Substrate concentrations were 1% (w/v) glucose

Table 4. Summary of experimental design [a]

Microorganisms	Temperatures	Treatments [b]
M. elongata and *P. irregulare*	12 or 24°C	1% GLU; 1, 3 or 5% SBO; water-bath shaker; n=3
P. irregulare [c]	12 and 24°C	2% GLU; 2% SWS, 2% SMW or 2% SBO; water-bath shaker; n=2
P. irregulare	12 and 24°C	All combinations of 2, 3, or 4% SBO + 0.1, 0.2, or 0.4% TW80; orbital shaker; n=3
P. irregulare	12, 18, and 24°C	1, 2, or 3% GLU + SBO + TW80 [c]; orbital shaker; n=3
		1, 2, or 3% SMW + SBO + TW80 [c]; orbital shaker; n=3
		1 or 3% SWS + SBO + TW80 [c]; orbital shaker; n=3

[a] All experiments conducted with 3 replications except the second experiment (2 replications) for each treatment

[b] Abbreviations: SBO, crude soybean oil; TW80, Tween 80; SWS, sucrose waste stream; SMW, soymeal waste; GLU, glucose

[c] Determined from the previous experiment

(control) and 1, 3, 5% (w/v) soybean oil (SBO).

Experiment 2. The purpose of this experiment was to investigate the utilization of crude soybean oil and two food processing byproducts by the best EPA producer in the water-bath shaker at 12 and 24°C. The concentration applied of each substrate, GLU, SBO, SWS, or SMW, was 2% (w/v).

Experiment 3. In early experiments, there was a problem with agglomeration of soybean oil in aqueous media. Therefore, an emulsifier (Tween 80) was added to allow better utilization of soybean oil as a major carbon source in culture media in the form of an oil-in-water or O/W emulsion. To find out the best combination of crude soybean oil and Tween 80 for EPA and ARA production by *P. irregulare*, 2, 3, 4, and 5% (w/v) SBO was tested in combination with 0.1, 0.2, and 0.4% (w/v) Tween 80 (TW80). Flasks were incubated in a temperature controlled orbital shaker (300 rpm).

Experiment 4. To investigate maximum EPA production by *P. irregulare*, the combination of SBO and TW80 resulting in maximum EPA production in experiment 3 was added to three different concentrations (1, 2, and 3%, w/v) of GLU, SWS or SMW, each being as an additional carbon source. EPA production was investigated at 3 temperatures, 12, 18 and 24°C. The orbital shaker was maintained at the same speed as experiment 3. Four control groups included 2% GLU, 2% SMW, 4% SBO, and 4% SBO+0.2%TW80.

MICROORGANISMS AND CULTIVATION

Mortierella elongata NRRL 5513 and *Pythium irregulare* ATCC 10951 cultures were purchased from American Type Culture Collection (ATCC, Rockville, Maryland) These fungi were maintained in our stock and transferred every three months through slant tubes of corn meal agar (CMA).

Fermentation experiments were conducted in shaker flasks in an orbital shaker or shaking water bath. 45-mL of culture broth (substrates added to basal media) in 250 mL flasks were autoclaved at 121°C under pressure (1.1 kg/cm^2 or 15 psi) for 15 min before inoculation with 2 mL of blended inoculation culture. The inoculation culture was created by adding 1 cm^2 of culture grown on potato dextrose agar (PDA) to 45 mL of 2% glucose and shaking at 24°C for 2 days. The flasks were incubated with shaking in a water bath (120 strokes/min) or in an orbital shaker (300 rpm) for 8 days. The incubation of 12°C groups was 2 days at 24°C followed by 6 days at 12°C; the 24°C groups were maintained at 24°C for the entire 8 days. This design was based on a previously mentioned finding by Kendrick and Ratledge (1992b) that the lipid accumulation is triggered by exhaustion of nutrients.

In all experiments a basal media (0.5% yeast extract and 0.1 g/L phosphate) was adjusted to a pH of 6.0 with acetic acid and NaOH. The only exception was that neither yeast extract nor phosphate was added to the soymeal waste samples, because they already contained sufficient amounts of nitrogen. Fermentation substrates were added at levels as stated.

EXTRACTION OF LIPIDS

The methods of Hara and Radin (1978) and Lepage and Roy (1984) were modified and used in this study. Fungal biomass was harvested by centrifugation at 4,500 rpm for 10 min. The wet mycelium was washed with 10 mL of distilled water to remove leftover substrates, and dried in a freeze-dryer for at least 17 h. Samples grown on soybean oil were additionally washed three times (5-mL petroleum ether for each wash). After that, 14 mL of hexane-isopropanol solution (HIP, 3:2) was applied to an aliquot of 0.1 g biomass in a centrifuge tube, homogenized with a Polytron for 1 min, and centrifuged at 4,500 rpm for 10 min. The homogenization and centrifugation steps were repeated twice using 3 mL of HIP with the residue. The three washed supernatants were poured into the same tube. The supernatant was then filtered through Whatman No.1 filter paper on a Coors No. 3 Buchner funnel with slight suction. Afterwards, the filtrate was transferred to a 45-mL glass tube. Then, 13.3 mL of 0.47 M sodium sulfate was added to break the emulsion. The upper phase (hexane layer) containing purified lipid was poured into a tared 20-mL vial and evaporated to dryness under a gentle stream of nitrogen with slight heat (40-50°C) in a water bath. The dry weight of the residue was then determined as the weight of total lipids.

PREPARATION OF METHYL ESTERS FOR GC ANALYSES

A rapid transmethylation method, based on Christie (1982), was applied in this study. Before GC analysis, extracted triglycerides and phospholipids were trans-

esterified rapidly in anhydrous methanol in the presence of a basic catalyst, sodium methoxide, which facilitated the exchange between glycerol and methanol to form fatty acid methyl esters (FAME). An amount of 1.95-mL hexane was added to the dried sample from the extraction. Then, 50 µl of C17:0-BHT solution (made by mixing 1.5 mL of 10 mg/mL heptadecanoic acid with 15-mg BHT for prevention of oxidation of PUFAs) was added. To make methyl esters, 40-µL methyl acetate and 40 µL of 1 M sodium methoxide in dry methanol were added for catalyzing esterification. The solution immediately became cloudy as sodium-glycerol derivatives were precipitated. After 5 min at room temperature, the reaction was stopped by adding 60 µL of 1 M acetic acid. The top 1.5-mL solution was collected (avoiding bottom sodium acidate precipitate) to a 2-mL brown vial, and a 1-µL aliquot was taken directly for GC analysis.

GC Analysis and Yield Calculation

Gas Chromatography

A gas chromatographic (GC) unit was coupled to a flame ionization detector (FID) with a WCOT fused silica capillary column (30 m × 320 µm i.d. × 0.25 µm nominal)(model No.: ChromPack 8843 with stationary phase CP-Wax 52 CB, Chrompack Inc., Netherlands). A Hewlett Packard 6890 GC system equipped with an enhanced integrator and HP 3365 ChemStation software was used to determine the percent of each fatty acid. Helium was used as a carrier gas with a flow rate of 2.5 mL/min; the hydrogen flow rate was 40.0 mL/min; and the airflow rate was 380 mL/min.

Both injector temperature and detector temperature were 250°C. The initial oven temperature was 120°C, and it was raised to a final temperature of 230°C through temperature programming (Table A1). Hexane was used as the solvent, and 1 μL of solution was injected. The split to splitless ratio was 10:1. To analyze those PUFAs, correction factors of authentic standards (FAME 37) and an internal standard method were used. Those fatty acids were identified by comparing their retention times from GC analysis with FAME 37.

The operating parameters and FAME 37 for the GC are outlined in Appendix A. Fatty acids quantified were palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1n9), linoleic acid (C18:2n6), α-linolenic acid (C18:3n3), arachidonic acid (C20:4n6), and EPA (C20:5n3). The resolution of ARA peak and EPA peak in authentic standards was obtained by the equation (Pomeranz and Meloan, 1987):

$$R = \frac{2(\Delta t_R)}{W_1 + W_2}$$

Δt_R = difference of two retention times
W_1 = width of ARA peak
W_2 = width of EPA peak

PUFA Yield Calculation

The corrected area normalization of individual PUFAs and the internal standard method (Rounds and Nielsen, 1994) were used for yield calculation. The calculation of PUFA levels was based on the following equations:

$$Yield = (\%PUFA)(Lipid_{aliquot})\left(\frac{X_{total}}{X_{aliquot}}\right)\left(\frac{1}{Broth}\right)$$

$$\%PUFA = \frac{\frac{Area_i}{C_i}}{\sum\left(\frac{Area_i}{C_i}\right)}$$

in which PUFA *Yield* (mg/L) is expressed as mg per litter of culture broth; $Lipid_{aliquot}$ is the lipid content (mg) harvested from biomass aliquot ($X_{aliquot}$); X is biomass weight (mg); *Broth* is the total volume of culture broth (L). *Percentage PUFA* (w/w) is based on the $Area_i$ (the peak area of individual PUFA in the sample) and the correction factor (C_i). Correction factors of FAME 37 were calculated as:

$$C_i = \frac{\frac{Area_i}{Conc_i}}{\frac{Area_{is}}{Conc_{is}}}$$

$Area_i$ = the peak area of individual PUFA of FAME 37
$Area_{is}$ = the peak area of internal standard of FAME 37
$Conc$ = % PUFA (w/w) of FAME 37
i = individual PUFA of FAME 37
is = internal standard

STATISTICAL ANALYSIS

A complete randomized design (CRD) with a 3×3×3 factorial and three replications (two replications for the second experiment) was applied for statistical purposes. The data were analyzed by Statistical Analysis System (SAS Institute Inc., 1990). The analysis of least squares means (LSMEANS) of PUFA yields among different temperatures, media, concentrations, and their interactions were determined by Proc Mixed using observed marginals (OM) and BYLEVEL options so that the

LSMEANS could be estimated even with missing treatment combinations involving 2% SWS. In the case of significant F values, the Tukey test was used to separate the treatment LSMEANS.

CHAPTER IV

RESULTS AND DISCUSSION

EXPERIMENT 1: *MORTIERELLA ELONGATA* VERSUS *PYTHIUM IRREGULARE*

EPA, ARA and Biomass Yields

Figures 6 and 7 show the comparison of EPA, ARA and biomass yields between the two microorganisms (see Appendix B for statistical grouping). Since many studies indicate *M. elongata* NRRL 5513 prefers lower temperatures in producing PUFAs (Neidleman, 1987; Shimizu *et al.*, 1989b; Bajpai *et al.*, 1991a,b,c,d,f), a temperature of 12°C (2 days at 24°C followed by 6 days at 12°C) was applied in all experiments when this microorganism was used. When comparing SBO with GLU, the greatest production of both ARA and EPA (grams per liter of culture broth) from *M. elongata* NRRL 5513 was achieved with glucose ($P < 0.05$). In SBO samples, the ARA levels were about 130-160 mg/L, while EPA levels remained very low, 16-32 mg/L. The EPA content was too low for *M. elongata* to be a good producer from SBO. Figure 6 also illustrates the total dried biomass yield was only 1.2 to 2.0 g/L over the 8 day growing period. The ARA to EPA ratio decreased from 10 to 4 with increased SBO concentrations. Typical values for this ratio lie between 2 and 20 for most *Mortierella* species (Shimizu *et al.*, 1989b).

Bajpai *et al.* (1991b) found that the ARA/EPA ratios for *M. elongata* NRRL 5513

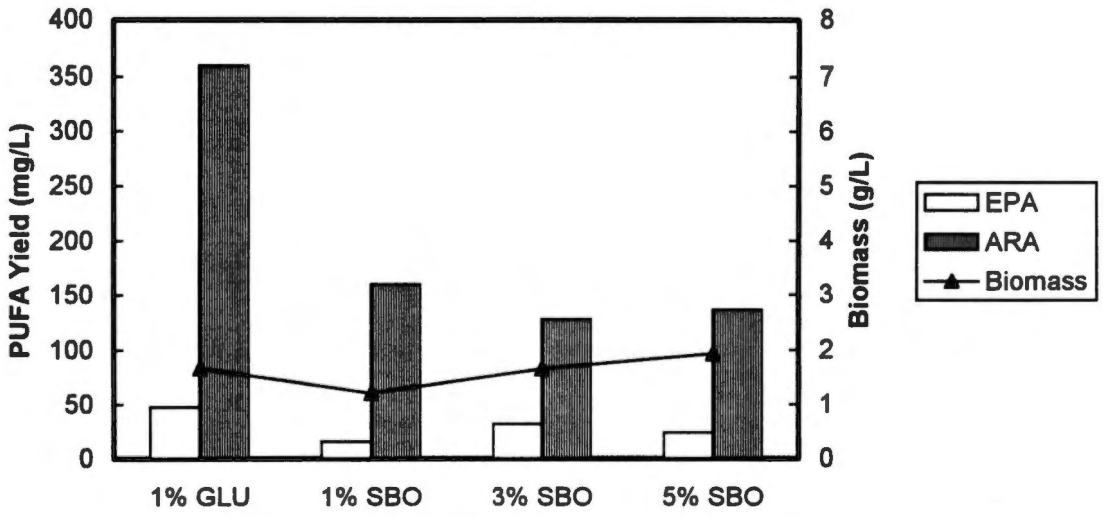


Figure 6. Effect of crude soybean oil concentrations on EPA, ARA and biomass production by *M. elongata* NRRL 5513 in water-bath shaker for 2 days at 24°C followed by 6 days at 12°C (see Table B1 for statistical grouping)

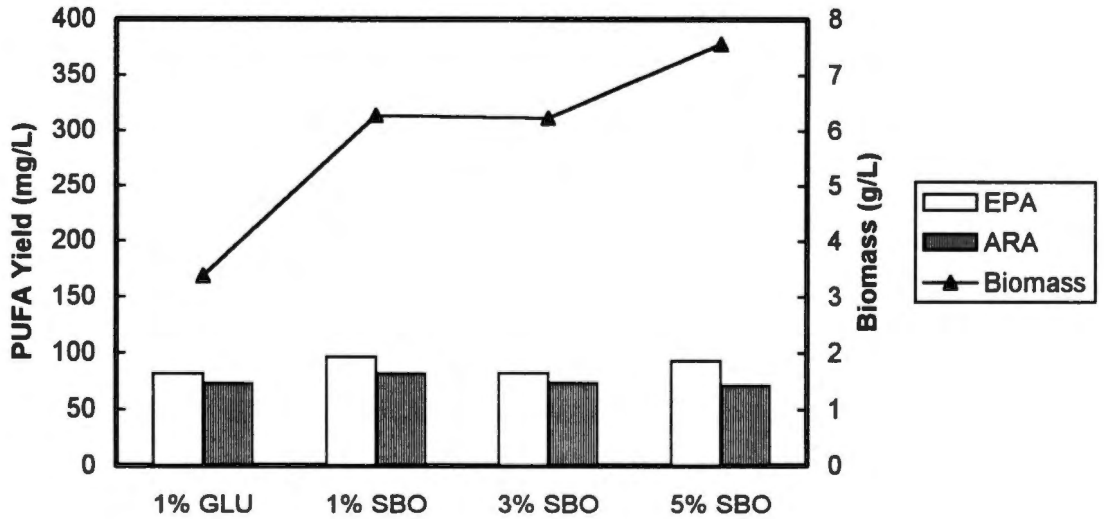


Figure 7. Effect of crude soybean oil concentrations on EPA, ARA and biomass production by *P. irregulare* ATCC 10951 in water-bath shaker for 8 days at 24°C (see Table B2 for statistical grouping)

grown at 11°C were much higher with 4% glucose (2.3) than 4% linseed oil (0.86) and a combination of 2% of each substrate yielded an intermediate ratio of 1.5. Also production of lipids were highest in 4% linseed oil. In experiment 1 *M. elongata* yielded high ARA/EPA ratios. A 2% glucose solution produced a ratio of 7.5, while a 3% soybean oil solution had a ratio of 4.0; however, the product yields were much lower for soybean oil.

P. irregulare cultivated at room temperature (24°C) for 8 days produced both ARA and EPA at approximately the same levels (Figure 7). The ARA/EPA ratio ranged from 0.8 to 0.9 (Appendix B). SBO at these three concentrations yielded productivities that were not different from those of the glucose (control) ($P > 0.05$).

pH Effect

The pH stayed in the optimal range of 6.0-8.5 in the soybean oil samples (SBO) and dropped below that level in the glucose samples (Figure 8). A possible reason is that the choline group of lecithin functioned as a buffer from crude soybean oil. The pH effect is a likely explanation for the reduced fungal growth in glucose, compared to SBO, as the fermentation progressed.

