Biotechnology Research and Innovation (2019) 3, 103–119

Biotechnology
Research & Innovation

http://www.journals.elsevier.com/biotechnology-research-and-innovation/

REVIEW ARTICLE

Industrial production, patent landscape, and market trends of arachidonic acid-rich oil of *Mortierella alpina*

Luis Daniel Goyzueta Mamani[a], **Antonio Irineudo Magalhães Jr.**[a], **Zhenhua Ruan**[b], **Júlio Cesar de Carvalho**[a,*], **Carlos Ricardo Soccol**[a]

[a] *Federal University of Paraná, Department of Bioprocess Engineering and Biotechnology, Curitiba, PR, Brazil*
[b] *Department of Biosystems and Agricultural Engineering, Michigan State University, East Lansing, USA*

Received 22 October 2018; accepted 17 February 2019
Available online 15 March 2019

KEYWORDS
ARA;
Mortierella alpina;
PUFA;
Downstream;
Industrial production;
Market

Abstract Polyunsaturated fatty acids are essential, health-promoting nutrients that are widely used in medicine, pharmaceutics, cosmetics, nutrition, and other fields. Suitable plant and animal sources for these lipids are limited, but alternatives have been actively researched and developed in the last three decades, especially those produced microbiologically. Arachidonic acid (ARA) is one of the most valuable among nutraceutical lipids, being associated with the good development of the nervous central system and enhancement of immune response. Currently, microbial sources of ARA are used for industrial production due to their rapid and controllable production, as compared to animal (fish) and plant sources. Microbial sources are also eco-friendly and reduce the pressure on marine life. The fungus *Mortierella alpina* is one of the most important microbial lipid sources, but there are few accounts of industrial and market information regarding this microorganism.

At the current pace, the industrial production of ARA is projected to reach 410 thousand tons by 2025, which will not be enough to meet the demand. This will keep stimulating research for intensified production, which can be optimized in terms of concentration and yields (through bioprospection and metabolic and biomolecular engineering), productivity and economics (through media optimization and byproduct use), and formulation. This work reviews aspects such as the important considerations for industrial production of arachidonic acid-rich oil by *Mortierella alpina*, based on patents and studies, and presents a global market analysis and forecast.

Introduction

The inclusion of essential fatty acids (EFA) in human diets, especially for infants, has become popular in recent decades

* Corresponding author.
E-mail: jccarvalho@ufpr.br (J.C. Carvalho).

https://doi.org/10.1016/j.biori.2019.02.002
2452-0721/© 2019 Sociedade Brasileira de Biotecnologia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

and this is fueling their production around the world. Polyunsaturated fatty acids (PUFA) are of great interest because of their beneficial properties to human health. There are several well-studied PUFA, such as arachidonic acid (ARA) and docosahexaenoic acid (DHA), which have been biotechnologically produced from the oleaginous fungus *Mortierella alpina* since 2001, after having been granted the generally recognized as safe (GRAS) status by the Food and Drug Administration (FDA). Nowadays, several food and nutraceutical products include PUFA as ingredients or additives. ARA can be used in infant and geriatric formulas, food supplements, cosmetics, and drugs.

ARA is a long-chain (20:4 Δ5,8,11,14 $n-6$) polyunsaturated fatty acid of the $\omega-6$ class. This fatty acid can be found in the blood, brain, glandular organs, and liver. ARA plays a role in maintaining hippocampal neuron membrane fluidity (Higashiyama, Fujikawa, Park, & Shimizu, 2002) and is synthesized in animals and humans by the desaturation and elongation of linoleic acid (Oregon State and University, 2014). It is a precursor of several mediators of essential processes, acting in the modulation of inflammation, cytokine release, platelet aggregation, immune response, allergic phenomena, and thrombosis (Uauy, Mena, & Rojas, 2000). It is also important as a second messenger in the central nervous system and plays a role in the expression of long-term potentiation (Nishizaki, Nomura, Matsuoka, & Tsujishita, 1999).

ARA-rich oil is commonly used in food formulation to enhance the development and health of the brain, vascular systems, and the nervous system. ARA and DHA comprise over 90% of the brain essential fatty acids (Nisha & Venkateswaran, 2011). ARA is used in formulas to reach erythrocytes and blood lipid fatty acid profiles that are equivalent to those in breast-fed infants (Hoffman et al., 2000). Its mixture with DHA helps to improve visual function, cognitive development, and normal blood pressure (Forsyth et al., 2003). In 2014, about 120 thousand tons of ARA were produced (QY Research Center, 2015).

Industrial production of ARA relies on the cultivation of lipid-rich biomass in fermentation processes as well as the extraction and purification of the oil. The aim of this paper is to present the main steps of the industrial processing of ARA by *M. alpina*, as well as the innovations and world market trends: it describes the industrial aspects of productivity, ARA production versus growth conditions, processing of biomass into ARA-rich oil, and presents an overview of commercial products, the market for this valuable fatty acid and promising paths for further research and development.

Arachidonic acid produced by *M. alpina*

The genus *Mortierella* (family *Mortierellaceae*, order *Mortierellales*, class *Zygomycetes*) was first described in the 19th century and the first mention of the species *M. alpina* dates back to 1913 (Saccardo, Traverso, & Trotter, n.d.). The specie *alpina* is the most important industrially. Several wild and genetically modified strains of *M. alpina* have been evaluated to optimize industrial processes for PUFA-rich oil production. Table 1 presents literature productivity data for several strains of *M. alpina* and shows significant differences between various producers, with final ARA concentrations ranging from 1 to 20 g L^{-1} and productivities ranging from 0.2 to 1.8 g L^{-1} day^{-1}. In the last decade, *M. alpina* has been genetically modified to produce alternatives polyunsaturated fatty acids such as dihomo-y-linolenic acid (disrupting Δ5-desaturase gene) (Kikukawa et al., 2016), linoleic acid or oleic acid (expressing Δ12-desaturase gene derived from *Coprinopsis cinereal)* (Sakamoto et al., 2017). An alternative fatty acid from the Omega-3 family, eicosatetraenoic acid, was also produced transforming and expressing the endogenous ω3-desaturase gene or the heterologous *Saprolegnia diclina* D17 (sdd17m) desaturase gene at 28 °C, this fatty acid was only produced under low-temperature conditions (Okuda et al., 2015). A recent review by Kikukawa et al. (2018) details mutant breeding and recent advances in the production of PUFAs by molecular engineering techniques.