Effect of SBO on Lipid Production

Lipid production was substantially different in the SBO samples. Samples with 5% SBO in experiment 1 had the highest lipid production in biomass -- 24% by *M. elongata* and 28% by *P. irregulare* (Appendix B). The high amount of lipid came from the high level of SBO in the broth. It is possible that microorganisms utilize and store SBO inside the cells or SBO attached outside the cells was not completely washed by

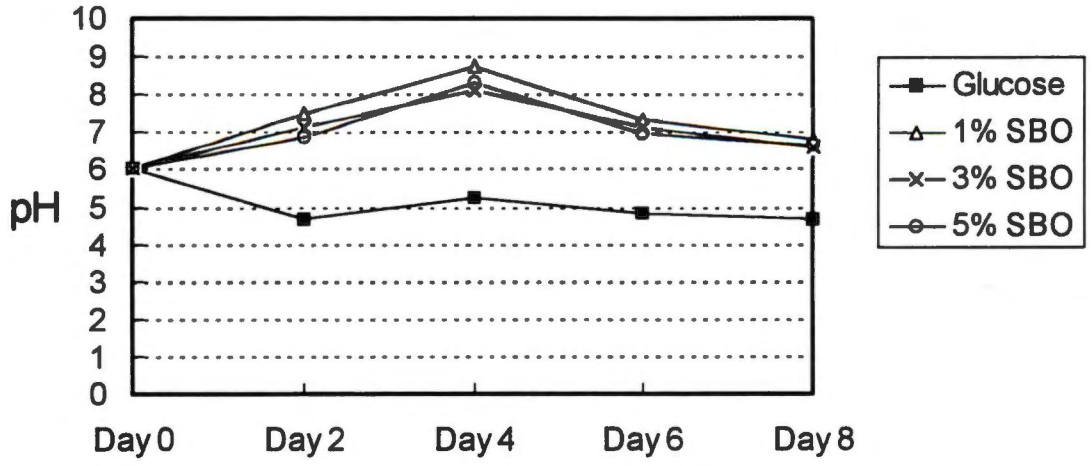


Figure 8. pH changes of culture broth of *M. elongata* NRRL 5513 during experiment 1

petroleum ether. Therefore, residual SBO inflated the amount of total lipids and PUFAs detected. This effect will also be shown in the results of experiments 3 and 4.

EXPERIMENT 2: EFFECT OF SBO AND FOOD WASTE STREAMS – SWS AND SMW

High yields of EPA and ARA were obtained from the fermentation of *P. irregulare* with the two industrial waste streams and glucose (Figures 9, 10; see Appendix C for statistical grouping). Soybean oil was a statistically ($P < 0.05$) inferior medium at 12°C with regard to biomass and EPA production. While the SBO alone under low temperatures is generally considered to be a poor substrate for *P. irregulare*, the sucrose waste stream (SWS) had an EPA and ARA production of 470 mg/L and 210 mg/L, respectively, which were not different ($P > 0.05$) from those of the control group at

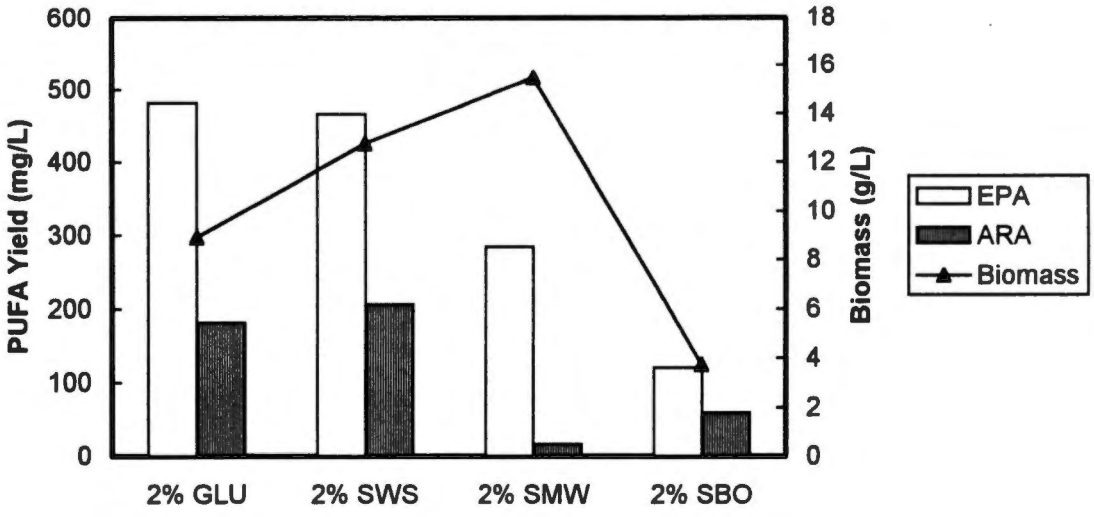


Figure 9. Effect of different media on EPA, ARA and biomass production by *P. irregulare* ATCC 10951 at 12°C (24°C for 2 days followed by 12°C for 6 days) (see Table C1 for statistical grouping)

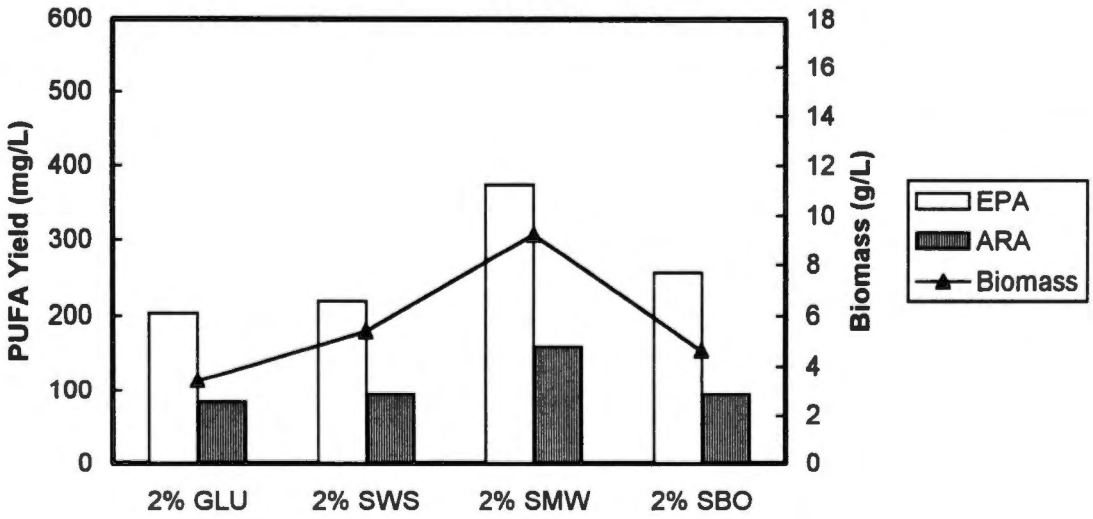


Figure 10. Effect of different media on EPA, ARA and biomass production by *P. irregulare* at 24°C for 8 days (see Table C1 for statistical grouping)

the same temperature (EPA, 480 mg/L; ARA, 180 mg/L). It is well known that cultivation of many fungi at reduced temperatures increases lipid unsaturation (Neidleman, 1987). There was a similar tendency in this study with the SWS and GLU samples, in which PUFA production was better at 12°C than at 24°C. However, a treatment with 2% SBO at room temperature for 8 days produced 256 mg/L EPA, by *P. irregulare*, compared to 20 mg/L EPA by *M. alpina* at 28°C for 6 days from Shimizu *et al.* (1989b). There were no significant differences among ARA levels (Appendix C).

Comparison of Experiments 1 and 2

From the results of first 2 experiments, it was found that *P. irregulare* was a better EPA producer than *M. elongata*, though *M. elongata* produced ARA at a much greater rate than EPA. EPA production by *M. elongata* in the shaker flasks was 10 to 25% that of ARA, and was much lower than those reported by previous researchers using linseed oil (Table 2); therefore, *P. irregulare* was used in all subsequent experiments. Another finding was that both 2%GLU and 2%SWS had great EPA production at 12°C, while both 2% SBO and 2% SMW had high amounts of EPA at both temperatures.

Moreover, soybean oil was not mixed well with culture broth. A reliable and economical emulsifier or surfactant was needed to alleviate this problem, since the addition of surfactant would allow much greater dispersion of the oil in the aqueous media. The oil would become more accessible for the fungi, which should stimulate faster growth and optimize substrate utilization. After the related literature was reviewed (deMan, 1990), Tween 80 was then used. In addition, soybean oil residue on biomass

affected the accuracy of PUFA quantification; therefore, a dependable solvent washing method was necessary. Petroleum ether was then used for this purpose.

The optimal initial pH of 6.0 was well established from preliminary experiments and many other studies (Bajpai *et al.*, 1991d; Jareonkitmongkol *et al.*, 1993b; Shimizu *et al.*, 1989b; Shirasaka *et al.*, 1995). The pH values during fermentation varied within 6.0-8.0 when using soybean oil, which has a unique characteristic of stabilizing pH. The significant findings of experiments 1 and 2 are outlined in Table 5.

Table 5. Significant results summarized from experiments 1 and 2 [a]

Fungi	Temp.	Major media [b]	Comments
M. elongata [c]	12°C	1, 3, 5% SBO	Poor EPA production SBO stabilized pH
P. irregulare [c]	24°C	1, 3, 5% SBO	Better EPA producer Agglomerated oil
P. irregulare [d]	12 or 24°C	2%SMW, 2%SWS, 2%SBO	SMW and SBO good at both temperatures SWS and GLU: highest EPA production at 12°C

[a] Compositions of basal media are defined in text
[b] Abbreviations: SBO, crude soybean oil; SWS, sucrose waste stream; SMW, soymeal waste; GLU, glucose
[c] Conducted with 3 replications
[d] Condusted with 2 replications

EXPERIMENT 3: EMULSION EFFECT

A major problem with the SBO samples in previous experiments was that the oil tended to agglomerate. This study involved the use of an emulsifier to solve this problem. It was assumed that the addition of emulsifier would allow much greater dispersion of the oil in the aqueous media; thus, the purpose of the experiment was to determine the optimal amount of Tween 80. The greatest EPA production occurred with 3-4% SBO and 0.2% Tween 80 ($P < 0.05$)(Table 6). Although EPA content was not detectable from any 5% SBO samples. This was probably due to unexplained experimental error. Higher amounts of SBO should be tested in the future.

Table 6. Effect of combinations of crude soybean oil and Tween 80 on EPA production (mg/L) by *P. irregulare* 10951 at 24°C [a]

	Crude Soybean Oil			
Tween 80	2%	3%	4%	5%
0.1%	540 [bc]	270 [d]	380 [d]	ND [f]
0.2%	230 [d]	650 [b]	700 [b]	ND
0.4%	410 [cd]	40 [e]	330 [d]	ND

[a] Control group (2% GLU) had an average EPA production of 550 mg/L

[bcde] LSMEANS (n=3) of EPA and ARA yield with unlike superscripts are different ($P < 0.05$)

[f] ND=Not detectable

EXPERIMENT 4: SUGAR AND OIL COMBINATIONS

This experiment was based on the hypothesis that the addition of a sugar/carbon source to the SBO medium may stimulate fungal growth and maintenance of the organism and may allow the organism to convert the high contents of linoleic and α-linolenic acids in SBO to EPA. Previously used sucrose, soymeal, and glucose sugar sources were used at 1, 2 and 3% in combination with 4% SBO and 0.2% Tween 80. Figures 11 and 12 illustrate EPA and ARA production from the experiment with sugar and oil combinations.

Water-bath versus Orbital Shaker

The orbital shaker was expected to provide better mixing and temperature control than the previously used water-bath shaker. However, statistical comparison of results can only be made with the SBO control ($P < 0.05$). Using the water-bath shaker, SBO groups had about 90 mg/L of EPA (Table B2); however, the same microorganism (P. irregulare) at the same temperature (24°C) produced about 300 mg/L of EPA from SBO using the orbital shaker (Table D3).

Glucose, Soymeal, and Soybean Oil Controls. The orbital shaker did not increase ARA and EPA levels from 2% GLU ($P > 0.05$) at any temperatures applied. The 2% SMW without SBO had very low yields. However, the orbital shaker did significantly ($P < 0.05$) affect both EPA and ARA yields from SBO control (4% SBO).

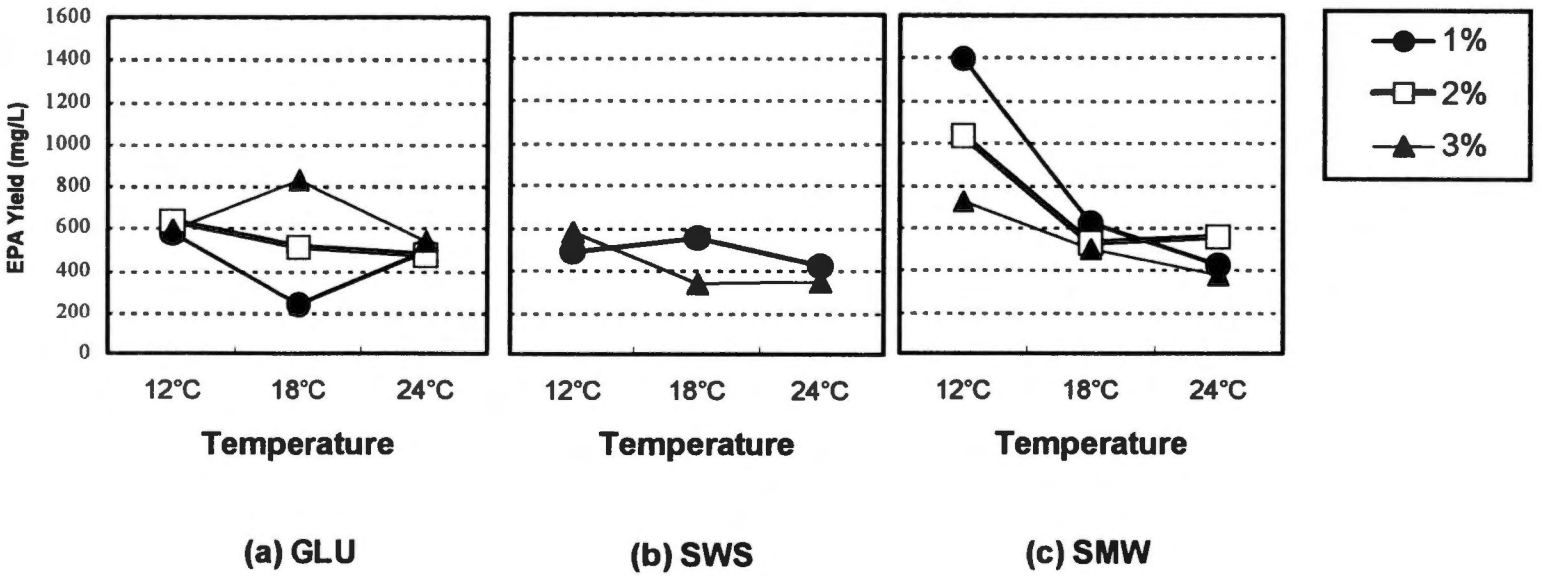


Figure 11. Experiment 4: EPA production from SBO plus additional sugars: (a) GLU, (b) SWS, and (c) SMW (See Tables 7, 8, and 9 for statistical grouping)

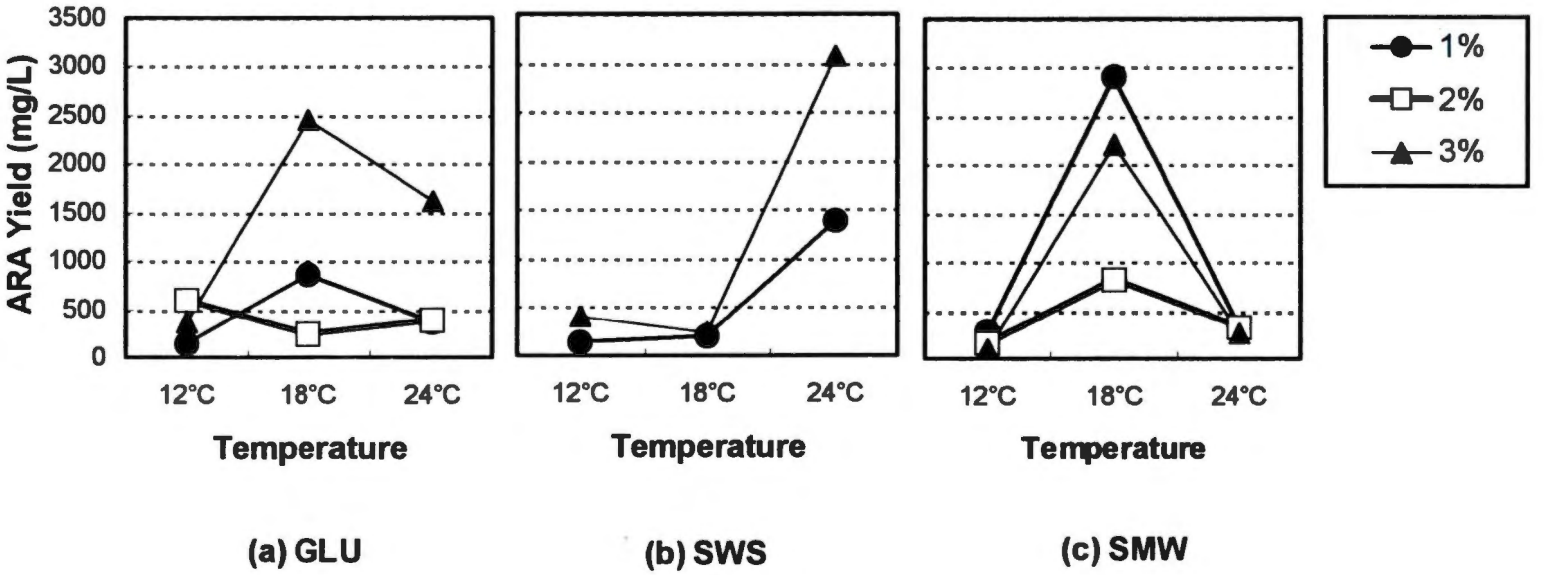


Figure 12. Experiment 4: ARA production from SBO plus additional sugars: (a) GLU, (b) SWS, and (c) SMW (See Tables 7, 8, and 9 for statistical grouping)

The SBO control produced 300-560 mg/L of EPA and 320-2000 mg/L of ARA (Appendix D) with the orbital shaker, while the water-bath shaker produced less than 100 mg/L of EPA or ARA from the 1, 3 and 5% SBO groups (Figure 7).