The biosynthesis and accumulation of lipids in oleaginous microorganisms depend on the ability to produce acetyl-CoA (precursor for fatty acid synthetase – FAS) and NADPH. Nitrogen depletion induces the formation of acetyl-CoA through the cleavage of citrate in the cytosol by the ATP:citrate:lyase (ACL) (Ratledge, 2004). The unsaturated fatty acids synthesis starts from a C-16 backbone produced by FAS. This backbone is elongated and unsaturated in successive enzymatic steps to form two important families of PUFAs: ω3 and ω6. For the formation of arachidonic acid, a ω6-PUFA, palmitic acid (C16:0) is elongated forming stearic acid (C18:0), which is then sequentially unsaturated at the Δ9, Δ12 and Δ6 positions, forming gamma-linolenic acid. This is further elongated, and unsaturated by Δ5-desaturase to form ARA (Certik & Shimizu, 1999). These desaturases are oxygen-dependent and require cytochrome b5 as a co-factor (Michaelson, Lazarus, Griffiths, Napier, & Stobart, 1998). A variety of mutants with enhanced desaturase (Δ9, Δ12, Δ6, Δ5, and $\omega-3$) and elongase (EL1) activity have been derived from *M. alpina* 1S-4 (Saeree Jareonkitmongkol, Kawashima, Shimizu, & Yamada, 1992). Several mutants derived from 1S-4 and their characteristics are summarized in Table 2.

M. alpina strains ME-1 and 1S-4 have been used for industrial production and as a model for lipogenesis studies due to their high oleaginous capacity, lipogenesis regulation, PUFA production, and capacity of absorption and transformation of exogenous fatty acids (Shimizu & Jareonkitmongkol, 1995). The composition of fatty acids in *M. alpina* can be manipulated by varying fermentation conditions (e.g., agitation, oxygenation, pH). The lipid concentration in the fungus may reach 50% of the biomass (Singh & Ward, 1997), with levels of ARA between 30% and 70% of the total fatty acids (Amano et al., 1992).

Two types of cultivation are used for industrial-scale production: submerged and solid-state. Submerged cultivation is preferred because of its easier scale-up, biomass recovery, higher ARA production yield, and fermentation time (solid-state fermentation needs a cultivation period of over 20 days). High yields of arachidonic acid have been reported in over 10 g L^{-1} of liquid culture (Higashiyama, Yaguchi, Akimoto, Fujikawa, & Shimizu, 1998; Jin et al., 2008; Singh & Ward, 1997), with productivity of approximately 1 g L^{-1} per day. The final ARA concentration and the productivity of multiliter or pilot-scale cultures of *M. alpina* presented in Table 1 show that most of the best-producing strains are mutants, which have distinctive features in their fatty acid

Table 1 Arachidonic acid and total lipid production using *M. alpina* in multiliter or pilot-scales.

Strain	Assay volume (L)	ARA ($g\,L^{-1}$)	Time (days)	Productivity ($g\,L^{-1}\,day^{-1}$)	Reference
			Submerged culture – Flask		
ME-1	0.250	11.4	–	–	Ji et al. (2014)
ATC 32222	0.250	11	11	1	Singh and Ward (1997)
Wuji-H4	0.250	3.9	5	0.8	Ho and Chen (2008)
			Submerged culture – Fermenter		
ME-1	3	19.8	11	1.8	Jin et al. (2008)
	3.5	9.2	7	1.3	Peng et al. (2010)
1S-4	10	13	10	1.3	Higashiyama, Yaguchi, Akimoto, Fujikawa, and Shimizu (1998)
	5	3	8	0.4	Higashiyama, Yaguchi, Akimoto, Fujikawa, and Shimizu (1998)
ATCC 32221	500	11	16	0.7	Totani, Someya, and Oba (1992)
ATCC 16266	45	6.5	11	0.6	Fischer et al. (2013)
UW-1	20	5.5	8	0.7	Li, Lu, Yadwad, and Ward (1995)
LPM 301	30	4.5	8	0.6	Eroshin, Satroutdinov, Dedyukhina, and Chistyakova (2000)
	6	4.5	8	0.6	Eroshin et al. (2000)
ATCC 42430	20	4.1	6	0.7	Kyle (2001)
DSA-12	500	3.3	6	0.6	Park et al. (1999)
M18	6	1.41	7	0.2	Yu et al. (2003)
CBS 528.72	–	1.39	7	0.2	Nisha et al. (2011)
CBS 343.66	5	1.0	6	0.2	Lindberg and Molin (1993)
			Solid culture – Flask		
IFO 8568	–	13[a]	20	0.65	Totani et al. (1987)
CCF 185	0.300	36[a]	21	1.42	Stredanská and Šajbidor (1993)

[a] $g\,kg^{-1}$ medium.

synthesis pathway. Therefore, understanding the biosynthesis of fatty acids in *M. alpina* is the key to enhancing lipid production.

Influences on the fermentation process

The parameters that influence the biotechnological production of ARA are essential to achieve high productivity and yield. The suitable conditions for an improved ARA production according to several authors are: glucose as a carbon source; yeast extract as a nitrogen source; supplementation with calcium, magnesium, manganese, iron, copper, zinc, and potassium; at 25 °C, pH 6.00, and 15 ppm of oxygen. Most literature investigating *Mortierella* cultures describe its production on a small scale. In this section, we describe the main factors that can affect the production of ARA by *M. alpina*.

Conservation of *M. alpina*

In industrial processes, it is essential that the strains maintain viability and lipid production capacity, especially the ARA yield. Most species of *M. alpina* do not sporulate in laboratory conditions. The mutant strain 1S-4u (uridine-requiring auxotrophic mutant) is an exception (Lounds, Eagles, Carter, MacKenzie, & Archer, 2007). Since 1S-4u is mostly used in industrial fermentations, its preservation is important for maintaining reproducible ARA yields. Higashiyama (2005), developed a conservation technique based on the spores produced by this mutant strain grown in Czapeck medium (with pH adjusted to 4–7). The spores are harvested with sterilized water, resulting in a stock solution adjusted to 10^6 spores mL^{-1}. Then, a 10% cryoprotectant is added, usually, glycerin (Morris, Smith, & Coulson, 1988), and the solution is stored in ultra-low temperatures (between −85 and −50 °C) for extended periods. The microorganism conserved produces ARA reliably for 5 years. The average ARA yield with inoculants produced from spore suspensions is reduced by 3%, which is better than the 7% reduction observed when refrigerated slants with mycelium are used. For short periods (<3 months), a preparation of spore/mycelia suspension from slants of PDA and Czapeck agar can be stored at 5 °C (Zhu, Yu, Liu, & Xu, 2004). Due to the poor sporulation of *M. alpina*, a mycelial suspension may be used as an inoculum in fermentation scale-up after partial mechanical disruption using a blade mill (Rocky-salimi, Hamidi-esfahani, & Abbasi, 2011).

Medium components

The strain, carbon sources, and fermentation conditions must be selected based on the fatty acid of interest. For arachidonic acid production, the most frequently

Table 2 Derivations of mutants from *M. alpina* 1S-4.

Mutant	Characteristic	Reference
△5 desaturase-defective	High dihomo-gamma-linolenic (DHGLA) and low ARA level production. Does not need an inhibitor for DHGLA production; high yields of ARA (4.1 g/L, 42% in oil). Commercial strain.	Jareonkitmongkol et al. (1993)
△12 desaturase-defective	$n-6$, $n-3$, and $n-9$ PUFAs absent from their mycelia. Produces an oil rich in mead acid. Produces ARA and eicosapentaenoic acid (EPA) when $n-6$ or $n-3$ fatty acids are added (i.e., J180, M-209-7). Used for production of EPA rich-oil with low ARA quantities.	Jareonkitmongkol et al. (1993)
Double defective in both △12 and △6	Accumulation of 20:2$n-9$ in large quantities and α-linolenic acid inhibits the conversion of oleic acid to 20:2 $n-9$ which is converted in 20:4 $n-3$.	Kamada et al. (1999), Kawashima et al. (1998)
△6 desaturase-defective	High linoleic acid synthesis and low concentration of GLA, DHGLA, and arachidonic acid. Characterized by the production of eicosadienoic acid (20:2$n-6$) and eicosatrienoic acid (20:3$n-6$ (△5)). Capable of producing methylene-interrupted $n-3$ eicosatrienoic acid (20:4$n-3$ (△5)) from α-linolenic.	Jareonkitmongkol et al. (1993)
$n-3$ desaturase-defective	Unable to synthesize $n-3$ PUFAs at low temperature (<20°). High quantities of ARA at 20 °C, but part of it is converted to EPA (i.e., Y11, Y135, Y61).	Sakuradani et al. (2004)
△9 desaturase-defective	Stearic acid is the main fatty acid produced (up to 40%). This enzyme is not completely blocked since a minimum activity is needed for cell viability (introduction of the first double bond).	Jareonkitmongkol et al. (1994)
Elongase (EL1 for the conversion of 16:0 to 18:0)-defective mutants	Produces high levels of palmitic acid (16:0) and palmitoleic acid (16:1$n-7$), with low amounts of $n-7$ and $n-4$.	Sakuradani et al. (2004)
Mutants with enhanced desaturase activities	The mutant 209-7 with the 1.4-fold of elevated △6 desaturase activity. JT-180 is another mutant with elevated △5 desaturase activity, which produces a large quantity of mead acid (49% in oil).	Sakuradani et al. (2002), Sakuradani et al. (2013)
Diacylglycerol-accumulating	The percentage of triacylglycerols is about 90% of the total lipids in the mutant *M. alpina* 1S-4. KY1 accumulates 30% of diacylglycerol and is expected to be used to produce diacylglycerols rich in C20 PUFAs.	Sakuradani et al. (2004)

used carbon source is glucose (Jang, Lin, & Yang, 2005), probably because it directly feeds the glycolytic pathway (Wynn, Hamid, Li, & Ratledge, 2001). Shinmen and Shimizu (1989), tested glucose and other carbon sources – fructose, maltose, soluble starch, and corn starch – using *M. alpina* 1S-4. These authors obtained the same amount of biomass among all carbon sources assays but higher ARA yields when glucose was used as the carbon source. Stredanská and Šajbidor (1993), also affirmed that glucose was the most suitable source of carbon for biomass and total lipid production, compared to dextrins. Nisha, Rastogi, and Venkateswaran (2011), achieved 40.41% of total PUFA using rhamnose. However, this is an expensive sugar and is possibly not feasible for use in producing ARA on an industrial scale. These authors ranked the carbon sources in the following order: rhamnose > glucose > mannose > fructose > lactose > raffinose > starch.

Koike, Jie Cai, Higashiyama, Fujikawa, and Park (2001), affirms that when glucose concentration increases from 2.0% to 12.0% in *M. alpina* production media, the fatty acid content tends to increase. However, the ARA yield shows the opposite behavior. More than 20% of glucose (w/v) in culture media increases the osmotic pressure, hindering lipid and biomass production (Totani, Someya, & Oba, 1992). Starch can be an option to use instead of high glucose concentrations. In a study conducted by Jang et al. (2005), arachidonic acid comprised 57.3–64.8% of the total PUFA, with soluble corn starch as the carbon source. Alkane hydrocarbons

such as decane and alcohols such as hexadecanol were also tested in the search for alternative carbon sources. A total of 14 g L^{-1} of biomass and 5 g L^{-1} of total fatty acids was obtained using decane (Yokochi, Kamisaka, Nakahara, & Suzuki, 1995), showing an increment of the biosynthesis of linolenic acid (47% of the total fatty acids) (Xian et al., 2001).

Nitrogen is essential for protein synthesis, while the C/N ratio affects the cellular composition. In general, low C/N ratios – i.e., nitrogen-rich media – favor the production of biomass, while high C/N ratios favor the synthesis of substances for energy reserve, such as carbohydrates and lipids. The depletion of nitrogen during cultivation is a prerequisite for fatty acid accumulation, but it is not favorable for biomass growth. Koike et al. (2001), evaluated different C/N ratios and showed that the optimum ratio was in the range of 15 to 20 (weight basis). ARA production in a medium with a C/N ratio below 15 is constant, above which increases proportionally to the C/N ratio, up to 20. Šajbidor, Dobroňová, and Čert'ik (1990), reported the same range as being optimum for ARA production in *Mortierella* sp. S-17. Packter (1981); Totani, Suzaki, and Kudo (1992); Wynn et al. (2001), affirmed that organic nitrogen sources (amino acids and peptides) are preferable for *M. alpina* growth. Yeast extract was found to be the most suitable nitrogen source for biomass, lipid, and ARA production (due to the presence of micronutrients). Organic sources of nitrogen affect lipogenesis regulation and increase acetyl-CoA carboxylase (ACC) activity. Oleaginicity depends on ACC, which synthesizes malonyl-CoA – the key building block for lipid synthesis. Malic enzyme (ME) activity also increases when organic nitrogen sources are present (Certik, Megova, & Horenitzky, 1999). After studying the use of yeast extract and soybean meal, Higashiyama, Yaguchi, Akimoto, Fujikawa, and Shimizu (1998), concluded that yeast extract is effective as a source of nitrogen. Park, Koike, Higashiyama, Fujikawa, and Okabe (1999), investigated several nitrogen sources (yeast extract, corn steep liquor, pharmamedia, fishmeal, and gluten meal) and obtained better results with yeast extract than with other sources. These authors also established a relationship between ARA production and morphology, affirming the efficacy of feather-like pellets.