Temperature Effect

The temperature did affect EPA and ARA production (Figure 13a). Among the averaged yields across each temperature, EPA LSMEAN yield at 12°C (750 mg/L) was significantly greater than the production at 18°C (510 mg/L) and the production at 24°C (450 mg/L) ($P < 0.05$). Nevertheless, the best temperature for ARA was 18°C (1300 mg/L), the second was 24°C (970 mg/L), and the last was 12°C (280 mg/L). All differences among the three ARA LSMEAN yields were significant ($P < 0.05$).

Moreover, the highest EPA production appeared at 12°C (1% SMW, 4% SBO)(Figure 11c). At 12°C EPA yields ranged from 480 mg/L (1% SWS, 4% SBO) to 1400 mg/L (1% SMW, 4% SBO). The second highest EPA production occurred at 18°C, in which EPA yields ranged from 230 mg/L (1% GLU, 4% SBO) to 830 mg/L (3% GLU, 4% SBO). At 24°C EPA ranged from 340 mg/L (3% SWS, 4% SBO) to 550 mg/L (2% SMW, 4% SBO).

Media Effect

The media affected only EPA production in this study (Figure 13b). SMW+SBO had a EPA LSMEAN yield of 680 mg/L, which was greater than those from GLU+SBO (540 mg/L) and SWS+SBO (450 mg/L) ($P < 0.05$). SWS+SBO and GLU+SBO produced the same level of EPA ($P > 0.05$). EPA yields of SMW ranged from 380 mg/L (3%, 24°C) to 1400 mg/L (1%, 12°C), while those of GLU range from 230 mg/L (1%,

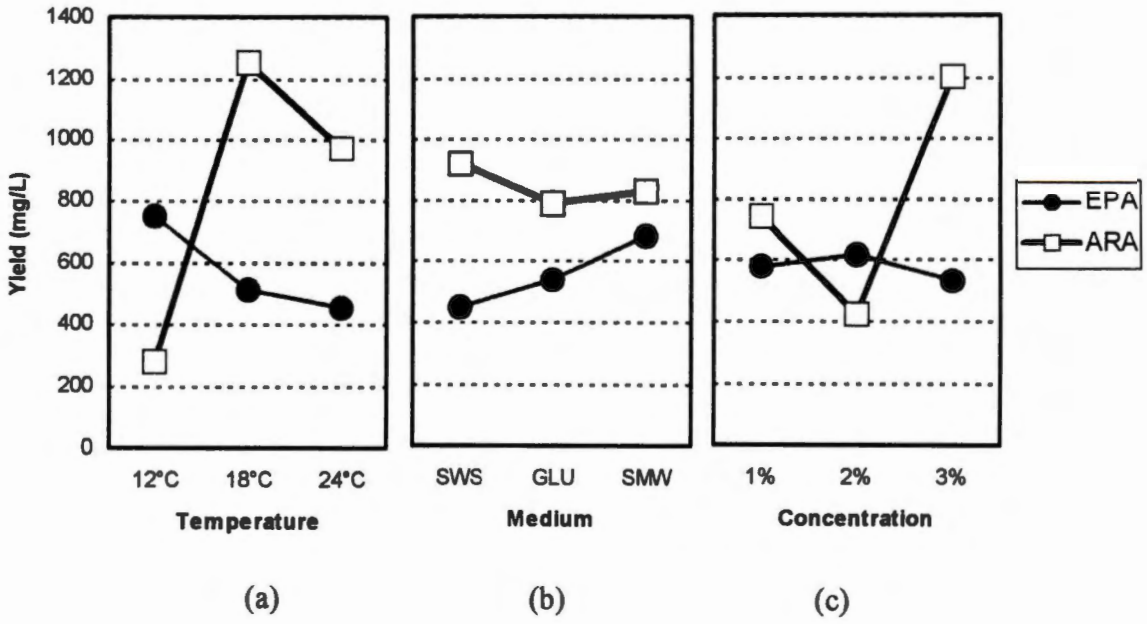


Figure 13. Effects of (a) temperature, (b) media, or (c) medium concentration alone on EPA and ARA LSMEAN yields (mg/L) for experiment 4

18°C) to 830 mg/L (3%, 18°C). There were no significant differences among ARA contents affected by the different types of media.

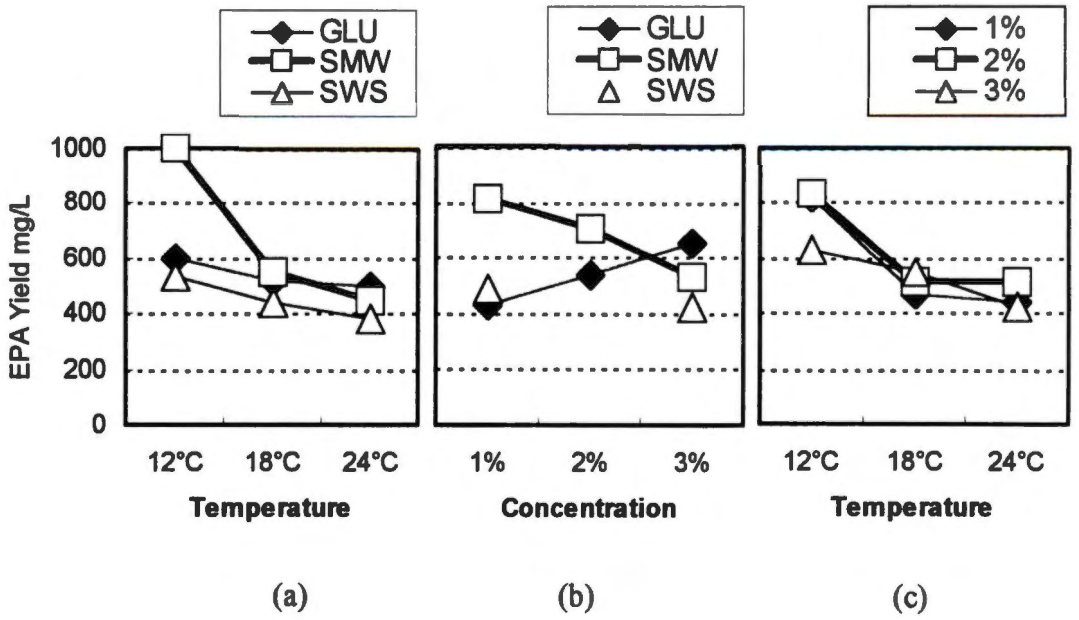
Concentration Effect

Media concentration did not affect EPA production ($P > 0.05$) (Figure 13c); that is, there were not significant differences between any two of the three concentrations – 1, 2 and 3%. However, 3% of any medium, in general, produced a higher ($P < 0.05$) ARA amount than the 1% or 2% medium.

Interactions of Media, Concentration, and Temperature

EPA Production. Three significant two-way interactions ($P < 0.05$) existed in EPA yields: media-temperature, media-concentration, and temperature-concentration (Appendix E). The interaction of media-temperature can be illustrated by comparing GLU+SBO to SMW+SBO. While EPA levels of GLU+SBO remained at the same level (ca 540 mg/L) with increase in temperature (12, 18, to 24°C), the yields decreased from 1050 mg/L for SMW-12°C, to 545 mg/L for SMW-18°C, to 450 mg/L for SMW-24°C (Figure 14a).

As for media-concentration interactions on EPA levels, Figure 14b shows that EPA from GLU+SBO increased with increase in GLU concentration, while EPA from SMW decreased with increasing SMW concentration ($P < 0.05$), and SWS+SBO's EPA levels remained at the same level ($P > 0.05$). Table 14c also shows that 12°C was the best temperature for EPA production ($P < 0.05$), no matter which of the three concentrations was used ($P > 0.05$).

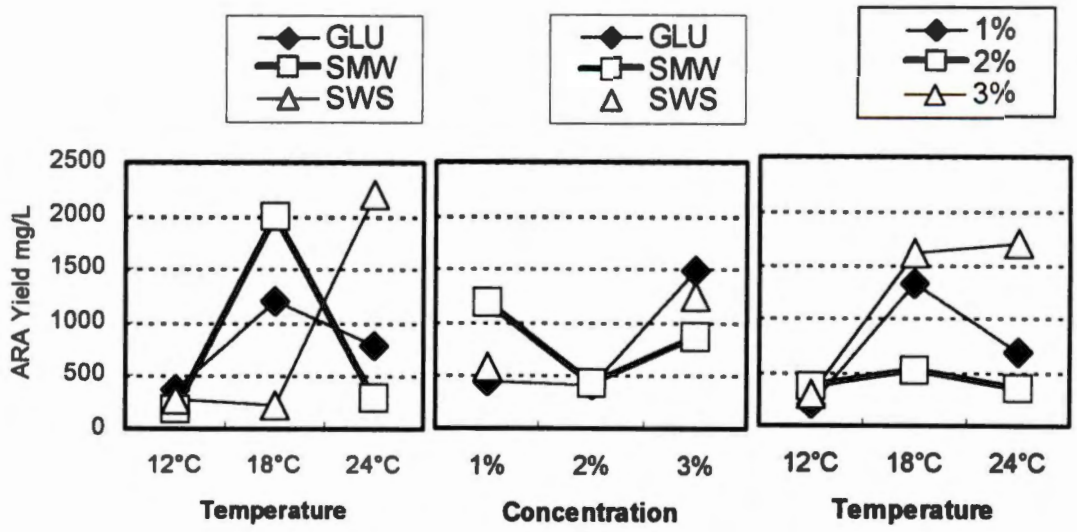


(a) (b) (c)

Figure 14. Two-way interactions of EPA LSMEAN yields: (a) media-temperature, (b) media-concentration, and (c) temperature-concentration ($P < 0.05$) (See Appendix E for statistical analysis and grouping)

ARA Production. Figure 15 illustrates the significant two-way interactions of ARA LSMEAN yields. Though ARA production had no temperature, concentration, or media effect alone, there were three two-way interactions ($P < 0.05$) found: media-temperature, media-concentration, and temperature-concentration (Appendix D). The highest ARA production occurred at 18°C-SMW+SBO with a LSMEAN 2000 mg/L followed by 18°C-GLU+SBO (1200 mg/L), 24°C-GLU+SBO (790 mg/L), and 12°C-GLU+SBO (370 mg/L) and 24°C-SMW+SBO (300 mg/L)(Figure 15a). Both GLU+SBO and SMW+SBO had the highest yields at 18°C, which increased from 12 to 18°C and then decreased at 24°C ($P < 0.05$). At 12°C, both media had the same lowest ARA levels. However, ARA production from SMW+SBO was higher than that from GLU+SBO at 18°C ($P < 0.05$), while the production from GLU+SBO was greater than that from SMW+SBO at 24°C ($P < 0.05$). Moreover, EPA production from SMW+SBO decreased more sharply than that from GLU+SBO with increased temperature from 18 to 24°C ($P < 0.05$).

The media-concentration interaction is illustrated in Figure 15b. 3% GLU, 3% SWS and 1% SMW produced high amounts of ARA (1500, 1200, and 1200 mg/L, respectively). Production of ARA decreased with concentration for SMW but increased for GLU and SWS. Although there was no data available for the 2% SWS, it was also found that the temperature-concentration interaction had significance ($P < 0.05$) (Figure 15c). An increased ARA production occurred with 3% substrate when the temperature was 24°C.



(a) (b) (c)

Figure 15. Two-way interactions of ARA LSMEAN yields: (a) media-temperature, (b) media-concentration, and (c) temperature-concentration ($P < 0.05$) (See Appendix E for statistical analysis and grouping)

SMW (1%+4%SBO+0.2%TW80) at 12°C produced the greatest amount of EPA. This combination of substrate and temperature also had a very high ARA yield. ARA yield could be increased somewhat with a slightly higher temperature.

Production of Other Fatty Acids

The treatment of 24°C-2%GLU+SBO produced the highest ($P < 0.05$) amount of C18:3n3 (4700 mg/L) (Tables 7, 8 and 9). There were no significant differences among other treatments (Appendix D). In general, all samples treated with SBO had great amounts of C16:0 and C-18 fatty acids, which came from the SBO residue in fungal biomass. The 12°C-3%SMW+SBO samples produced significantly higher yields of C14:0 (1500 mg/L) and C16:1 (2400 mg/L) than any other substrate/temperature combinations ($P < 0.05$). There were no significant differences among those yields for all the other treatments ($P > 0.05$).

Table 10 shows the effects of temperature, media, or concentration alone on the LSMEAN yields (mg/L) of fatty acids other than EPA and ARA for experiment 4. The combination of SMW+SBO yielded significantly higher amounts of C-18 PUFAs ($P < 0.05$) than the other substrate combinations. In general, samples from 3% media at 12°C had higher production of on C16:0 and C16:1 fatty acids ($P < 0.05$).

Table 7. Fatty acid profile of biomass from *P. irregulare* ATCC 10951 treated with crude soybean oil, Tween 80 and additional carbon sources in an orbital shaker at 12°C [a]

Fatty Acid [b]	Control Group				Additional Carbon Source							
					GLU			SMW			SWS	
	2% GLU	2% SMW	4% SBO	4% SBO + 0.2% TW80	1%	2%	3%	1%	2%	3%	1%	3%
C14:0	270 ^{e} (9.9)	30 ^{e} (4.7)	130 ^{e} (0.9)	290 ^{e} (2.0)	190 ^{e} (0.9)	470 ^{e} (2.2)	460 ^{e} (2.2)	150 ^{e} (0.6)	180 ^{e} (0.7)	1500 ^{d} (5.5)	390 ^{e} (2.5)	300 ^{e} (1.4)
C16:0	740 ^{hi} (27)	110 ^{i} (14)	1200 ^{hi} (7.8)	1600 ^{ghi} (11)	2300 ^{fghi} (11)	3200 ^{defgh} (15)	4700 ^{def} (23)	1900 ^{ghi} (8.6)	1700 ^{ghi} (7.7)	4300 ^{defg} (16)	2400 ^{fghi} (16)	2400 ^{fghi} (11)
C16:1	ND ^{j}	ND	ND	70 ^{f} (0.5)	ND	ND	160 ^{f} (0.8)	100 ^{f} (0.5)	140 ^{f} (0.7)	2400 ^{d} (8.2)	730 ^{e} (4.8)	10 ^{f} (0.03)
C18:0	ND	ND	260 (1.7)	640 (4.8)	160 (0.7)	ND	ND	670 (3.3)	ND	ND	ND	60 (0.3)
C18:1n9	90 ^{g} (3.4)	120 ^{g} (18)	3400 ^{de} (23)	2800 ^{def} (18)	4000 ^{d} (18)	870 ^{efg} (4.1)	2900 ^{def} (14)	4200 ^{d} (19)	4600 ^{d} (20)	1210 ^{efg} (4.4)	690 ^{fg} (4.8)	4900 ^{d} (22)
C18:2n6	520 ^{ghi} (19)	200 ^{hi} (28)	6300 ^{defg} (42)	5900 ^{efgh} (40)	8500 ^{def} (40)	11000 ^{de} (53)	7300 ^{def} (36)	8500 ^{def} (39)	9300 ^{def} (41)	12000 ^{d} (43)	7300 ^{def} (46)	8800 ^{def} (40)
C18:3n3	50 ^{e} (2.0)	ND	610 ^{e} (4.0)	860 ^{e} (5.8)	1100 ^{e} (5.3)	1700 ^{e} (7.6)	1100 ^{e} (5.4)	1200 ^{e} (5.5)	1300 ^{e} (5.7)	2000 ^{e} (7.0)	1300 ^{e} (8.3)	1500 ^{e} (6.9)
C20:4n6	190 ^{i} (6.9)	50 ^{i} (7.9)	570 ^{hi} (3.8)	260 ^{i} (1.8)	150 ^{i} (0.7)	590 ^{hi} (2.8)	370 ^{i} (1.8)	290 ^{i} (1.4)	290 ^{i} (1.5)	100 ^{i} (0.4)	150 ^{i} (1.0)	420 ^{i} (1.9)
C20:5n3	360 ^{fg} (13)	110 ^{gh} (16)	480 ^{fg} (3.2)	490 ^{fg} (3.4)	570 ^{f} (2.6)	630 ^{ef} (3.0)	600 ^{ef} (2.9)	1400 ^{d} (6.4)	1000 ^{de} (5.4)	720 ^{ef} (2.6)	480 ^{fg} (3.1)	560 ^{f} (2.6)

[a] Growing conditions described in text

[b] Fatty acid expressed as mg/L culture broth followed by % fatty acid (in parentheses) in total lipids

[c] Standard error of LSMEAN for each fatty acid shown in Table 9

^{defghi} LSMEANS in the same row with unlike superscripts are different ($P < 0.05$)

[j] ND=Not detectable

Table 8. Fatty acid profile of biomass from *P. irregulare* ATCC 10951 treated with crude soybean oil, Tween 80 and additional carbon sources in an orbital shaker at 18°C [a]