Nitrogen sources also affect the mycelia morphology and ARA production of *Mortierella* (Park et al., 1999). This is important because the morphology affects oxygen transfer and nutrient diffusion due to the availability of a greater transfer area (Park, Tamura, Koike, Toriyama, & Okabe, 1997). Koike et al. (2001), noted that the C/N ratio influenced the ARA production and pellet morphology of *M. alpina*. Natural sources of nitrogen may also change the morphology of *M. alpina* in submerged fermentation. Nisha and Venkateswaran (2011), reported morphology variations using yeast extract, which resulted in fluffy circular pellets (feather-like pellets), while other nitrogen sources resulted in filamentous mycelia growth. Totani, Hyodo, and Ueda (2000), tested salts as nitrogen sources, including ammonium nitrate, sodium nitrate, ammonium acetate, and ammonium sulfate, and obtained low biomass production yield. Yeast extract at 1% (w/v) concentration was considered optimum for ARA production.

Lu, Peng, Ji, You, and Cong (2011), tested several organic and inorganic sources for ARA production by *M. alpina*, also finding that organic nitrogen (yeast extract, peptones) is generally superior to nitrate or urea for cell growth and lipid production.

Micronutrients, such as manganese, calcium, iron, copper, and zinc, are also important in the medium composition for ARA fermentation. Sajbidor, Kozelouhova, and Certik (1992). investigated the effect of selected metal ions for better ARA production in *Mortierella* sp. S-17. They found that biomass production was not affected, but ARA yields were maximized with the addition of 2 mg L^{-1} manganese. There are metal ions with positive effects on mycelial growth but negative effects in fatty acid production. Totani et al. (2000), found that manganese, calcium chloride, and iron are essential for lipid production. Kyle (1997a,1997b), concluded that iron, copper, and zinc stimulate ARA production in *M. alpina* while Nagamuna, Uzuka, and Tanaka (1985), observed the beneficial effect of calcium (100 mg L^{-1}) on lipid overproduction. The use of bivalent cations is important because of their role as a cofactor of acetyl-CoA carboxylase and other enzyme complexes that are essential in the first stage of fatty acid synthesis. Higashiyama, Yaguchi, Akimoto, Fujikawaa, and Shimizu (1998), studied the influence of minerals in morphology, showing that mycelia dispersion is possibly suppressed by an increase in the ionic strength, which induces pellet formation. Pellets with 1–2 mm of diameter gave the best results with higher ARA concentrations, compared to pellets of different sizes (e.g., 0–1 mm and 2–4 mm). This could also explain the positive effect of NaCl reported by Ho and Chen (2008).

Since arachidonic acid is a secondary metabolite, its production may be enhanced using metabolic engineering techniques. The use of precursors, inducers, and other additives may drive the metabolism toward enhanced lipid and ARA content. Several oils have been tested as main carbon sources or culture media supplements. Presumably, when the culture media is supplemented with oils, the mold produces lipases, cleaves fats into fatty acids and glycerol, and incorporates these residues as lipid structures/skeletons (Akhtar, Mirza, Nawazish, & Chughtai, 1983). This could favorably affect the production of unsaturated fatty acids. Jang et al. (2005), tested different fats and oils (linseed, sunflower, soybean, lard, peanut, and corn), and the best results, in terms of biomass production and ARA content, came from using 1% linseed oil. They also reported a positive influence of sunflower oil in DHA production. Their results showed an increment of eicosapentaenoic acid (EPA) and linoleic acid as well as a decrement in the degree of unsaturation. These fatty acids are added to long-chain fatty acids as precursors. Shinmen and Shimizu (1989), also tested different oils to increase the ARA production of *M. alpina* 1S-4. Their results showed an increase of 2.8 times when soybean oil was applied as a supplement. However, the use of oils as carbon sources, as tested by A Nisha and Venkateswaran (2011), gave unsatisfactory results: the oils were not assimilated as easily as carbohydrates. The nature of the oil and the presence of minor components could have inhibition/repression power over desaturases and elongases. For example, the presence of sesamine in sesame oil inhibits Δ5 desaturase; therefore the conversion of dihomo-gamma-linolenic (DHGLA) into ARA is blocked, resulting in higher amounts of DHGLA (Wynn & Ratledge, 2005). There is no consensus among researchers about the most suitable oil

Table 3 Additives for fatty acid production using *M. alpina* strains.

Additive	Effects	Reference
NaCl 2%	Added after 3 days of cultivation. A stimulation of diacylglycerol acyl transferase was reported, increasing total fatty acids (TFAs) and TAG content.	Ho and Chen (2008)
Octadecanol 2%	Directly transformed into oleic acid by desaturation.	Xian et al. (2001)
Ethanol	Applied after 5 days of cultivation. It increases NADPH levels, which is available for desaturase activity. Ethanol is converted directly into acetyl-CoA avoiding the glycolytic way.	Sijtsma and Anderson (2010)
Glutamate ($0.8\,g\,L^{-1}$)	Activates Acetyl-CoA carboxylase, catalyzing the formation of malonyl-CoA (the substrate for fatty acid synthesis and elongation).	Yu et al. (2003)
Soybean and linseed oil	Used by the microorganism as a backbone to the formation of ARA. The linoleic, oleic, and linolenic acids present in these oils are converted into GLA by $\triangle$6, $\triangle$5 desaturase, and EL2 to form DHGLA and finally ARA.	Ho et al. (2007)
Citrates	They are cleaved for the lipid synthesis. It is cleaved by ACL (ATP:citrate:lyase) to give acetyl-CoA (principal precursor of fatty acid synthesis).	Papanikolaou and Aggelis (2011), Ratledge and Wynn (2002)
Glycerol	It is phosphorylated, oxidized and converted into 3-P-glyceraldehyde. It enters the glycolytic pathway and results in acetyl-CoA.	Papanikolaou et al. (2008)
Octadecanol, Hexadecanol	Alcohols are considered primary intermediates in the oxidation and dissimilation of alkanes toward aldehyde-alcohol dehydrogenase (n-6-route).	Xian et al. (2002)

for producing arachidonic acid due to the variable concentrations and yields obtained using different oils. Table 3 summarizes the effects of several additives tested for *M. alpina* cultivation.