	Control Group				Additional Carbon Source							
Fatty	2%	2%	4%	4% SBO +	GLU			SMW			SWS	
Acid [b]	GLU	SMW	SBO	0.2% TW80	1%	2%	3%	1%	2%	3%	1%	3%
C14:0	100 ^{e} (8.2)	18 ^{e} (6.4)	130 ^{e} (1.0)	180 ^{e} (1.3)	ND	ND	270 ^{e} (1.1)	130 ^{e} (0.6)	90 ^{e} (0.4)	290 ^{e} (1.2)	230 ^{e} (1.3)	195 ^{e} (0.9)
C16:0	170 ^{i} (13.2)	40 ^{i} (14)	330 ^{hi} (5.4)	2600 ^{efghi} (18)	1700 ^{ghi} (6.6)	5200 ^{de} (19)	1400 ^{hi} (5.7)	1900 ^{ghi} (9.1)	1000 ^{hi} (4.8)	1100 ^{hi} (4.7)	5800 ^{d} (32)	1700 ^{ghi} (8.0)
C16:1	120 ^{f} (10)	30 ^{f} (9.3)	ND	ND	ND	ND	ND	230 ^{f} (1.0)	ND	ND	ND	ND
C18:0	ND	ND	260 (1.9)	350 (2.4)	ND	ND	570 (2.3)	760 (3.5)	ND	780 (3.4)	440 (2.4)	760 (3.4)
C18:1n9	150 ^{g} (12)	30 ^{g} (11)	2100 ^{efg} (15)	2100 ^{efg} (16)	3300 ^{de} (13)	3300 ^{de} (12)	3900 ^{d} (15)	2300 ^{defg} (10)	3600 ^{d} (16)	3500 ^{de} (15)	2000 ^{efg} (11)	3800 ^{d} (17)
18:2n6	180 ^{hi} (14)	50 ^{i} (19)	6390 ^{def} (47)	5900 ^{efgh} (41)	12000 ^{d} (48)	11000 ^{de} (40)	8500 ^{def} (34)	6700 ^{def} (30)	9000 ^{def} (41)	9600 ^{def} (41)	5900 ^{efgh} (32)	11000 ^{de} (50)
C18:3n3	ND	ND	1400 ^{e} (10)	690 ^{e} (4.8)	1300 ^{e} (5.2)	890 ^{e} (3.6)	960 ^{e} (3.8)	690 ^{e} (3.0)	1000 ^{e} (4.6)	1500 ^{e} (6.5)	540 ^{e} (3.0)	1300 ^{e} (6.0)
C20:4n6	110 ^{i} (8.6)	40 ^{i} (15)	320 ^{i} (2.4)	230 ^{i} (1.6)	870 ^{ghi} (3.4)	250 ^{i} (1.0)	2500 ^{de} (9.7)	3000 ^{d} (13)	820 ^{ghi} (3.8)	2200 ^{def} (9.4)	210 ^{i} (1.1)	250 ^{i} (1.2)
C20:5n3	150 ^{gh} (12)	30 ^{h} (14)	560 ^{fg} (4.1)	280 ^{fg} (2.0)	230 ^{gh} (0.9)	500 ^{fg} (2.0)	830 ^{e} (3.3)	620 ^{ef} (2.8)	530 ^{fg} (2.4)	500 ^{fg} (2.1)	550 ^{fg} (3.1)	340 ^{fg} (1.6)

[a] See Table 7 for footnotes

Table 9. Fatty acid profile of biomass from *P. irregulare* ATCC 10951 treated with crude soybean oil, Tween 80 and additional carbon sources in an orbital shaker at 24°C [a]

	Control Group				Additional Carbon Source								
					GLU			SMW			SWS		
Fatty Acid [b]	2% GLU	2% SMW	4% SBO	4% SBO + 0.2% TW80	1%	2%	3%	1%	2%	3%	1%	3%	SE [c]
C14:0	220 [e] (8.6)	14 [e] (4.0)	22 [e] (0.2)	68 [e] (0.5)	160 [e] (0.9)	230 [e] (1.1)	140 [e] (0.7)	80 [e] (0.4)	160 [e] (0.7)	500 [e] (2.0)	120 [e] (0.6)	60 [e] (0.4)	30
C16:0	480 [hi] (19)	60 [i] (16)	560 [hi] (3.2)	660 [hi] (4.7)	1600 [ghi] (8.6)	1700 [ghi] (8.3)	1100 [hi] (5.2)	1200 [hi] (5.8)	1500 [hi] (7.3)	1800 [ghi] (7.6)	920 [hi] (5.1)	720 [hi] (4.3)	150
C16:1	180 [f] (7.0)	9 [f] (2.6)	18 [f] (0.1)	17 [f] (0.1)	42 [f] (0.3)	120 [f] (0.5)	220 [f] (1.2)	120 [f] (0.6)	22 [f] (0.1)	36 [f] (0.1)	63 [f] (0.4)	11 [f] (0.1)	40
C18:0	52 (2.1)	7 (2.0)	170 (1.1)	220 (1.7)	49 (0.2)	55 (0.3)	280 (1.5)	160 (0.8)	42 (0.2)	520 (2.3)	890 (5.4)	220 (1.2)	35
C18:1n9	403 [fg] (16)	46 [g] (13)	2000 [efg] (11)	1500 [efg] (11)	3100 [de] (18)	3000 [def] (15)	450 [fg] (2.3)	4800 [d] (22)	4000 [d] (20)	3800 [d] (16)	2700 [def] (16)	2400 [defg] (14)	160
C18:2n6	380 [hi] (15)	77 [i] (21)	8500 [def] (48)	4900 [fghi] (35)	8700 [def] (47)	6200 [defg] (32)	10000 [def] (50)	10000 [def] (50)	9700 [def] (47)	11000 [de] (47)	7300 [def] (41)	7300 [def] (41)	370
C18:3n3	40 [e] (1.6)	22 [e] (6.0)	1500 [e] (8.9)	1600 [e] (11)	1300 [e] (7.0)	4700 [d] (24)	1500 [e] (7.9)	1200 [e] (5.4)	1200 [e] (5.9)	1500 [e] (6.1)	890 [e] (5.0)	640 [e] (3.6)	97
C20:4n6	150 [i] (6.1)	41 [i] (12)	2000 [ef] (11)	1800 [ef] (13)	360 [i] (2.1)	390 [i] (1.9)	1600 [efg] (9.0)	230 [i] (1.5)	330 [i] (1.6)	260 [i] (1.1)	1400 [fgh] (8.3)	3100 [d] (17)	85
C20:5n3	170 [gh] (7.2)	33 [h] (9.7)	300 [fg] (1.7)	460 [fg] (3.1)	490 [fg] (2.7)	470 [fg] (2.4)	530 [fg] (2.9)	420 [fg] (2.0)	550 [fg] (2.7)	370 [fg] (1.6)	420 [fg] (2.4)	340 [fg] (1.9)	27

[a] See Table 7 for footnotes

Table 10. Effects of temperature, media, or concentration alone on the LSMEAN yields (mg/L) of fatty acids other than EPA and ARA for experiment 4

	Temperature			Media			Concentration		
	12°C	18°C	24°C	GLU	SWS	SMW	1%	2%	3%
C14:0	450 [a]	150 [b]	180 [b]	210 [a]	220 [a]	340 [a]	160 [b]	190 [b]	410 [a]
C16:0	2900 [a]	2500 [a]	1300 [b]	2500 [a]	2300 [ab]	1800 [b]	2200 [a]	2400 [a]	2100 [a]
C16:1	440 [a]	30 [b]	80 [b]	60 [b]	130 [b]	340 [a]	140 [b]	47 [c]	320 [a]
C18:0	110 [b]	410 [a]	280 [ab]	120 [b]	400 [a]	330 [ab]	350 [a]	16 [b]	350 [a]
C18:1n9	2900 [a]	3200 [a]	3000 [a]	2700 [b]	2700 [b]	3600 [a]	3000 [a]	3200 [a]	3000 [a]
C18:2n6	9100 [a]	9100 [a]	8800 [a]	9300 [ab]	7900 [b]	9500 [a]	8300 [a]	9400 [a]	9500 [a]
C18:3n3	1400 [a]	1000 [a]	1600 [a]	1600 [a]	1000 [a]	1300 [a]	1100 [b]	1800 [a]	1300 [ab]

[abc] LSMEANS (n=3) in the same row of each treatment category with unlike superscripts are different ($P < 0.05$)

CHAPTER V

CONCLUSIONS

Four experiments were conducted in this study to determine optimal conditions for fungal production of EPA. The results of experiment 1 showed that *M. elongata* was not a good producer of EPA. The use of *P. irregulare* was chosen because it produced high amounts of EPA at both low and high temperatures. That is, *P. irregulare* was a better EPA producer than *M. elongata* in terms of EPA yield and cultivation conditions. An advantage of *P. irregulare* was its ability to produce EPA at room temperature, which is economical and convenient for industrial applications. Soybean oil also had a unique characteristic as a pH stabilizer, which was critical for fungal growth. The optimal initial pH was 6.0.

High yields of EPA and ARA were obtained from the fermentation with the two industrial waste streams and glucose (experiment 2). The sucrose waste stream (SWS) produced EPA around 400 mg/L and ARA at 200 mg/L at 12°C, and this was not statistically different than the control. This result showed that cultivation of *P. irregulare* treated with SWS or GLU at reduced temperatures increases lipid unsaturation. However, both 2% SBO and 2% SMW in combination produced high amounts of EPA at both temperatures (12 and 24°C).

In experiment 3, the emulsifier, Tween 80, was used to solve the agglomeration problem with soybean oil. Tween 80 allowed much greater dispersion of the oil in aqueous broth, increased substrate utilization, and stimulated fungal growth. The greatest

EPA production occurred with 3-4% SBO and 0.2% Tween 80. The combination of 4% SBO and 0.2% Tween 80 was used for the final experiment.

Sugar and oil combinations (experiment 4) greatly increased PUFA production. The addition of soybean oil increased the bioconversion of linoleic acid (ca. 50% of the total lipids in soybean oil, see Table 3) and α-linolenic acid to EPA. The orbital shaker (300 rpm) provided better mixing of soybean oil and Tween 80 in the culture broth. The greatest EPA production (1400 mg/L) was obtained at 12°C (1% SMW, 4% SBO) and ARA production was 300 mg/L with those conditions. EPA yields of SMW+SBO were significantly higher ($P < 0.05$) than those of GLU+SBO, while SWS+SBO had no differences ($P > 0.05$) from SMW+SBO. There were also no significant differences among ARA contents among the different substrates. Substrate concentrations did not affect EPA production; however, 3% of any substrate produced a higher ARA amount than the 1% substrate.

As mentioned previously, the ideal ratio of n-6 and n-3 PUFAs in the diet is not known; however, based on the ratio in maternal milk in human, the ARA/EPA ratio ranges from 1 to 7 (Leaf and Weber, 1987). The ARA/EPA ratio in experiment 4 ranged from 0.2 to 4.0; therefore, production of PUFAs from the proposed substrates may be a feasible commercial source for an additive to infant formula.

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APPENDIXES

APPENDIX A

GC OPERATING PARAMETERS AND FAME 37AUTHENTIC STANDARD

Table A1. Operating parameters for gas chromatographic analysis

Item	Setting
Split carrier gas, flow rate	He 25 mL/min (column head 15 psi; tank 30 psi)
Column carrier gas, flow rate	He 2.5 mL/min
Split/splitless ratio	10:1
Make-up carrier gas, flow rate	He 20.5 mL/min
Hydrogen gas, flow rate	40.0 mL/min (tank 18 psi)
Air flow rate	380 mL/min (tank 42 psi)
Injector temperature	250°C
Detector temperature	250°C
Initial oven temperature	120°C
Final oven temperature	230°C
Temperature program rate	0°C/min × 2min, +10°C/min × 6min, +2°C/min × 16.5min, and +10°C/min × 1.7min
Sample injected	1μL

Table A2. GC analysis parameters for FAME 37 Standard

FAME	Symbol	Area	Conc, %	Area/Conc	Correction factor
Palmitic acid methyl ester	C16:0	464.23	6	77.372	1.01
Palmitoleic acid methyl ester	C16:1	157.70	2	78.850	1.03
Heptadecanoic acid methyl ester	C17:0	153.53	2	76.765	1.00
Stearic acid methyl ester	C18:0	308.76	4	77.190	1.01
Oleic acid methyl ester	C18:1n9	161.75	2	80.875	1.05
Linoleic acid methyl ester	C18:2n6	623.00	8	77.875	1.01
Linolenic acid methyl ester	C18:3n3	157.62	2	78.810	1.03
5,8,11,14--*cis*-Arachidonic acid methyl ester [a]	C20:4n6	152.92	2	76.460	1.00
5,8,11,14,17-*cis*-Eicosapentaenoic acid methyl ester [b]	C20:5n3	151.00	2	75.500	0.98

[ab] Resolution value = 2.7, which is greater than 1.5 (>99% separation); width of ARA is 0.0605 min and width of EPA is 0.1203 min

APPENDIX B

EXPERIMENT 1: EPA AND ARA PRODUCTION (n=3) BY *MORTIERELLA ELONGATA* NRRL 5513 VERSUS *PYTHIUM IRREGULARE* ATCC 10951

Table B1. EPA and ARA production from crude soybean oil by *M. elongata* NRRL 5513 in shaker flasks for 2 days at 24°C followed by 6 days at 12°C [a]

Medium [b]	Dry biomass (g/L)	Total lipids in biomass (% w/w)	EPA			ARA		
			in lipids (% w/w)	in biomass (mg/g)	yield (mg/L)	in lipids (% w/w)	in biomass (mg/g)	yield (mg/L)
1% GLU	1.7	9.4	28	27	48 [c]	230	220	360 [c]
1% SBO	1.2	11	11	12	16 [c]	120	130	160 [d]
3% SBO	1.7	14	13	18	32 [c]	58	80	130 [e]
5% SBO	2.0	24	5.1	13	24 [c]	29	72	140 [e]

[a] All data conducted as the average of triplicates of each treatment

[b] Culture media treated as % (w/v) of glucose (GLU) or soybean oil (SBO) in 45 mL of basal media; compositions of basal media defined in text

[cde] LSMEANS in the same column of EPA and ARA yield in Tables B1 and B2 with unlike superscripts are different ($P < 0.05$)

Table B2. EPA and ARA production from crude soybean oil by *P. irregulare* ATCC 10951 in shaker flasks for 8 days at 24°C [a]

Medium [b]	Dry biomass (g/L)	Total lipids in biomass (% w/w)	EPA			ARA		
			in lipids (% w/w)	in biomass (mg/g)	yield (mg/L)	in lipids (% w/w)	in biomass (mg/g)	yield (mg/L)
1% GLU	3.4	8.8	27	24	82 [c]	24	21	73 [e]
1% SBO	6.3	16	94	15	96 [c]	8.3	13	82 [e]
3% SBO	6.2	28	4.6	13	82 [c]	4.2	11	73 [e]
5% SBO	7.6	29	4.3	12	93 [c]	3.3	9.4	71 [e]

[a] See Table B1 for footnotes

APPENDIX C

EXPERIMENT 2: MEDIA EFFECT ON EPA AND ARA PRODUCTION (n=2) BY *P. IRREGULARE* ATCC 10951 AT 12 AND 24°C

Table C1. Effect of different media on EPA and ARA production by *P. irregulare* ATCC 10951at 12 and 24°C [a]

Medium [b]	Temp. (°C)	Dry biomass (g/L)	Total lipids in biomass (% w/w)	EPA				ARA			
				in lipids (% w/w)	in biomass (mg/g)	yield (mg/L)	productivity (mg/L/d)	in lipids (% w/w)	in biomass (mg/g)	yield (mg/L)	productivity (mg/L/d)
2% GLU	12°C	8.9	7.7	70	54	480 [c]	60	26	20	180 [c]	23
	24°C	3.4	7.7	78	59	200 [d]	25	33	25	85 [c]	11
2% SMW	12°C	15	33	5.5	18	280 [c]	35	0.3	1.1	17 [c]	2.1
	24°C	9.2	20	20	41	370 [c]	47	8.6	17	160 [c]	20
2% SWS	12°C	12.8	21	17	36	470 [c]	58	7.6	16	210 [c]	26
	24°C	5.4	14	29	41	220 [d]	27	12	18	95 [c]	12
2% SBO	12°C	3.7	59	5.4	32	120 [e]	15	2.6	16	59 [c]	7.4
	24°C	4.6	73	7.1	52	260 [d]	32	2.8	20	94 [c]	12

[a] All data conducted as the average of 2 replicates of each treatment

[b] Culture media treated as % (w/v) in 150 mL of basal media: 2% glucose (GLU), 2% soymeal waste (SMW), 2% sucrose waste stream (SWS) and 2% soybean oil (SBO); compositions of basal media are defined in text

[cde] LSMEANS in the same column of EPA and ARA yield with unlike superscripts are different ($P < 0.05$)

APPENDIX D

EXPERIMENT 4: EFFECTS OF ADDITIONAL SUGARS ON EPA AND ARA PRODUCTION (n=3)

Table D1. Effect of additional carbon sources on EPA and ARA production of crude soybean oil from *P. irregulare* ATCC 10951 at 12°C [a]

Medium [b]		Dry biomass (g/L)	Total lipids in biomass (% w/w)	EPA				ARA			
				in lipids (% w/w)	in biomass (mg/g)	yield (mg/L)	productivity (mg/L/d)	in lipids (% w/w)	in biomass (mg/g)	yield (mg/L)	productivity (mg/L/d)
Control Groups											
2% GLU		6.3	43	13	57	360 ^{fg}	45	6.9	30	190 ^{i}	23
2% SMW		16	4.5	16	6.8	110 ^{gh}	13	7.9	3.4	54 ^{i}	6.8
4% SBO		23	64	3.2	21	480 ^{fg}	60	3.8	25	570 ^{hi}	71
4% SBO + 0.2% TW80		26	56	3.4	19	490 ^{fg}	61	1.8	10	260 ^{i}	32
Groups of Additional Carbon Sources [c] (combined with SBO and Tween 80)											
GLU	1%	35	62	2.6	17	570 ^{f}	71	0.68	4.3	150 ^{i}	18
	2%	30	70	3.0	21	630 ^{ef}	78	2.8	20	590 ^{hi}	74
	3%	31	67	2.9	20	600 ^{ef}	75	1.8	12	370 ^{i}	46
SMW	1%	33	67	6.4	42	1400 ^{d}	170	1.4	9.5	300 ^{i}	37
	2%	40	55	5.4	29	1000 ^{de}	130	1.5	8.2	290 ^{i}	36
	3%	55	51	2.6	13	720 ^{ef}	90	0.37	1.9	100 ^{i}	12
SWS	1%	31	51	3.1	16	480 ^{fg}	60	0.97	4.9	150 ^{i}	19
	3%	34	64	2.6	17	560 ^{f}	72	1.9	12	420 ^{i}	52

[a] All data conducted as the average of 3 replicates of each treatment

[b] Culture media treated as % (w/v) in 45 mL of basal media: glucose (GLU), soymeal waste (SMW), sucrose waste stream (SWS) and soybean oil (SBO); compositions of basal media are defined in text

[c] Additional carbon sources (GLU, SMW, SWS) added with 4% SBO and 0.2% Tween 80 (TW80)

^{defghi} LSMEANS in the same column of EPA or ARA yield in Tables 7, 8 and 9 with unlike superscripts are different ($P < 0.05$)

Table D2. Effect of additional carbon sources on EPA and ARA production of crude soybean oil from *P. irregulare* ATCC 10951 at 18°C [a]

Medium [b]		Dry biomass (g/L)	Total lipids in biomass (% w/w)	EPA				ARA			
				in lipids (% w/w)	in biomass (mg/g)	yield (mg/L)	productivity (mg/L/d)	in lipids (% w/w)	in biomass (mg/g)	yield (mg/L)	productivity (mg/L/d)
Control Groups											
2% GLU		7.8	16	12	19	150 ^{gh}	19	8.6	14	110 ^{i}	13
2% SMW		9.2	2.8	14	3.7	34 ^{h}	4.2	15	4.3	41 ^{i}	5.2
4% SBO		21	66	4.1	27	560 ^{fg}	70	2.3	15	320 ^{i}	40
4% SBO + 0.2% TW80		26	55	2.0	11	280 ^{fg}	35	1.5	8.7	230 ^{i}	28
Groups of Additional Carbon Sources [c] (combined with SBO and Tween 80)											
GLU	1%	33	76	0.9	7.0	230 ^{gh}	29	3.4	26	870 ^{ghi}	110
	2%	35	76	2.0	15	500 ^{fg}	63	1.0	7.3	250 ^{i}	31
	3%	35	73	3.3	24	830 ^{e}	100	9.7	71	2500 ^{de}	310
SMW	1%	35	63	2.8	18	620 ^{ef}	77	13	84	2900 ^{d}	360
	2%	38	56	2.4	14	520 ^{fg}	66	3.8	21	820 ^{ghi}	100
	3%	51	46	2.1	9.7	490 ^{fg}	62	9.3	44	2200 ^{def}	280
SWS	1%	30	62	3.1	19	550 ^{fg}	69	1.1	7.0	210 ^{i}	26
	3%	36	61	1.5	9.4	340 ^{fg}	42	1.1	7.0	250 ^{i}	31

[a] See Table D1 for footnotes

Table D3. Effect of additional carbon sources on EPA and ARA production of crude soybean oil from *P. irregulare* ATCC 10951 at 24°C [a]

Medium [b]		Dry biomass (g/L)	Total lipids in biomass (% w/w)	EPA				ARA			
				in lipids (% w/w)	in biomass (mg/g)	yield (mg/L)	productivity (mg/L/d)	in lipids (% w/w)	in biomass (mg/g)	yield (mg/L)	productivity (mg/L/d)
Control Groups											
2% GLU		6.1	41	7.2	28	170 ^{gh}	21	6.0	24	150 ^{i}	18
2% SMW		17	2.1	9.7	1.9	33 ^{h}	41	12	2.4	41 ^{i}	5.1
4% SBO		27	67	1.7	11	300 ^{fg}	37	11	75	2000 ^{ef}	250
4% SBO + 0.2% TW80		25	57	3.1	18	460 ^{fg}	57	13	74	1800 ^{ef}	230
Groups of Additional Carbon Sources [c] (combined with SBO and Tween 80)											
GLU	1%	29	60	2.7	16	490 ^{fg}	61	2.1	12	360 ^{i}	45
	2%	29	69	2.4	16	470 ^{fg}	59	1.9	13	390 ^{i}	48
	3%	28	67	2.9	19	530 ^{fg}	67	9.0	59	1600 ^{efg}	200
SMW	1%	33	65	1.9	13	420 ^{fg}	52	1.5	10	320 ^{i}	40
	2%	35	58	2.7	16	550 ^{fg}	69	1.6	9.5	330 ^{i}	41
	3%	40	60	1.6	9.4	370 ^{fg}	47	1.1	6.6	260 ^{i}	33
SWS	1%	30	59	2.4	14	420 ^{fg}	52	8.3	48	1400 ^{fgh}	170
	3%	32	55	1.9	11	340 ^{fg}	42	17	95	3100 ^{d}	380

[a] See Table D1 for footnotes

APPENDIX E

EXPERIMENT 4: LEVELS OF LEAST SQUARES MEANS (n=3) OF MAJOR FATTY ACIDS IN HARVESTED DRIED BIOMASS

Table E1. Analyses of LSMEANS (n=3) for yields (mg/L) of major fatty acids in biomass by Mixed procedure

Dependent Variable: C14:0

Effect	TEMP	MEDIA	CONC	LSMEAN	Std Error	DF	t	Pr > |t|
TEMP	12			452.86208333	55.60760829	48	8.14	0.0001
TEMP	18			150.85250000	55.60760829	48	2.71	0.0092
TEMP	24			180.99333333	55.60760829	48	3.25	0.0021
MEDIA		GLU		213.21296296	52.42735587	48	4.07	0.0002
MEDIA		SMW		339.94037037	52.42735587	48	6.48	0.0001
MEDIA		SWS		216.54722222	64.21013523	48	3.37	0.0015
CONC			1%	161.80962963	52.42735587	48	3.09	0.0034
CONC			2%	186.60500000	64.21013523	48	2.91	0.0055
CONC			3%	411.30518519	52.42735587	48	7.85	0.0001
TEMP*MEDIA	12	GLU		373.50111111	90.80684408	48	4.11	0.0002
TEMP*MEDIA	12	SMW		603.23111111	90.80684408	48	6.64	0.0001
TEMP*MEDIA	12	SWS		346.35000000	111.21521657	48	3.11	0.0031
TEMP*MEDIA	18	GLU		91.39111111	90.80684408	48	1.01	0.3193
TEMP*MEDIA	18	SMW		169.34555556	90.80684408	48	1.86	0.0683
TEMP*MEDIA	18	SWS		212.30500000	111.21521657	48	1.91	0.0623
TEMP*MEDIA	24	GLU		174.74666667	90.80684408	48	1.92	0.0602
TEMP*MEDIA	24	SMW		247.24444444	90.80684408	48	2.72	0.0090
TEMP*MEDIA	24	SWS		90.98666667	111.21521657	48	0.82	0.4173
TEMP*CONC	12		1%	244.09000000	90.80684408	48	2.69	0.0099
TEMP*CONC	12		2%	322.96666667	111.21521657	48	2.90	0.0056
TEMP*CONC	12		3%	748.23111111	90.80684408	48	8.24	0.0001
TEMP*CONC	18		1%	119.50888889	90.80684408	48	1.32	0.1944
TEMP*CONC	18		2%	45.81166667	111.21521657	48	0.41	0.6822
TEMP*CONC	18		3%	252.22333333	90.80684408	48	2.78	0.0078
TEMP*CONC	24		1%	121.83000000	90.80684408	48	1.34	0.1860
TEMP*CONC	24		2%	191.03666667	111.21521657	48	1.72	0.0923
TEMP*CONC	24		3%	233.46111111	90.80684408	48	2.57	0.0133
MEDIA*CONC		GLU	1%	117.60888889	90.80684408	48	1.30	0.2015
MEDIA*CONC		GLU	2%	231.33111111	90.80684408	48	2.55	0.0141
MEDIA*CONC		GLU	3%	290.69888889	90.80684408	48	3.20	0.0024
MEDIA*CONC		SMW	1%	120.99555556	90.80684408	48	1.33	0.1890
MEDIA*CONC		SMW	2%	141.87888889	90.80684408	48	1.56	0.1248
MEDIA*CONC		SMW	3%	756.94666667	90.80684408	48	8.34	0.0001
MEDIA*CONC		SWS	1%	246.82444444	90.80684408	48	2.72	0.0091
MEDIA*CONC		SWS	3%	186.27000000	90.80684408	48	2.05	0.0457
TEMP*MEDIA*CONC	12	GLU	1%	194.12000000	157.28206762	48	1.23	0.2231
TEMP*MEDIA*CONC	12	GLU	2%	466.74666667	157.28206762	48	2.97	0.0047
TEMP*MEDIA*CONC	12	GLU	3%	459.63666667	157.28206762	48	2.92	0.0053
TEMP*MEDIA*CONC	12	SMW	1%	149.44666667	157.28206762	48	0.95	0.3468
TEMP*MEDIA*CONC	12	SMW	2%	179.18666667	157.28206762	48	1.14	0.2602
TEMP*MEDIA*CONC	12	SMW	3%	1481.0600000	157.28206762	48	9.42	0.0001
TEMP*MEDIA*CONC	12	SWS	1%	388.70333333	157.28206762	48	2.47	0.0171
TEMP*MEDIA*CONC	12	SWS	3%	303.99666667	157.28206762	48	1.93	0.0592
TEMP*MEDIA*CONC	18	GLU	1%	0.00000000	157.28206762	48	0.00	1.0000
TEMP*MEDIA*CONC	18	GLU	2%	0.00000000	157.28206762	48	0.00	1.0000
TEMP*MEDIA*CONC	18	GLU	3%	274.17333333	157.28206762	48	1.74	0.0877
TEMP*MEDIA*CONC	18	SMW	1%	128.60333333	157.28206762	48	0.82	0.4176
TEMP*MEDIA*CONC	18	SMW	2%	91.62333333	157.28206762	48	0.58	0.5629
TEMP*MEDIA*CONC	18	SMW	3%	287.81000000	157.28206762	48	1.83	0.0735
TEMP*MEDIA*CONC	18	SWS	1%	229.92333333	157.28206762	48	1.46	0.1503
TEMP*MEDIA*CONC	18	SWS	3%	194.68666667	157.28206762	48	1.24	0.2218
TEMP*MEDIA*CONC	24	GLU	1%	158.70666667	157.28206762	48	1.01	0.3180
TEMP*MEDIA*CONC	24	GLU	2%	227.24666667	157.28206762	48	1.44	0.1550
TEMP*MEDIA*CONC	24	GLU	3%	138.28666667	157.28206762	48	0.88	0.3837
TEMP*MEDIA*CONC	24	SMW	1%	84.93666667	157.28206762	48	0.54	0.5917
TEMP*MEDIA*CONC	24	SMW	2%	154.82666667	157.28206762	48	0.98	0.3299
TEMP*MEDIA*CONC	24	SMW	3%	501.97000000	157.28206762	48	3.19	0.0025
TEMP*MEDIA*CONC	24	SWS	1%	121.84666667	157.28206762	48	0.77	0.4423
TEMP*MEDIA*CONC	24	SWS	3%	60.12666667	157.28206762	48	0.38	0.7039

Continued

Table E1 continued

Dependent Variable: C16:0

Effect	TEMP	MEDIA	CONC	LSMEAN	Std Error	DF	t	Pr > |t|
TEMP	12			2870.7529167	186.27048771	48	15.41	0.0001
TEMP	18			2481.6375000	186.27048771	48	13.32	0.0001
TEMP	24			1314.4229167	186.27048771	48	7.06	0.0001
MEDIA		GLU		2548.2862963	175.61749999	48	14.51	0.0001
MEDIA		SMW		1825.5414815	175.61749999	48	10.39	0.0001
MEDIA		SWS		2328.3427778	215.08663244	48	10.83	0.0001
CONC			1%	2187.9825926	175.61749999	48	12.46	0.0001
CONC			2%	2390.5350000	215.08663244	48	11.11	0.0001
CONC			3%	2144.3837037	175.61749999	48	12.21	0.0001
TEMP*MEDIA	12	GLU		3423.4711111	304.17843269	48	11.25	0.0001
TEMP*MEDIA	12	SMW		2625.4444444	304.17843269	48	8.63	0.0001
TEMP*MEDIA	12	SWS		2409.6383333	372.54097542	48	6.47	0.0001
TEMP*MEDIA	18	GLU		2780.1022222	304.17843269	48	9.14	0.0001
TEMP*MEDIA	18	SMW		1335.9311111	304.17843269	48	4.39	0.0001
TEMP*MEDIA	18	SWS		3752.5000000	372.54097542	48	10.07	0.0001
TEMP*MEDIA	24	GLU		1441.2855556	304.17843269	48	4.74	0.0001
TEMP*MEDIA	24	SMW		1515.2488889	304.17843269	48	4.98	0.0001
TEMP*MEDIA	24	SWS		822.89000000	372.54097542	48	2.21	0.0320
TEMP*CONC	12		1%	2209.5066667	304.17843269	48	7.26	0.0001
TEMP*CONC	12		2%	2480.3966667	372.54097542	48	6.66	0.0001
TEMP*CONC	12		3%	3792.2366667	304.17843269	48	12.47	0.0001
TEMP*CONC	18		1%	3106.3000000	304.17843269	48	10.21	0.0001
TEMP*CONC	18		2%	3121.7416667	372.54097542	48	8.38	0.0001
TEMP*CONC	18		3%	1430.2388889	304.17843269	48	4.70	0.0001
TEMP*CONC	24		1%	1248.1411111	304.17843269	48	4.10	0.0002
TEMP*CONC	24		2%	1569.4666667	372.54097542	48	4.21	0.0001
TEMP*CONC	24		3%	1210.6755556	304.17843269	48	3.98	0.0002
MEDIA*CONC		GLU	1%	1852.2400000	304.17843269	48	6.09	0.0001
MEDIA*CONC		GLU	2%	3378.0766667	304.17843269	48	11.11	0.0001
MEDIA*CONC		GLU	3%	2414.5422222	304.17843269	48	7.94	0.0001
MEDIA*CONC		SMW	1%	1671.4877778	304.17843269	48	5.50	0.0001
MEDIA*CONC		SMW	2%	1402.9933333	304.17843269	48	4.61	0.0001
MEDIA*CONC		SMW	3%	2402.1433333	304.17843269	48	7.90	0.0001
MEDIA*CONC		SWS	1%	3040.2200000	304.17843269	48	9.99	0.0001
MEDIA*CONC		SWS	3%	1616.4655556	304.17843269	48	5.31	0.0001
TEMP*MEDIA*CONC	12	GLU	1%	2306.4200000	526.85249998	48	4.38	0.0001
TEMP*MEDIA*CONC	12	GLU	2%	3224.3433333	526.85249998	48	6.12	0.0001
TEMP*MEDIA*CONC	12	GLU	3%	4739.6500000	526.85249998	48	9.00	0.0001
TEMP*MEDIA*CONC	12	SMW	1%	1885.6100000	526.85249998	48	3.58	0.0008
TEMP*MEDIA*CONC	12	SMW	2%	1736.4500000	526.85249998	48	3.30	0.0018
TEMP*MEDIA*CONC	12	SMW	3%	4254.2733333	526.85249998	48	8.07	0.0001
TEMP*MEDIA*CONC	12	SWS	1%	2436.4900000	526.85249998	48	4.62	0.0001
TEMP*MEDIA*CONC	12	SWS	3%	2382.7866667	526.85249998	48	4.52	0.0001
TEMP*MEDIA*CONC	18	GLU	1%	1669.9366667	526.85249998	48	3.17	0.0027
TEMP*MEDIA*CONC	18	GLU	2%	5239.3033333	526.85249998	48	9.94	0.0001
TEMP*MEDIA*CONC	18	GLU	3%	1431.0666667	526.85249998	48	2.72	0.0092
TEMP*MEDIA*CONC	18	SMW	1%	1886.6500000	526.85249998	48	3.58	0.0008
TEMP*MEDIA*CONC	18	SMW	2%	1004.1800000	526.85249998	48	1.91	0.0626
TEMP*MEDIA*CONC	18	SMW	3%	1116.9633333	526.85249998	48	2.12	0.0392
TEMP*MEDIA*CONC	18	SWS	1%	5762.3133333	526.85249998	48	10.94	0.0001
TEMP*MEDIA*CONC	18	SWS	3%	1742.6866667	526.85249998	48	3.31	0.0018
TEMP*MEDIA*CONC	24	GLU	1%	1580.3633333	526.85249998	48	3.00	0.0043
TEMP*MEDIA*CONC	24	GLU	2%	1670.5833333	526.85249998	48	3.17	0.0026
TEMP*MEDIA*CONC	24	GLU	3%	1072.9100000	526.85249998	48	2.04	0.0472
TEMP*MEDIA*CONC	24	SMW	1%	1242.2033333	526.85249998	48	2.36	0.0225
TEMP*MEDIA*CONC	24	SMW	2%	1468.3500000	526.85249998	48	2.79	0.0076
TEMP*MEDIA*CONC	24	SMW	3%	1835.1933333	526.85249998	48	3.48	0.0011
TEMP*MEDIA*CONC	24	SWS	1%	921.85666667	526.85249998	48	1.75	0.0866
TEMP*MEDIA*CONC	24	SWS	3%	723.92333333	526.85249998	48	1.37	0.1758