Fermentation conditions

M. alpina growth was observed from 8 to 28 °C, with an optimal range of 20–25 °C (Bajpai & Bajpai, 1992). Hansson and Dostálek (1988), evaluated several species of *Mortierella*, reporting that 25 °C was the optimal temperature for biomass production. Bajpai, Bajpai, and Ward (1991), reported a decrease in arachidonic acid and biomass production of *M. alpina* ATCC 32222 when the temperature was shifted from 25 to 28 °C, while Shimizu, Kawashima, Shinmen, Akimoto, and Yamada (1988), observed a 26% decrease in ARA production when comparing cultures from 25 to 20 °C. The fatty acid profile varies with temperature: the unsaturation level increases as the temperature decreases. In most species, the highest levels of ARA were observed at 25 °C, while EPA prevailed at lower temperatures (Hansson & Dostálek, 1988; Nisha & Venkateswaran, 2011). This may have been due to an adaptation of the PUFAs for membrane stabilization, because of the stress caused by colder conditions. However, although PUFA content increases in lower temperatures, biomass production, and ARA production are indeed highest in the range of 20° to 25 °C (Peng et al., 2010; Shimizu et al., 1988).

The pH generally shows a positive influence on the production of saturated and monounsaturated fatty acids when it is close to neutral. Nisha et al. (2011), reported an important effect of pH on biomass growth and ARA production: biomass growth ceased at pH levels outside of the range from 4.5 to 8.0. The optimum pH level for ARA production was at the range of 6–6.5 (46% of total lipids produced) (Nisha & Venkateswaran, 2011). The initial pH influences the fungal mycelial morphology, which is a critical factor for metabolite formation (Shu & Lung, 2004) that allows the capability of cell aggregation due to the production of hydrophobic proteins to coordinate the adherence of hyphae (Feofilova, 2010).

Elongation and desaturation reactions give rise to PUFAs. These are aerobic reactions, so the dissolved oxygen concentration is an important factor (Davies, Holdsworth, & Reader, 1990). At lower temperatures, the microorganism adjusts its membrane fluidity by increasing the desaturation of its lipids (Cohen, Vonshak, & Richmond, 1987). Lower temperatures may increase the unsaturation of fatty acids because of the higher availability of oxygen for desaturase enzymes (Hunter & Rose, 1972). The oxygen concentration can also be controlled by increasing the pressure or oxygen concentration of the gas fed to the reactor. Higashiyama, Yaguchi, Akimoto, Fujikawa, and Shimizu (1998), studied the effect of oxygen in air under normal atmospheric pressure, with or without oxygen enrichment. They found that 15–20 ppm of oxygen concentration is an adequate level for biomass and ARA production (1.6-fold better than at 7 ppm). A morphological change was observed using an oxygen enrichment

method from filaments to pellets (20–50 ppm), and a dramatic reduction of ARA yield was noted because of low transfer rates of oxygen into the pellets.

Industrial production

The industrial production of bioproducts must ensure high productivity and reproducibility. Therefore, multiple variables must be evaluated when scaling up from a small laboratory scale to maximize throughput and profit. Huong, Slugen, and Sajbidor (1998), compared solid and submerged cultivation at a small scale. Their solid medium was composed of milled sesame seeds and resulted in 9-fold more DHGLA than submerged cultures provide. Totani, Suzaki, & Kudo (1992), assert that submerged fermentation is preferred for producing ARA-rich oils because it provides optimal growth conditions. As seen before, many aspects of the culture can affect the microorganism development and lipid production. However, optimized conditions at the laboratory scale are not necessarily replicated in the scaling-up, requiring further adaptation. Multiliter-scale data are valuable and rare in the literature. Table 4 shows the conditions for multiliter cultivation, as retrieved from patent literature.

The downstream process, represented in Fig. 1, is similar among these patents listed in Table 4. Biomass is harvested after the fermentation process by solid-liquid separation (i.e., filtration or centrifugation). A prior pasteurization step is recommended (60–65 °C) to deactivate lipases that could degrade lipids in the final stage of fermentation, avoiding alterations in quality, appearance, odor, and taste in the final product (Ratledge & Hopkins, 2006). The filtered biomass is washed with water and is usually dried and ground. The drying process can be done using conventional air-drying or advanced methods such as with a spray dryer. After this step, the biomass cake that is formed can be pressed, loosened (by extrusion), and/or crushed. The next step is to extract the lipids, which can be done using wet or dried biomass, using organic solvents such as hexane, chloroform, ethanol, methanol, and petroleum ether. The use of wet biomass may complicate the extraction because of the three phases that may form – solids, solvent and water micelles. The solvent must be of low polarity (Ono, Aki, & Higashiyama, 2011a). The most common solvent for lipid extraction in the industry is hexane. Extraction may be improved with an extra step for the residual lipids in the biomass using a chloroform-methanol-water layer system, as in (Dueppen, Zeller, Diltz, & Driver, 2005). Although batch solvent extraction is the most common method reported, the lipids can also be obtained by less conventional methods, such as counter-current extraction of the dry biomass using commercial units such as an immersion extractor or a supercritical fluid extraction using CO_2 or N_2O (Sajbidor et al., 1992). The industrial units used for SCO extraction and recovery are relatively small, compared with those used for vegetable oils. The solvent must be evaporated from the crude oil. This crude oil resulting from the extraction step can be cloudy due to the presence of biomass particles, lecithins, and free fatty acids (FFAs). Therefore, clarification is usually done, improving the aspect of the product. Lipid oxidation during the whole process can be avoided by using an inert atmosphere, e.g., nitrogen.

There are five steps in the refining process of edible oils when using the classical alkaline method (Bijl & Wolf, 2004). These steps consist first of a degumming process (using membrane filtration and supercritical extraction, in which water is used to remove hydratable phospholipids and metals) (Yan et al., 2014). Then, 85% phosphoric acid is added in a proportion of 1.5 g of the acid solution per kg of oil to convert the remaining non-hydratable phospholipids into hydratable phospholipids (Bijl & Wolf, 2004). Third, the FFA is neutralized using a sodium hydroxide solution at 15% to wash out the soap and the remaining hydrated phospholipids (De Greyt, 2013). Next, there is a bleaching process with natural or acid-activated clay that absorbs colored components (Brooks, Berbesi, & Hodgson, 2011); another adsorbent option is silica gel (1% w/v). Finally, a deodorizing process is done at high temperatures (180–220 °C) and low pressures (2–6 mbar) to remove volatile components such as ketones and aldehydes (De Greyt, 2013).