Continued

Table E1 continued

Dependent Variable: C16:1

Effect	TEMP	MEDIA	CONC	LSMEAN	Std Error	DF	t	Pr > |t|
TEMP	12			444.94541667	25.68085250	48	17.33	0.0001
TEMP	18			28.87166667	25.68085250	48	1.12	0.2665
TEMP	24			79.40541667	25.68085250	48	3.09	0.0033
MEDIA		GLU		59.52000000	24.21213994	48	2.46	0.0176
MEDIA		SMW		342.05074074	24.21213994	48	14.13	0.0001
MEDIA		SWS		135.27388889	29.65369421	48	4.56	0.0001
CONC			1%	143.49925926	24.21213994	48	5.93	0.0001
CONC			2%	46.59666667	29.65369421	48	1.57	0.1227
CONC			3%	317.18962963	24.21213994	48	13.10	0.0001
TEMP*MEDIA	12	GLU		52.69666667	41.93665653	48	1.26	0.2150
TEMP*MEDIA	12	SMW		888.01555556	41.93665653	48	21.18	0.0001
TEMP*MEDIA	12	SWS		368.71333333	51.36170501	48	7.18	0.0001
TEMP*MEDIA	18	GLU		-0.00000000	41.93665653	48	-0.00	1.0000
TEMP*MEDIA	18	SMW		76.99111111	41.93665653	48	1.84	0.0726
TEMP*MEDIA	18	SWS		0.00000000	51.36170501	48	0.00	1.0000
TEMP*MEDIA	24	GLU		125.86333333	41.93665653	48	3.00	0.0043
TEMP*MEDIA	24	SMW		61.14555556	41.93665653	48	1.46	0.1513
TEMP*MEDIA	24	SWS		37.10833333	51.36170501	48	0.72	0.4735
TEMP*CONC	12		1%	276.44333333	41.93665653	48	6.59	0.0001
TEMP*CONC	12		2%	69.94166667	51.36170501	48	1.36	0.1796
TEMP*CONC	12		3%	863.45000000	41.93665653	48	20.59	0.0001
TEMP*CONC	18		1%	76.99111111	41.93665653	48	1.84	0.0726
TEMP*CONC	18		2%	-0.00000000	51.36170501	48	-0.00	1.0000
TEMP*CONC	18		3%	-0.00000000	41.93665653	48	-0.00	1.0000
TEMP*CONC	24		1%	77.06333333	41.93665653	48	1.84	0.0723
TEMP*CONC	24		2%	69.84833333	51.36170501	48	1.36	0.1802
TEMP*CONC	24		3%	88.11888889	41.93665653	48	2.10	0.0409
MEDIA*CONC		GLU	1%	14.16444444	41.93665653	48	0.34	0.7370
MEDIA*CONC		GLU	2%	39.17888889	41.93665653	48	0.93	0.3549
MEDIA*CONC		GLU	3%	125.21666667	41.93665653	48	2.99	0.0044
MEDIA*CONC		SMW	1%	151.76666667	41.93665653	48	3.62	0.0007
MEDIA*CONC		SMW	2%	54.01444444	41.93665653	48	1.29	0.2039
MEDIA*CONC		SMW	3%	820.37111111	41.93665653	48	19.56	0.0001
MEDIA*CONC		SWS	1%	264.56666667	41.93665653	48	6.31	0.0001
MEDIA*CONC		SWS	3%	5.98111111	41.93665653	48	0.14	0.8872
TEMP*MEDIA*CONC	12	GLU	1%	0.00000000	72.63641981	48	0.00	1.0000
TEMP*MEDIA*CONC	12	GLU	2%	-0.00000000	72.63641981	48	-0.00	1.0000
TEMP*MEDIA*CONC	12	GLU	3%	158.09000000	72.63641981	48	2.18	0.0345
TEMP*MEDIA*CONC	12	SMW	1%	98.93666667	72.63641981	48	1.36	0.1795
TEMP*MEDIA*CONC	12	SMW	2%	139.88333333	72.63641981	48	1.93	0.0601
TEMP*MEDIA*CONC	12	SMW	3%	2425.2266667	72.63641981	48	33.39	0.0001
TEMP*MEDIA*CONC	12	SWS	1%	730.39333333	72.63641981	48	10.06	0.0001
TEMP*MEDIA*CONC	12	SWS	3%	7.03333333	72.63641981	48	0.10	0.9233
TEMP*MEDIA*CONC	18	GLU	1%	0.00000000	72.63641981	48	0.00	1.0000
TEMP*MEDIA*CONC	18	GLU	2%	-0.00000000	72.63641981	48	-0.00	1.0000
TEMP*MEDIA*CONC	18	GLU	3%	-0.00000000	72.63641981	48	-0.00	1.0000
TEMP*MEDIA*CONC	18	SMW	1%	230.97333333	72.63641981	48	3.18	0.0026
TEMP*MEDIA*CONC	18	SMW	2%	-0.00000000	72.63641981	48	-0.00	1.0000
TEMP*MEDIA*CONC	18	SMW	3%	-0.00000000	72.63641981	48	-0.00	1.0000
TEMP*MEDIA*CONC	18	SWS	1%	0.00000000	72.63641981	48	0.00	1.0000
TEMP*MEDIA*CONC	18	SWS	3%	0.00000000	72.63641981	48	0.00	1.0000
TEMP*MEDIA*CONC	24	GLU	1%	42.49333333	72.63641981	48	0.59	0.5613
TEMP*MEDIA*CONC	24	GLU	2%	117.53666667	72.63641981	48	1.62	0.1122
TEMP*MEDIA*CONC	24	GLU	3%	217.56000000	72.63641981	48	3.00	0.0043
TEMP*MEDIA*CONC	24	SMW	1%	125.39000000	72.63641981	48	1.73	0.0907
TEMP*MEDIA*CONC	24	SMW	2%	22.16000000	72.63641981	48	0.31	0.7616
TEMP*MEDIA*CONC	24	SMW	3%	35.88666667	72.63641981	48	0.49	0.6235
TEMP*MEDIA*CONC	24	SWS	1%	63.30666667	72.63641981	48	0.87	0.3878
TEMP*MEDIA*CONC	24	SWS	3%	10.91000000	72.63641981	48	0.15	0.8812

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Table E1 continued

Dependent Variable: C18:0

Effect	TEMP	MEDIA	CONC	LSMEAN	Std Error	DF	t	Pr > |t|
TEMP	12			110.03083333	65.14185497	48	1.69	0.0977
TEMP	18			413.66125000	65.14185497	48	6.35	0.0001
TEMP	24			277.32625000	65.14185497	48	4.26	0.0001
MEDIA		GLU		123.40407407	61.41632985	48	2.01	0.0501
MEDIA		SMW		326.16592593	61.41632985	48	5.31	0.0001
MEDIA		SWS		393.66944444	75.21933500	48	5.23	0.0001
CONC			1%	347.65444444	61.41632985	48	5.66	0.0001
CONC			2%	16.28111111	75.21933500	48	0.22	0.8296
CONC			3%	353.50777778	61.41632985	48	5.76	0.0001
TEMP*MEDIA	12	GLU		52.67888889	106.37620372	48	0.50	0.6227
TEMP*MEDIA	12	SMW		222.15111111	106.37620372	48	2.09	0.0421
TEMP*MEDIA	12	SWS		27.87833333	130.28370994	48	0.21	0.8315
TEMP*MEDIA	18	GLU		190.62222222	106.37620372	48	1.79	0.0794
TEMP*MEDIA	18	SMW		514.36777778	106.37620372	48	4.84	0.0001
TEMP*MEDIA	18	SWS		597.16000000	130.28370994	48	4.58	0.0001
TEMP*MEDIA	24	GLU		126.91111111	106.37620372	48	1.19	0.2387
TEMP*MEDIA	24	SMW		241.97888889	106.37620372	48	2.27	0.0274
TEMP*MEDIA	24	SWS		555.97000000	130.28370994	48	4.27	0.0001
TEMP*CONC	12		1%	274.83000000	106.37620372	48	2.58	0.0129
TEMP*CONC	12		2%	0.00000000	130.28370994	48	0.00	1.0000
TEMP*CONC	12		3%	18.58555556	106.37620372	48	0.17	0.8620
TEMP*CONC	18		1%	400.96444444	106.37620372	48	3.77	0.0004
TEMP*CONC	18		2%	0.00000000	130.28370994	48	0.00	1.0000
TEMP*CONC	18		3%	702.13222222	106.37620372	48	6.60	0.0001
TEMP*CONC	24		1%	367.16888889	106.37620372	48	3.45	0.0012
TEMP*CONC	24		2%	48.84333333	130.28370994	48	0.37	0.7094
TEMP*CONC	24		3%	339.80555556	106.37620372	48	3.19	0.0025
MEDIA*CONC		GLU	1%	69.14111111	106.37620372	48	0.65	0.5188
MEDIA*CONC		GLU	2%	18.41777778	106.37620372	48	0.17	0.8633
MEDIA*CONC		GLU	3%	282.65333333	106.37620372	48	2.66	0.0107
MEDIA*CONC		SMW	1%	530.93111111	106.37620372	48	4.99	0.0001
MEDIA*CONC		SMW	2%	14.14444444	106.37620372	48	0.13	0.8948
MEDIA*CONC		SMW	3%	433.42222222	106.37620372	48	4.07	0.0002
MEDIA*CONC		SWS	1%	442.89111111	106.37620372	48	4.16	0.0001
MEDIA*CONC		SWS	3%	344.44777778	106.37620372	48	3.24	0.0022
TEMP*MEDIA*CONC	12	GLU	1%	158.03666667	184.24898955	48	0.86	0.3953
TEMP*MEDIA*CONC	12	GLU	2%	-0.00000000	184.24898955	48	-0.00	1.0000
TEMP*MEDIA*CONC	12	GLU	3%	0.00000000	184.24898955	48	0.00	1.0000
TEMP*MEDIA*CONC	12	SMW	1%	666.45333333	184.24898955	48	3.62	0.0007
TEMP*MEDIA*CONC	12	SMW	2%	0.00000000	184.24898955	48	0.00	1.0000
TEMP*MEDIA*CONC	12	SMW	3%	0.00000000	184.24898955	48	0.00	1.0000
TEMP*MEDIA*CONC	12	SWS	1%	-0.00000000	184.24898955	48	-0.00	1.0000
TEMP*MEDIA*CONC	12	SWS	3%	55.75666667	184.24898955	48	0.30	0.7635
TEMP*MEDIA*CONC	18	GLU	1%	0.00000000	184.24898955	48	0.00	1.0000
TEMP*MEDIA*CONC	18	GLU	2%	0.00000000	184.24898955	48	0.00	1.0000
TEMP*MEDIA*CONC	18	GLU	3%	571.86666667	184.24898955	48	3.10	0.0032
TEMP*MEDIA*CONC	18	SMW	1%	763.19666667	184.24898955	48	4.14	0.0001
TEMP*MEDIA*CONC	18	SMW	2%	0.00000000	184.24898955	48	0.00	1.0000
TEMP*MEDIA*CONC	18	SMW	3%	779.90666667	184.24898955	48	4.23	0.0001
TEMP*MEDIA*CONC	18	SWS	1%	439.69666667	184.24898955	48	2.39	0.0210
TEMP*MEDIA*CONC	18	SWS	3%	754.62333333	184.24898955	48	4.10	0.0002
TEMP*MEDIA*CONC	24	GLU	1%	49.38666667	184.24898955	48	0.27	0.7898
TEMP*MEDIA*CONC	24	GLU	2%	55.25333333	184.24898955	48	0.30	0.7656
TEMP*MEDIA*CONC	24	GLU	3%	276.09333333	184.24898955	48	1.50	0.1406
TEMP*MEDIA*CONC	24	SMW	1%	163.14333333	184.24898955	48	0.89	0.3803
TEMP*MEDIA*CONC	24	SMW	2%	42.43333333	184.24898955	48	0.23	0.8188
TEMP*MEDIA*CONC	24	SMW	3%	520.36000000	184.24898955	48	2.82	0.0069
TEMP*MEDIA*CONC	24	SWS	1%	888.97666667	184.24898955	48	4.82	0.0001
TEMP*MEDIA*CONC	24	SWS	3%	222.96333333	184.24898955	48	1.21	0.2322

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Table E1 continued

Dependent Variable: C18:1n9

Effect	TEMP	MEDIA	CONC	LSMEAN	Std Error	DF	t	Pr > |t|
TEMP	12			2911.3600000	180.43922108	48	16.13	0.0001
TEMP	18			3198.2341667	180.43922108	48	17.72	0.0001
TEMP	24			3031.9166667	180.43922108	48	16.80	0.0001
MEDIA		GLU		2734.8788889	170.11972909	48	16.08	0.0001
MEDIA		SMW		3557.3218519	170.11972909	48	20.91	0.0001
MEDIA		SWS		2750.3800000	208.35326572	48	13.20	0.0001
CONC			1%	3004.8262963	170.11972909	48	17.66	0.0001
CONC			2%	3227.7538889	208.35326572	48	15.49	0.0001
CONC			3%	2969.1251852	170.11972909	48	17.45	0.0001
TEMP*MEDIA	12	GLU		2566.3600000	294.65601415	48	8.71	0.0001
TEMP*MEDIA	12	SMW		3333.5811111	294.65601415	48	11.31	0.0001
TEMP*MEDIA	12	SWS		2795.5283333	360.87844215	48	7.75	0.0001
TEMP*MEDIA	18	GLU		3465.3955556	294.65601415	48	11.76	0.0001
TEMP*MEDIA	18	SMW		3120.4844444	294.65601415	48	10.59	0.0001
TEMP*MEDIA	18	SWS		2914.1166667	360.87844215	48	8.08	0.0001
TEMP*MEDIA	24	GLU		2172.8811111	294.65601415	48	7.37	0.0001
TEMP*MEDIA	24	SMW		4217.9000000	294.65601415	48	14.31	0.0001
TEMP*MEDIA	24	SWS		2541.4950000	360.87844215	48	7.04	0.0001
TEMP*CONC	12		1%	2944.3044444	294.65601415	48	9.99	0.0001
TEMP*CONC	12		2%	2736.7783333	360.87844215	48	7.58	0.0001
TEMP*CONC	12		3%	2994.8033333	294.65601415	48	10.16	0.0001
TEMP*CONC	18		1%	2529.9400000	294.65601415	48	8.59	0.0001
TEMP*CONC	18		2%	3434.8150000	360.87844215	48	9.52	0.0001
TEMP*CONC	18		3%	3708.8077778	294.65601415	48	12.59	0.0001
TEMP*CONC	24		1%	3540.2344444	294.65601415	48	12.01	0.0001
TEMP*CONC	24		2%	3511.6683333	360.87844215	48	9.73	0.0001
TEMP*CONC	24		3%	2203.7644444	294.65601415	48	7.48	0.0001
MEDIA*CONC		GLU	1%	3430.2644444	294.65601415	48	11.64	0.0001
MEDIA*CONC		GLU	2%	2380.3655556	294.65601415	48	8.08	0.0001
MEDIA*CONC		GLU	3%	2394.0066667	294.65601415	48	8.12	0.0001
MEDIA*CONC		SMW	1%	3776.1255556	294.65601415	48	12.82	0.0001
MEDIA*CONC		SMW	2%	4075.1422222	294.65601415	48	13.83	0.0001
MEDIA*CONC		SMW	3%	2820.6977778	294.65601415	48	9.57	0.0001
MEDIA*CONC		SWS	1%	1808.0888889	294.65601415	48	6.14	0.0001
MEDIA*CONC		SWS	3%	3692.6711111	294.65601415	48	12.53	0.0001
TEMP*MEDIA*CONC	12	GLU	1%	3956.0800000	510.35918726	48	7.75	0.0001
TEMP*MEDIA*CONC	12	GLU	2%	867.12333333	510.35918726	48	1.70	0.0958
TEMP*MEDIA*CONC	12	GLU	3%	2875.8766667	510.35918726	48	5.64	0.0001
TEMP*MEDIA*CONC	12	SMW	1%	4184.4266667	510.35918726	48	8.20	0.0001
TEMP*MEDIA*CONC	12	SMW	2%	4606.4333333	510.35918726	48	9.03	0.0001
TEMP*MEDIA*CONC	12	SMW	3%	1209.8833333	510.35918726	48	2.37	0.0218
TEMP*MEDIA*CONC	12	SWS	1%	692.40666667	510.35918726	48	1.36	0.1812
TEMP*MEDIA*CONC	12	SWS	3%	4898.6500000	510.35918726	48	9.60	0.0001
TEMP*MEDIA*CONC	18	GLU	1%	3270.0733333	510.35918726	48	6.41	0.0001
TEMP*MEDIA*CONC	18	GLU	2%	3270.6300000	510.35918726	48	6.41	0.0001
TEMP*MEDIA*CONC	18	GLU	3%	3855.4833333	510.35918726	48	7.55	0.0001
TEMP*MEDIA*CONC	18	SMW	1%	2303.6766667	510.35918726	48	4.51	0.0001
TEMP*MEDIA*CONC	18	SMW	2%	3599.0000000	510.35918726	48	7.05	0.0001
TEMP*MEDIA*CONC	18	SMW	3%	3458.7766667	510.35918726	48	6.78	0.0001
TEMP*MEDIA*CONC	18	SWS	1%	2016.0700000	510.35918726	48	3.95	0.0003
TEMP*MEDIA*CONC	18	SWS	3%	3812.1633333	510.35918726	48	7.47	0.0001
TEMP*MEDIA*CONC	24	GLU	1%	3064.6400000	510.35918726	48	6.00	0.0001
TEMP*MEDIA*CONC	24	GLU	2%	3003.3433333	510.35918726	48	5.88	0.0001
TEMP*MEDIA*CONC	24	GLU	3%	450.66000000	510.35918726	48	0.88	0.3816
TEMP*MEDIA*CONC	24	SMW	1%	4840.2733333	510.35918726	48	9.48	0.0001
TEMP*MEDIA*CONC	24	SMW	2%	4019.9933333	510.35918726	48	7.88	0.0001
TEMP*MEDIA*CONC	24	SMW	3%	3793.4333333	510.35918726	48	7.43	0.0001
TEMP*MEDIA*CONC	24	SWS	1%	2715.7900000	510.35918726	48	5.32	0.0001
TEMP*MEDIA*CONC	24	SWS	3%	2367.2000000	510.35918726	48	4.64	0.0001