The intracellular lipids produced by *M. alpina* can be secreted to the medium by induction using surfactants or by mutation (Fukui, 1995). Akimoto, Kawashima, and Shimizu (2011) patented a process to produce, recover, and encapsulate extracellular lipids through a process in which the biomass is separated using a centrifuge at $1500 \times g$ and washed with sterile water to prepare the lipid vesicles. These vesicles were added to powdered milk to reach an ARA concentration similar to that of breast milk.

In the patent developed by Kyle (1997a,1997b) for the formulation of the baby formula Similac®, a lipid-rich biomass was freeze-dried and then extracted using hexane in a 5:1 solvent to biomass proportion with continuous stirring for 2 h. After biomass separation, the miscella (filtrate) was evaporated to recover the oils. An additional extraction step was done with the residual biomass using ethanol in a proportion of (50:1) for 1 h, evaporating the solvent to recover it, yielding 26 g of crude oil from 100 g of biomass with an ARA concentration of 30–35%. This oil was added to the formula dropwise (1 g L^{-1}).

Sodium ARA salt is another product form of *M. alpina* oil that is approved for infant formula supplementation. This salt is produced by Jost Chemical Company and is presented as a powder. The product received FDA-GRAS status in 2014; it contains a minimum of 15% of ARA, which is a lower concentration than those of known commercial oils available in the market (usually >40% of ARA, such as ARASCO, SUNTGA, and RAO). The manufacturing process of Jost Sodium ARA consists of three phases: first, oil that is rich in ARA is saponified into FFA using water and sodium hydroxide at 50–80 °C in a nitrogen atmosphere. Diluted sulfuric acid is added to recover the FFAs into an organic phase; the aqueous phase carries the residual glycerol. Second, FFA is converted back into sodium salts by the addition of sodium hydroxide and finally, other ingredients are added to create a commercial blend (antioxidants, phosphates, maltodextrins, ascorbates, citrates, and caseinates) (Vandamme & Revuelta, 2016), which is dried. In the case of baby and geriatric milk formulas, a pool of vitamins is added to preserve the final product; some of the vitamins also work as a stabilizer and antioxidant agents that protect the ARA structure from degradation. In the case of ARA, when it is not just a

Table 4 Compilations of industrial patents developed for pilot-scale production of ARA.

Strain	Company	Culture Medium	Conditions	P_{ARA} ($g L^{-1} day^{-1}$)	ARA ($g L^{-1}$)	Reference
M. alpina IFO 8568	Lion Corporation	Potato extract 1 $kg L^{-1}$; glucose 30 $g L^{-1}$	20 days; 25 °C; pH 6.0; batch	0.29	5.7[a]	Totani, Suzaki, & Kudo (1992)
M. alpina 200012201-12-2-2	Wuan Polytechnic University	Glucose 80 $g L^{-1}$; corn flour 20 $g L^{-1}$; yeast extract 2 $g L^{-1}$; peptone 1 $g L^{-1}$; KH_2PO_4 1 $g L^{-1}$; $MgSO_4$ 0.1 $g L^{-1}$; cottonseed oil 10 $g L^{-1}$	6 days; 28 °C, pH 6.5; 50 rpm, 1 vvm; 50 L; batch	2.52	15.1	Dongping et al. (2014)
M. alpina LU166	Xiamen University (UYXI-C)	Glucose 50 $g L^{-1}$; yeast extract 8 $g L^{-1}$; corn meal 3 $g L^{-1}$; KH_2PO_4 2 $g L^{-1}$; glutamic acid 1 $g L^{-1}$; $MgSO_4.7H_2O$ 0.1 $g L^{-1}$; $ZnSO_4.7H_2O$ 2 $g L^{-1}$; $CaCO_3$ 0.05 $g L^{-1}$; trace elements 1 $mL L^{-1}$; malic acid 0.0025%	5 days; 28° C; 150 rpm; pH 6; batch	1.24	6.2	Ling et al. (2017)
M. alpina ATCC42430	Martek Corporation Suntory Holdings Limited	Dextrose 80 $g L^{-1}$, soy flour 16 $g L^{-1}$; $FeCl_3.6H_2O$ 30 $mg L^{-1}$; $ZnSO_4.7H_2O$ 1.5 $mg L^{-1}$; $CuSO_4.5H_2O$ 0.1 $mg L^{-1}$; biotin 1 $mg L^{-1}$; thiamine 2 $mg L^{-1}$; pantothenic acid 2 $mg L^{-1}$	10 days; 28 °C; pH > 7.3; 0.5 vvm; 11 psi; 3 $m s^{-1}$ agitation; 20 L; fed batch	0.53	5.29	Kyle (1997a,b)
M. alpina CBS 754.68		Glucose 2%; soybean oil 0.1%; soybean protein 1.5%	8 days; 24 °C, pH 6.0; 200 rpm; 1.0 vvm; 200 kPa; 50 L; fed batch	0.92	7.32	Higashiyama et al. (2005)
		Soybean powder 4%; soybean oil 0.1%, KH_2PO_4 0.3%; Na_2SO_4 0.1%; $CaCl_2.2H_2O$ 0.05%; $MgCl_2.6H_2O$ 0.05%; saccharified starch 30%	10 days, 25 °C, pH 6, 160 rpm; 50 L; fed batch	0.43	4.3	Ono et al. (2011b)
		Glucose 2%; soybean oil 0.1%; soybean protein 1.5%; KH_2PO_4 0.3%; $MgCl_2.6H_2O$ 0.05%; Na_2SO_4 0.1%; $CaCl_2.2H_2O$ 0.05%	8 days; 24 °C, pH 6.0; 200 rpm; 1.0 vvm; 200 kPa; 50 L; fed batch	1.13	9.04	Higashiyama et al. (2005)
M. alpina SAM 2241		Glucose 1.0%; KH_2PO_4 0.3%; $MgSO_4.7H_2O$ 0.02%; polypeptone 1.5%; NaCl 0.2%; yeast extract 0.1%	10 days; 24 °C; pH 5.0; 1.0 vvm; 10 L; fed batch	0.64	6.4	Akimoto et al. (2011)
M. alpina SAM 2197		Glucose 1.8%; soybean powder 4%; soybean oil 0.1%; KH_2PO_4 0.3%; Na_2SO_4 0.1%; $CaCl_2.2H_2O$ 0.05%; $MgCl_2.6H_2O$ 0.05%	10 days; 24 °C; 100 rpm; 50 L; fed batch	1.35	13.5	Ono et al. (2011a)
M. alpina 1 S-4		Glucose 2%; soybean powder 3.1%; glycerol 0.02%; soybean oil 0.1%; K_2HPO_4 0.3%; $MgSO_4.7H_2O$ 0.06%; $CaSO_4.2H_2O$ 0.06%	8 days; 26 °C; 1 vvm; 300 rpm, pH 6.3; 50 L; fed batch	0.90	7.17	Katano and Kawashima (2015)
Mutant strain	Jiabi Yousheng Bioengineering W. Co.L.	Sucrose 35 $g L^{-1}$; yeast extract 12 $g L^{-1}$	8 days; 28 °C; pH 7.0; 220 rpm, 1 vvm; 50 L; fed batch	2.81	22.5	Zhiming et al. (2014)

[a] $g kg^{-1}$.

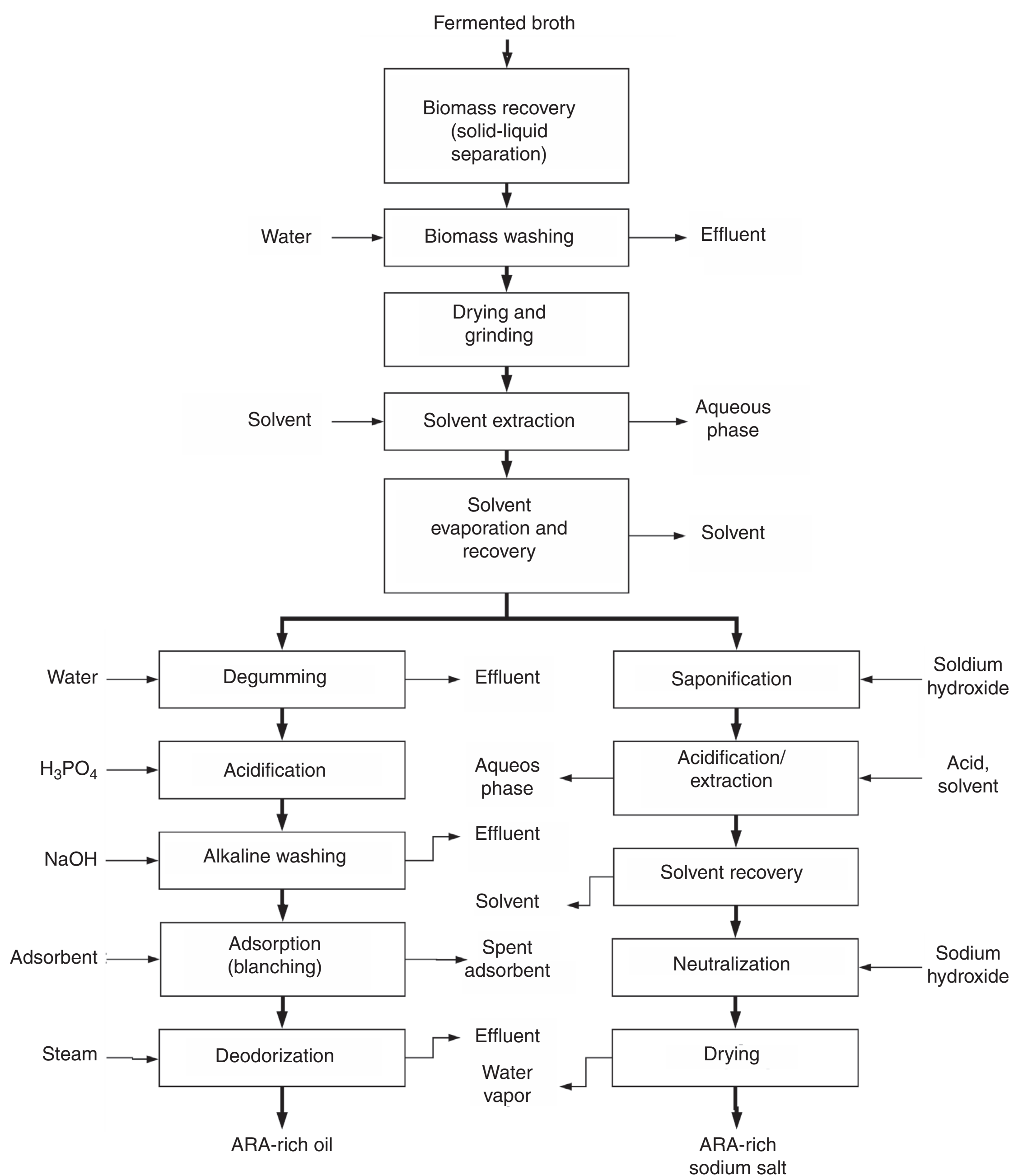

Figure 1 The process of ARA-rich oil production of *M. alpina* (for liquid and solid presentations).

complement of a formulation (i.e., capsules of the microbial oil), 500 ppm of vitamin E is added (Katano & Kawashima, 2015).

The presentation of the product, in Fig. 1 (i.e., oil SUNTGA40S and sodium salts of *M. alpina* oil), defines some of the products' physicochemical characteristics, such as solubility and final ARA concentration. The compositional fatty acid concentrations vary between the commercial oils (ARASCO®, SUNTGA 40S and RAO) and salts (sodium ARA), but the PUFA content of these commercial products is always superior to human milk and fish oil, which are both common sources of ARA (Table 5).

Arachidonic acid can be purified from the oil of *M. alpina* if required; several techniques with potential in industrial purification are available to do so, such as a selective esterification using microbial lipases (Yamauchi, Nagao, & Watanabe, 2005) consisting of three steps: (a) a non-selective hydrolysis of the oil using lipase from *Alcaligenes* sp. (1200 U g^{-1}) mixed with water (67%) at 40 °C for 48 h at 500 rpm under nitrogen atmosphere. After the reaction, the FFAs are extracted with n-hexane; (b) dissolving the FFA from the previous step in a solution of methanol, water, and urea, for elimination of long-chain, saturated fatty acids by urea adduct fractionation, and (c) a selective esterification of the FFA with lauryl alcohol (Shimada, Sugihara, Nakano, & Kuramoto, 1997) (1:2 mol mol^{-1}), catalyzed by a lipase from *Burkholderia cepacia* (20 units U g^{-1} of mixture of lipase-PS) at 30 °C for 16 h at 500 rpm.