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Table E1 continued

Dependent Variable: C18:2n6

Effect	TEMP	MEDIA	CONC	LSMEAN	Std Error	DF	t	Pr > |t|
TEMP	12			9102.5900000	420.87237617	48	21.63	0.0001
TEMP	18			9133.7800000	420.87237617	48	21.70	0.0001
TEMP	24			8832.7270833	420.87237617	48	20.99	0.0001
MEDIA		GLU		9260.7785185	396.80228161	48	23.34	0.0001
MEDIA		SMW		9544.6633333	396.80228161	48	24.05	0.0001
MEDIA		SWS		7883.9666667	485.98155935	48	16.22	0.0001
CONC			1%	8315.8100000	396.80228161	48	20.96	0.0001
CONC			2%	9353.8661111	485.98155935	48	19.25	0.0001
CONC			3%	9509.6988889	396.80228161	48	23.97	0.0001
TEMP*MEDIA	12	GLU		9016.9011111	687.28171230	48	13.12	0.0001
TEMP*MEDIA	12	SMW		9894.3655556	687.28171230	48	14.40	0.0001
TEMP*MEDIA	12	SWS		8043.4600000	841.74475234	48	9.56	0.0001
TEMP*MEDIA	18	GLU		10411.857778	687.28171230	48	15.15	0.0001
TEMP*MEDIA	18	SMW		8407.8022222	687.28171230	48	12.23	0.0001
TEMP*MEDIA	18	SWS		8305.6300000	841.74475234	48	9.87	0.0001
TEMP*MEDIA	24	GLU		8353.5766667	687.28171230	48	12.15	0.0001
TEMP*MEDIA	24	SMW		10331.822222	687.28171230	48	15.03	0.0001
TEMP*MEDIA	24	SWS		7302.8100000	841.74475234	48	8.68	0.0001
TEMP*CONC	12		1%	8098.5988889	687.28171230	48	11.78	0.0001
TEMP*CONC	12		2%	10262.738333	841.74475234	48	12.19	0.0001
TEMP*CONC	12		3%	9333.1488889	687.28171230	48	13.58	0.0001
TEMP*CONC	18		1%	8199.2322222	687.28171230	48	11.93	0.0001
TEMP*CONC	18		2%	9855.8216667	841.74475234	48	11.71	0.0001
TEMP*CONC	18		3%	9586.9666667	687.28171230	48	13.95	0.0001
TEMP*CONC	24		1%	8649.5988889	687.28171230	48	12.59	0.0001
TEMP*CONC	24		2%	7943.0383333	841.74475234	48	9.44	0.0001
TEMP*CONC	24		3%	9608.9811111	687.28171230	48	13.98	0.0001
MEDIA*CONC		GLU	1%	9710.8977778	687.28171230	48	14.13	0.0001
MEDIA*CONC		GLU	2%	9396.8033333	687.28171230	48	13.67	0.0001
MEDIA*CONC		GLU	3%	8674.6344444	687.28171230	48	12.62	0.0001
MEDIA*CONC		SMW	1%	8391.0600000	687.28171230	48	12.21	0.0001
MEDIA*CONC		SMW	2%	9310.9288889	687.28171230	48	13.55	0.0001
MEDIA*CONC		SMW	3%	10932.001111	687.28171230	48	15.91	0.0001
MEDIA*CONC		SWS	1%	6845.4722222	687.28171230	48	9.96	0.0001
MEDIA*CONC		SWS	3%	8922.4611111	687.28171230	48	12.98	0.0001
TEMP*MEDIA*CONC	12	GLU	1%	8476.2166667	1190.4068448	48	7.12	0.0001
TEMP*MEDIA*CONC	12	GLU	2%	11275.176667	1190.4068448	48	9.47	0.0001
TEMP*MEDIA*CONC	12	GLU	3%	7299.3100000	1190.4068448	48	6.13	0.0001
TEMP*MEDIA*CONC	12	SMW	1%	8498.6466667	1190.4068448	48	7.14	0.0001
TEMP*MEDIA*CONC	12	SMW	2%	9250.3000000	1190.4068448	48	7.77	0.0001
TEMP*MEDIA*CONC	12	SMW	3%	11934.150000	1190.4068448	48	10.03	0.0001
TEMP*MEDIA*CONC	12	SWS	1%	7320.9333333	1190.4068448	48	6.15	0.0001
TEMP*MEDIA*CONC	12	SWS	3%	8765.9866667	1190.4068448	48	7.36	0.0001
TEMP*MEDIA*CONC	18	GLU	1%	12003.263333	1190.4068448	48	10.08	0.0001
TEMP*MEDIA*CONC	18	GLU	2%	10749.336667	1190.4068448	48	9.03	0.0001
TEMP*MEDIA*CONC	18	GLU	3%	8482.9733333	1190.4068448	48	7.13	0.0001
TEMP*MEDIA*CONC	18	SMW	1%	6655.8533333	1190.4068448	48	5.59	0.0001
TEMP*MEDIA*CONC	18	SMW	2%	8962.3066667	1190.4068448	48	7.53	0.0001
TEMP*MEDIA*CONC	18	SMW	3%	9605.2466667	1190.4068448	48	8.07	0.0001
TEMP*MEDIA*CONC	18	SWS	1%	5938.5800000	1190.4068448	48	4.99	0.0001
TEMP*MEDIA*CONC	18	SWS	3%	10672.680000	1190.4068448	48	8.97	0.0001
TEMP*MEDIA*CONC	24	GLU	1%	8653.2133333	1190.4068448	48	7.27	0.0001
TEMP*MEDIA*CONC	24	GLU	2%	6165.8966667	1190.4068448	48	5.18	0.0001
TEMP*MEDIA*CONC	24	GLU	3%	10241.620000	1190.4068448	48	8.60	0.0001
TEMP*MEDIA*CONC	24	SMW	1%	10018.680000	1190.4068448	48	8.42	0.0001
TEMP*MEDIA*CONC	24	SMW	2%	9720.1800000	1190.4068448	48	8.17	0.0001
TEMP*MEDIA*CONC	24	SMW	3%	11256.606667	1190.4068448	48	9.46	0.0001
TEMP*MEDIA*CONC	24	SWS	1%	7276.9033333	1190.4068448	48	6.11	0.0001
TEMP*MEDIA*CONC	24	SWS	3%	7328.7166667	1190.4068448	48	6.16	0.0001

Continued

Table E1 continued

Dependent Variable: C18:3n3

Effect	TEMP	MEDIA	CONC	LSMEAN	Std Error	DF	t	Pr > |t|
TEMP	12			1391.5433333	171.83602036	48	8.10	0.0001
TEMP	18			1027.3008333	171.83602036	48	5.98	0.0001
TEMP	24			1604.9429167	171.83602036	48	9.34	0.0001
MEDIA		GLU		1620.7055556	162.00855367	48	10.00	0.0001
MEDIA		SMW		1270.6603704	162.00855367	48	7.84	0.0001
MEDIA		SWS		1028.0005556	198.41914523	48	5.18	0.0001
CONC			1%	1060.1974074	162.00855367	48	6.54	0.0001
CONC			2%	1780.7527778	198.41914523	48	8.97	0.0001
CONC			3%	1329.3337037	162.00855367	48	8.21	0.0001
TEMP*MEDIA	12	GLU		1303.2555556	280.60704621	48	4.64	0.0001
TEMP*MEDIA	12	SMW		1477.0088889	280.60704621	48	5.26	0.0001
TEMP*MEDIA	12	SWS		1395.7766667	343.67204073	48	4.06	0.0002
TEMP*MEDIA	18	GLU		1056.6144444	280.60704621	48	3.77	0.0005
TEMP*MEDIA	18	SMW		1067.6788889	280.60704621	48	3.80	0.0004
TEMP*MEDIA	18	SWS		922.76333333	343.67204073	48	2.69	0.0099
TEMP*MEDIA	24	GLU		2502.2466667	280.60704621	48	8.92	0.0001
TEMP*MEDIA	24	SMW		1267.2933333	280.60704621	48	4.52	0.0001
TEMP*MEDIA	24	SWS		765.46166667	343.67204073	48	2.23	0.0307
TEMP*CONC	12		1%	1211.5611111	280.60704621	48	4.32	0.0001
TEMP*CONC	12		2%	1469.4483333	343.67204073	48	4.28	0.0001
TEMP*CONC	12		3%	1519.5888889	280.60704621	48	5.42	0.0001
TEMP*CONC	18		1%	847.40222222	280.60704621	48	3.02	0.0040
TEMP*CONC	18		2%	943.89333333	343.67204073	48	2.75	0.0085
TEMP*CONC	18		3%	1262.8044444	280.60704621	48	4.50	0.0001
TEMP*CONC	24		1%	1121.6288889	280.60704621	48	4.00	0.0002
TEMP*CONC	24		2%	2928.9166667	343.67204073	48	8.52	0.0001
TEMP*CONC	24		3%	1205.6077778	280.60704621	48	4.30	0.0001
MEDIA*CONC		GLU	1%	1254.1988889	280.60704621	48	4.47	0.0001
MEDIA*CONC		GLU	2%	2409.0955556	280.60704621	48	8.59	0.0001
MEDIA*CONC		GLU	3%	1198.8222222	280.60704621	48	4.27	0.0001
MEDIA*CONC		SMW	1%	1014.8300000	280.60704621	48	3.62	0.0007
MEDIA*CONC		SMW	2%	1152.4100000	280.60704621	48	4.11	0.0002
MEDIA*CONC		SMW	3%	1644.7411111	280.60704621	48	5.86	0.0001
MEDIA*CONC		SWS	1%	911.56333333	280.60704621	48	3.25	0.0021
MEDIA*CONC		SWS	3%	1144.4377778	280.60704621	48	4.08	0.0002
TEMP*MEDIA*CONC	12	GLU	1%	1139.2333333	486.02566100	48	2.34	0.0233
TEMP*MEDIA*CONC	12	GLU	2%	1661.9133333	486.02566100	48	3.42	0.0013
TEMP*MEDIA*CONC	12	GLU	3%	1108.6200000	486.02566100	48	2.28	0.0270
TEMP*MEDIA*CONC	12	SMW	1%	1190.0433333	486.02566100	48	2.45	0.0180
TEMP*MEDIA*CONC	12	SMW	2%	1276.9833333	486.02566100	48	2.63	0.0115
TEMP*MEDIA*CONC	12	SMW	3%	1964.0000000	486.02566100	48	4.04	0.0002
TEMP*MEDIA*CONC	12	SWS	1%	1305.4066667	486.02566100	48	2.69	0.0099
TEMP*MEDIA*CONC	12	SWS	3%	1486.1466667	486.02566100	48	3.06	0.0036
TEMP*MEDIA*CONC	18	GLU	1%	1318.3766667	486.02566100	48	2.71	0.0092
TEMP*MEDIA*CONC	18	GLU	2%	892.20000000	486.02566100	48	1.84	0.0726
TEMP*MEDIA*CONC	18	GLU	3%	959.26666667	486.02566100	48	1.97	0.0542
TEMP*MEDIA*CONC	18	SMW	1%	686.45666667	486.02566100	48	1.41	0.1643
TEMP*MEDIA*CONC	18	SMW	2%	995.58666667	486.02566100	48	2.05	0.0460
TEMP*MEDIA*CONC	18	SMW	3%	1520.9933333	486.02566100	48	3.13	0.0030
TEMP*MEDIA*CONC	18	SWS	1%	537.37333333	486.02566100	48	1.11	0.2744
TEMP*MEDIA*CONC	18	SWS	3%	1308.1533333	486.02566100	48	2.69	0.0098
TEMP*MEDIA*CONC	24	GLU	1%	1304.9866667	486.02566100	48	2.69	0.0099
TEMP*MEDIA*CONC	24	GLU	2%	4673.1733333	486.02566100	48	9.62	0.0001
TEMP*MEDIA*CONC	24	GLU	3%	1528.5800000	486.02566100	48	3.15	0.0028
TEMP*MEDIA*CONC	24	SMW	1%	1167.9900000	486.02566100	48	2.40	0.0202
TEMP*MEDIA*CONC	24	SMW	2%	1184.6600000	486.02566100	48	2.44	0.0185
TEMP*MEDIA*CONC	24	SMW	3%	1449.2300000	486.02566100	48	2.98	0.0045
TEMP*MEDIA*CONC	24	SWS	1%	891.91000000	486.02566100	48	1.84	0.0727
TEMP*MEDIA*CONC	24	SWS	3%	639.01333333	486.02566100	48	1.31	0.1948

Continued

Table E1 continued

Dependent Variable: C20:4n6 (ARA)

Effect	TEMP	MEDIA	CONC	LSMEAN	Std Error	DF	t	Pr > |t|
TEMP	12			278.97833333	57.35751695	48	4.86	0.0001
TEMP	18			1249.4241667	57.35751695	48	21.78	0.0001
TEMP	24			968.18750000	57.35751695	48	16.88	0.0001
MEDIA		GLU		785.28592593	54.07718558	48	14.52	0.0001
MEDIA		SMW		825.08518519	54.07718558	48	15.26	0.0001
MEDIA		SWS		913.23000000	66.23075570	48	13.79	0.0001
CONC			1%	738.96296296	54.07718558	48	13.66	0.0001
CONC			2%	423.69611111	66.23075570	48	6.40	0.0001
CONC			3%	1197.7640741	54.07718558	48	22.15	0.0001
TEMP*MEDIA	12	GLU		370.07555556	93.66443295	48	3.95	0.0003
TEMP*MEDIA	12	SMW		184.04777778	93.66443295	48	1.96	0.0552
TEMP*MEDIA	12	SWS		284.72833333	114.71503389	48	2.48	0.0166
TEMP*MEDIA	18	GLU		1193.7577778	93.66443295	48	12.75	0.0001
TEMP*MEDIA	18	SMW		1985.3644444	93.66443295	48	21.20	0.0001
TEMP*MEDIA	18	SWS		229.01333333	114.71503389	48	2.00	0.0516
TEMP*MEDIA	24	GLU		792.02444444	93.66443295	48	8.46	0.0001
TEMP*MEDIA	24	SMW		305.84333333	93.66443295	48	3.27	0.0020
TEMP*MEDIA	24	SWS		2225.9483333	114.71503389	48	19.40	0.0001
TEMP*CONC	12		1%	197.94777778	93.66443295	48	2.11	0.0398
TEMP*CONC	12		2%	375.58166667	114.71503389	48	3.27	0.0020
TEMP*CONC	12		3%	295.60666667	93.66443295	48	3.16	0.0028
TEMP*CONC	18		1%	1329.2133333	93.66443295	48	14.19	0.0001
TEMP*CONC	18		2%	535.14166667	114.71503389	48	4.66	0.0001
TEMP*CONC	18		3%	1645.8233333	93.66443295	48	17.57	0.0001
TEMP*CONC	24		1%	689.72777778	93.66443295	48	7.36	0.0001
TEMP*CONC	24		2%	360.36500000	114.71503389	48	3.14	0.0029
TEMP*CONC	24		3%	1651.8622222	93.66443295	48	17.64	0.0001
MEDIA*CONC	GLU		1%	459.54000000	93.66443295	48	4.91	0.0001
MEDIA*CONC	GLU		2%	409.18111111	93.66443295	48	4.37	0.0001
MEDIA*CONC	GLU		3%	1487.1366667	93.66443295	48	15.88	0.0001
MEDIA*CONC	SMW		1%	1175.9200000	93.66443295	48	12.55	0.0001
MEDIA*CONC	SMW		2%	438.21111111	93.66443295	48	4.68	0.0001
MEDIA*CONC	SMW		3%	861.12444444	93.66443295	48	9.19	0.0001
MEDIA*CONC	SWS		1%	581.42888889	93.66443295	48	6.21	0.0001
MEDIA*CONC	SWS		3%	1245.0311111	93.66443295	48	13.29	0.0001
TEMP*MEDIA*CONC	12	GLU	1%	146.51666667	162.23155674	48	0.90	0.3710
TEMP*MEDIA*CONC	12	GLU	2%	594.01666667	162.23155674	48	3.66	0.0006
TEMP*MEDIA*CONC	12	GLU	3%	369.69333333	162.23155674	48	2.28	0.0272
TEMP*MEDIA*CONC	12	SMW	1%	295.53333333	162.23155674	48	1.82	0.0747
TEMP*MEDIA*CONC	12	SMW	2%	157.14666667	162.23155674	48	0.97	0.3376
TEMP*MEDIA*CONC	12	SMW	3%	99.46333333	162.23155674	48	0.61	0.5427
TEMP*MEDIA*CONC	12	SWS	1%	151.79333333	162.23155674	48	0.94	0.3541
TEMP*MEDIA*CONC	12	SWS	3%	417.66333333	162.23155674	48	2.57	0.0132
TEMP*MEDIA*CONC	18	GLU	1%	868.94333333	162.23155674	48	5.36	0.0001
TEMP*MEDIA*CONC	18	GLU	2%	246.21000000	162.23155674	48	1.52	0.1357
TEMP*MEDIA*CONC	18	GLU	3%	2466.1200000	162.23155674	48	15.20	0.0001
TEMP*MEDIA*CONC	18	SMW	1%	2911.7733333	162.23155674	48	17.95	0.0001
TEMP*MEDIA*CONC	18	SMW	2%	824.07333333	162.23155674	48	5.08	0.0001
TEMP*MEDIA*CONC	18	SMW	3%	2220.2466667	162.23155674	48	13.69	0.0001
TEMP*MEDIA*CONC	18	SWS	1%	206.92333333	162.23155674	48	1.28	0.2083
TEMP*MEDIA*CONC	18	SWS	3%	251.10333333	162.23155674	48	1.55	0.1282
TEMP*MEDIA*CONC	24	GLU	1%	363.16000000	162.23155674	48	2.24	0.0299
TEMP*MEDIA*CONC	24	GLU	2%	387.31666667	162.23155674	48	2.39	0.0210
TEMP*MEDIA*CONC	24	GLU	3%	1625.5966667	162.23155674	48	10.02	0.0001
TEMP*MEDIA*CONC	24	SMW	1%	320.45333333	162.23155674	48	1.98	0.0540
TEMP*MEDIA*CONC	24	SMW	2%	333.41333333	162.23155674	48	2.06	0.0453
TEMP*MEDIA*CONC	24	SMW	3%	263.66333333	162.23155674	48	1.63	0.1107
TEMP*MEDIA*CONC	24	SWS	1%	1385.5700000	162.23155674	48	8.54	0.0001
TEMP*MEDIA*CONC	24	SWS	3%	3066.3266667	162.23155674	48	18.90	0.0001

Continued

Table E1 continued

Dependent Variable: C20:5n3 (EPA)

Effect	TEMP	MEDIA	CONC	LSMEAN	Std Error	DF	t	Pr > |t|
TEMP	12			750.16958333	30.79561793	48	24.36	0.0001
TEMP	18			511.33208333	30.79561793	48	16.60	0.0001
TEMP	24			448.82416667	30.79561793	48	14.57	0.0001
MEDIA		GLU		538.99037037	29.03438702	48	18.56	0.0001
MEDIA		SMW		681.05888889	29.03438702	48	23.46	0.0001
MEDIA		SWS		450.36055556	35.55971660	48	12.66	0.0001
CONC			1%	574.42037037	29.03438702	48	19.78	0.0001
CONC			2%	618.07277778	35.55971660	48	17.38	0.0001
CONC			3%	533.82074074	29.03438702	48	18.39	0.0001
TEMP*MEDIA	12	GLU		598.19444444	50.28903349	48	11.90	0.0001
TEMP*MEDIA	12	SMW		1050.0233333	50.28903349	48	20.88	0.0001
TEMP*MEDIA	12	SWS		528.35166667	61.59123585	48	8.58	0.0001
TEMP*MEDIA	18	GLU		521.34555556	50.28903349	48	10.37	0.0001
TEMP*MEDIA	18	SMW		545.82888889	50.28903349	48	10.85	0.0001
TEMP*MEDIA	18	SWS		444.56666667	61.59123585	48	7.22	0.0001
TEMP*MEDIA	24	GLU		497.43111111	50.28903349	48	9.89	0.0001
TEMP*MEDIA	24	SMW		447.32444444	50.28903349	48	8.90	0.0001
TEMP*MEDIA	24	SWS		378.16333333	61.59123585	48	6.14	0.0001
TEMP*CONC	12		1%	816.63888889	50.28903349	48	16.24	0.0001
TEMP*CONC	12		2%	828.50166667	61.59123585	48	13.45	0.0001
TEMP*CONC	12		3%	631.47888889	50.28903349	48	12.56	0.0001
TEMP*CONC	18		1%	466.68444444	50.28903349	48	9.28	0.0001
TEMP*CONC	18		2%	515.22333333	61.59123585	48	8.37	0.0001
TEMP*CONC	18		3%	553.38555556	50.28903349	48	11.00	0.0001
TEMP*CONC	24		1%	439.93777778	50.28903349	48	8.75	0.0001
TEMP*CONC	24		2%	510.49333333	61.59123585	48	8.29	0.0001
TEMP*CONC	24		3%	416.59777778	50.28903349	48	8.28	0.0001
MEDIA*CONC		GLU	1%	430.03555556	50.28903349	48	8.55	0.0001
MEDIA*CONC		GLU	2%	534.14444444	50.28903349	48	10.62	0.0001
MEDIA*CONC		GLU	3%	652.79111111	50.28903349	48	12.98	0.0001
MEDIA*CONC		SMW	1%	810.11222222	50.28903349	48	16.11	0.0001
MEDIA*CONC		SMW	2%	702.00111111	50.28903349	48	13.96	0.0001
MEDIA*CONC		SMW	3%	531.06333333	50.28903349	48	10.56	0.0001
MEDIA*CONC		SWS	1%	483.11333333	50.28903349	48	9.61	0.0001
MEDIA*CONC		SWS	3%	417.60777778	50.28903349	48	8.30	0.0001
TEMP*MEDIA*CONC	12	GLU	1%	571.58333333	87.10316106	48	6.56	0.0001
TEMP*MEDIA*CONC	12	GLU	2%	625.81666667	87.10316106	48	7.18	0.0001
TEMP*MEDIA*CONC	12	GLU	3%	597.18333333	87.10316106	48	6.86	0.0001
TEMP*MEDIA*CONC	12	SMW	1%	1395.1866667	87.10316106	48	16.02	0.0001
TEMP*MEDIA*CONC	12	SMW	2%	1031.1866667	87.10316106	48	11.84	0.0001
TEMP*MEDIA*CONC	12	SMW	3%	723.69666667	87.10316106	48	8.31	0.0001
TEMP*MEDIA*CONC	12	SWS	1%	483.14666667	87.10316106	48	5.55	0.0001
TEMP*MEDIA*CONC	12	SWS	3%	573.55666667	87.10316106	48	6.58	0.0001
TEMP*MEDIA*CONC	18	GLU	1%	232.22666667	87.10316106	48	2.67	0.0104
TEMP*MEDIA*CONC	18	GLU	2%	505.57000000	87.10316106	48	5.80	0.0001
TEMP*MEDIA*CONC	18	GLU	3%	826.24000000	87.10316106	48	9.49	0.0001
TEMP*MEDIA*CONC	18	SMW	1%	617.91000000	87.10316106	48	7.09	0.0001
TEMP*MEDIA*CONC	18	SMW	2%	524.87666667	87.10316106	48	6.03	0.0001
TEMP*MEDIA*CONC	18	SMW	3%	494.70000000	87.10316106	48	5.68	0.0001
TEMP*MEDIA*CONC	18	SWS	1%	549.91666667	87.10316106	48	6.31	0.0001
TEMP*MEDIA*CONC	18	SWS	3%	339.21666667	87.10316106	48	3.89	0.0003
TEMP*MEDIA*CONC	24	GLU	1%	486.29666667	87.10316106	48	5.58	0.0001
TEMP*MEDIA*CONC	24	GLU	2%	471.04666667	87.10316106	48	5.41	0.0001
TEMP*MEDIA*CONC	24	GLU	3%	534.95000000	87.10316106	48	6.14	0.0001
TEMP*MEDIA*CONC	24	SMW	1%	417.24000000	87.10316106	48	4.79	0.0001
TEMP*MEDIA*CONC	24	SMW	2%	549.94000000	87.10316106	48	6.31	0.0001
TEMP*MEDIA*CONC	24	SMW	3%	374.79333333	87.10316106	48	4.30	0.0001
TEMP*MEDIA*CONC	24	SWS	1%	416.27666667	87.10316106	48	4.78	0.0001
TEMP*MEDIA*CONC	24	SWS	3%	340.05000000	87.10316106	48	3.90	0.0003

Table E2. Two-way interactions of LSMEANS (n=3) of yields (mg/L) of major fatty acids by *P. irregulare* treated with crude soybean oil and food byproducts and incubated in a shaker for 8 days.

Dependent Variable: C14:0

Media*Temperature

	Media		
Temp	GLU	SMW	SWS
12°C	370 ^{ab}	600 ^{a}	350 ^{ab}
18°C	90 ^{b}	170 ^{b}	210 ^{ab}
24°C	170 ^{b}	250 ^{ab}	90 ^{b}

Media*Concentration

	Media		
Conc.	GLU	SMW	SWS
1%	120 ^{b}	120 ^{b}	250 ^{b}
2%	230 ^{b}	140 ^{b}	-
3%	290 ^{b}	760 ^{a}	190 ^{b}

Temperature*Concentration

	Temp.		
Conc.	12°C	18°C	24°C
1%	240 ^{b}	120 ^{b}	120 ^{b}
2%	320 ^{ab}	46 ^{b}	190 ^{b}
3%	750 ^{a}	250 ^{b}	230 ^{b}

^{abcd} LSMEANS in the same table with unlike superscripts are different ($P < 0.05$)

^{e} ND=Not detectable

Continued

Table E2 continued

Dependent Variable: C16:0

Media*Temperature

	Media		
Temp.	GLU	SMW	SWS
12°C	3400 ^{a}	2600 ^{ab}	2400 ^{abc}
18°C	2800 ^{ad}	1300 ^{bc}	3800 ^{a}
24°C	1400 ^{cd}	1500 ^{cd}	820 ^{c}

Media*Concentration

	Media		
Conc.	GLU	SMW	SWS
1%	1900 ^{cb}	1700 ^{c}	3000 ^{ab}
2%	3400 ^{a}	1400 ^{c}	-
3%	2400 ^{abc}	2400 ^{abc}	1600 ^{c}

Temperature*Concentration

	Temp.		
Conc.	12°C	18°C	24°C
1%	2200 ^{bc}	3100 ^{ab}	1200 ^{c}
2%	2500 ^{abc}	3100 ^{ab}	1600 ^{bc}
3%	3800 ^{a}	1400 ^{c}	1200 ^{c}

Continued

Table E2 continued

Dependent Variable: C16:1

Media*Temperature

	Media		
Temp	GLU	SMW	SWS
12°C	53 ^{c}	890 ^{a}	370 ^{b}
18°C	ND	77 ^{c}	-
24°C	130 ^{c}	61 ^{c}	37 ^{c}

Media*Concentration

	Media		
Conc.	GLU	SMW	SWS
1%	14 ^{c}	150 ^{cb}	260 ^{b}
2%	39 ^{c}	54 ^{c}	-
3%	120 ^{cb}	820 ^{a}	6 ^{c}

Temperature*Concentration

	Temp.		
Conc.	12°C	18°C	24°C
1%	280 ^{b}	77 ^{c}	77 ^{bc}
2%	70 ^{bc}	ND	70 ^{bc}
3%	860 ^{a}	ND	88 ^{bc}

Continued

Table E2 continued

Dependent Variable: C18:0

Media*Temperature

	Media		
Temp	GLU	SMW	SWS
12°C	53 [a]	220 [a]	28 [a]
18°C	190 [a]	510 [a]	600 [a]
24°C	130 [a]	240 [a]	560 [a]

Media*Concentration

	Media		
Conc.	GLU	SMW	SWS
1%	69 [ab]	530 [a]	440 [ab]
2%	18 [b]	14 [b]	-
3%	280 [ab]	430 [ab]	340 [ab]

Temperature*Concentration

	Temp.		
Conc.	12°C	18°C	24°C
1%	270 [ab]	400 [ab]	370 [ab]
2%	ND	ND	49 [b]
3%	19 [b]	700 [a]	340 [ab]

Continued

Table E2 continued

Dependent Variable: C18:1n9

Media*Temperature

	Media		
Temp	GLU	SMW	SWS
12°C	2600 ^{b}	3300 ^{ab}	2800 ^{ab}
18°C	3500 ^{ab}	3100 ^{ab}	2900 ^{ab}
24°C	2200 ^{b}	4200 ^{a}	2500 ^{b}

Media*Concentration

	Media		
Conc.	GLU	SMW	SWS
1%	3400 ^{ab}	3800 ^{a}	1800 ^{c}
2%	2400 ^{bc}	4100 ^{a}	-
3%	2400 ^{bc}	2800 ^{abc}	3700 ^{ab}

Temperatures*Concentration

	Temp.		
Conc.	12°C	18°C	24°C
1%	2900 ^{ab}	2500 ^{ab}	3500 ^{ab}
2%	2700 ^{ab}	3400 ^{ab}	3500 ^{ab}
3%	3000 ^{ab}	3700 ^{a}	2200 ^{b}

Continued

Table E2 continued

Dependent Variable: C18:2n6

Media*Temperature

	Media		
Temp	GLU	SMW	SWS
12°C	9000 [a]	9900 [a]	8000 [a]
18°C	10000 [a]	8400 [a]	8300 [a]
24°C	8300 [a]	10000 [a]	7300 [a]

Media*Concentration

	Media		
Conc.	GLU	SMW	SWS
1%	9700 [ab]	8400 [ab]	6800 [b]
2%	9400 [ab]	9300 [ab]	-
3%	8700 [ab]	11000 [a]	8900 [ab]

Temperatures*Concentration

	Temp.		
Conc.	12°C	18°C	24°C
1%	8100 [a]	8200 [a]	8600 [a]
2%	10000 [a]	9900 [a]	7900 [a]
3%	9300 [a]	9600 [a]	9600 [a]

Continued

Table E2 continued

Dependent Variable: C18:3n3

Media*Temperature

	Media		
Temp	GLU	SMW	SWS
12°C	1300 ^{ab}	1500 ^{ab}	1400 ^{ab}
18°C	1100 ^{b}	1100 ^{b}	920 ^{b}
24°C	2500 ^{a}	1300 ^{ab}	760 ^{b}

Media*Concentration

	Media		
Conc.	GLU	SMW	SWS
1%	1300 ^{ab}	1000 ^{b}	910 ^{b}
2%	2400 ^{a}	1200 ^{ab}	-
3%	1200 ^{ab}	1600 ^{ab}	1100 ^{b}

Temperatures*Concentration

	Temp.		
Conc.	12°C	18°C	24°C
1%	1200 ^{b}	850 ^{b}	1100 ^{b}
2%	1500 ^{ab}	940 ^{b}	2900 ^{a}
3%	1500 ^{ab}	1300 ^{b}	1200 ^{b}

Continued

Table E2 continued

Dependent Variable: C20:4n6 (ARA)

Media*Temperature

	Media		
Temp	GLU	SMW	SWS
12°C	370 ^{cd}	180 ^{d}	290 ^{d}
18°C	1200 ^{b}	2000 ^{a}	230 ^{d}
24°C	790 ^{bc}	300 ^{d}	2200 ^{a}

Media*Concentration

	Media		
Conc.	GLU	SMW	SWS
1%	460 ^{cd}	1200 ^{ab}	580 ^{cd}
2%	410 ^{d}	440 ^{d}	-
3%	1500 ^{a}	860 ^{bc}	1200 ^{ab}

Temperatures*Concentration

	Temp.		
Conc.	12°C	18°C	24°C
1%	200 ^{c}	1300 ^{a}	690 ^{b}
2%	380 ^{bc}	530 ^{bc}	360 ^{bc}
3%	300 ^{bc}	1600 ^{a}	1700 ^{a}

Continued

Table E2 continued

Dependent Variable: C20:5n3 (EPA)

Media*Temperature

	Media		
Temp	GLU	SMW	SWS
12°C	600 ^{b}	1000 ^{a}	530 ^{b}
18°C	520 ^{b}	540 ^{b}	440 ^{b}
24°C	500 ^{b}	450 ^{b}	380 ^{b}

Media*Concentration

	Media		
Conc.	GLU	SMW	SWS
1%	430 ^{cd}	810 ^{a}	480 ^{bcd}
2%	530 ^{bcd}	700 ^{ab}	-
3%	650 ^{abc}	530 ^{bcd}	420 ^{d}

Temperatures*Concentrations

	Temp.		
Conc.	12°C	18°C	24°C
1%	820 ^{a}	470 ^{b}	440 ^{b}
2%	830 ^{a}	510 ^{b}	510 ^{b}
3%	630 ^{ab}	550 ^{b}	420 ^{b}

VITA

Ming-Huei Cheng was born on September 5, 1967, in Taiwan. In September 1982 he attended Taoyuan High School, Taoyuan, Taiwan, and graduated in July 1985. The following year, he studied animal sciences at National Pingtung Polytechnic Institute, Pingtung, Taiwan, and graduated in June 1989. In the same month, he started working as a laboratory assistant in the Department of Animal Science at National Taiwan University, Taipei, Taiwan, and then he joined the National Army in June 1990.

In January 1994, he enrolled at South Dakota State University, Brookings, South Dakota, and received a Bachelor of Science degree with high honors in Animal Science in August 1995. In January 1996, he began his graduate education in the Department of Food Science and Technology at the University of Tennessee, Knoxville, Tennessee, and he was awarded a graduate research assistantship. In August 1997, he received a Master of Science degree.

He is a member of the Institute of Food Technologists and Southern Association of Agricultural Scientists. This research under the direction of Dr. Gregory J. Hulbert was awarded a second place for the University of Tennessee chapter of *Sigma Xi*'s student paper competition in 1997.

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