Table 5 Fatty acids compositional profile and specifications of commercial ARA oils (from GRAS-certified *M. alpina*) and conventional sources.

Fatty acid	Sodium ARA (sodium salt of the fatty acids)	ARASCO (GRN 41 and GRN 80)	AA-rich oil SUNTGA 40S (GRN94)	RAO (GRN 326)	Anchovy and sardine oil[a]	Human milk[b]%w/w (Germany)
Arachidonic acid (%)	Minimum 15	42.69–44.26	38.60–42.40	41.15–45.55	2.00	0.35
Myristic acid (%)	0.10–0.50	0.34–0.58	0.40–0.60	0.23–0.35	7.00	8.75
Palmitic acid (%)	4.30–8.10	7.17–9.59	11.30–15.10	5.43–6.87	16.00	24.06
Palmitoleic acid (%)	0.00–0.40	–	–	0.02–0.04	8.00	0.50
Stearic acid (%)	4.20–7.60	7.70–10.20	7.50–8.90	4.48–5.98	3.00	0.63
Oleic acid (%)	3.40–9.50	15.95–23.35	6.20–6.70	4.33–5.76	10.00	29.95
Linoleic acid (%)	3.80–15.20	5.56–7.62	–	6.92–8.78	1.00	5.59
γ-Linolenic acid (%)	1.70–2.70	2.45–2.99	1.35–2.70	–	1.00	0.55
Arachidic acid (%)	0.60–1.00	0.83–0.96	0.70–0.80	0.69–0.80	<1.00	0.25
Homo-gamma-linolenic acid (%)	3.00–5.00	1.43–2.57	3.10–3.40	3.61–3.77	<1.00	0.19
Behenic acid (%)	2.50–4.10	1.98–2.02	2.20–3.00	3.00–3.24	<1.00	0.07
Lignoceric acid (%)	7.80–12.60	1.85–2.04	5.10–9.10	9.37–10.50	<1.00	Trace
Trans-fatty acids (%)	≤0.50	<1.00	–	0.04–0.51	–	–
Free fatty acids (%)	≤0.45	0.10–0.27	0.02–0.06	0.03–0.09	–	–
Solubility	Water soluble	Insoluble	Insoluble	Insoluble	Insoluble	–
Peroxide value (meq kg^{-1})	≤10.00	<5.00	≤5.00	≤2.00	≤2.00	–
Anisidine value (AV)	≤30.00	–	≤20.00	≤20.00	≤20.00	–
Free fatty acids (% oleic acid)	–	<0.40	≤0.20%	≤0.2%	–	–
Unsaponifiable matter (w/w %LOD = 0.3)	–	≤3.00	≤1.00	≤3.00	≤3.00	–
Lead (ppm)	≤0.10	≤0.10	≤0.10	≤0.10	≤0.10	–
Arsenic (ppm)	≤0.10	≤0.10	≤0.20	≤0.10	≤0.10	–
Cadmium (ppm)	≤0.10	≤0.20	≤0.10	≤0.10	≤0.10	–
Mercury (ppm)	≤0.05	≤0.20	≤0.10	≤0.05	≤0.10	–
Microbiological	Total plate count: <1000 cfu g^{-1}	Total plate count: <250 cfu mL^{-1}	Bacteria ≤10 cell g^{-1}	Total plate count: <10 cfu g^{-1}	–	–

[a] Srigley and Rader (2014).
[b] Koletzko et al. (1988).

Vali, Sheng, and Ju (2003), proposed a technique using commercial oil from *M. alpina* that also follows three steps: (i) first, commercial oil ARASCO® was saponified and fractionated by low-temperature solvent crystallization; (ii) then, a selective esterification was done with LA (2:1 $mol_{FA}\, mol_{LA}^{-1}$) catalyzed by a lipase from *Candida rugosa*, at 50 °C for 24 h and 400 rpm of agitation; and (iii) finally, a solvent extraction was done using acetonitrile. The use of high-performance liquid chromatography (HPLC) was included in a methodology developed by Yuan, Wang, and Yu (2007), which combined the use of a C_{18} preparative column (methanol and water as mobile phases) after a urea-inclusion step performed in the FFA (optimum conditions: ratio of FFA/urea/methanol was 1:2:8 wt/wt, urea inclusion reaction was −10 °C) for ARA purification. The purity of the ARA reached when using these three methodologies were 97%, 95.5%, and 99%, respectively.

Arachidonic acid market overview

Single-cell oils are highly valued products. The price of the oil can vary from \$65 kg^{-1} to over \$500 kg^{-1} or even more if it is composed of pure, specific, fatty acids (Gunstone,

2001). Because of the promise of sustainable and efficient production of SCO, the interest in research and development for the technology had significant contributions in the last 30 years (Ratledge, 2001). These fatty acids are currently in demand as dietary supplements for adults (in geriatric and bodybuilder supplements) and infants (baby milk formulas). According to Forsyth, Gautier, and Salem Jr. (2016), in low income regions in Africa, Asia, Caribbean and Central America and South America there is an intake significantly lower (64 mg/day) of ARA than current recommendation (102–258 mg/day) for young children (aged 6–36 months).

DHA and ARA-rich oils from microorganisms are gradually substituting fish oils in infant formulas. This substitution is beneficial because (1) reduces the risk of extinction for different fish species, and (2) microbial oils are intrinsically safer; toxic substances such as dioxins and heavy metals, that may bioaccumulate in fish (Vadivelan & Venkateswaran, 2014), are absent in microbial oils. Some of the most common oils produced are oils that are rich in arachidonic acid (ARASCO™) and docosahexaenoic acid (DHASCO™) (Wynn & Ratledge, 2005). This mixture is commercialized in Europe, Asia, Australia, and North America as an ingredient of infant formulas. The FDA gave the GRAS status to DHA/ARA-SCO in May 2001 for its use in formulas in the USA. An important increase (over 50%) of formulas in the USA has happened since the first fortified formula was sold in February of 2002. Over 95% of global production is destined for formula use; in 2006, the production did not meet demand.

The first companies to produce ARA by fermentation were Gist-brocades Co. (now DSM) and Suntory Ltd., while DSM had the exclusive rights to the ARA-rich oil produced by Martek Inc. (Ratledge & Hopkins, 2006). The ratio of the commercialized mixture is two parts of ARA to one of DHA (2:1 v/v). Until 2011, Martek Biosciences earned $450 million of oil sales annually before acquiring DSM acquisition (Ratledge & Hopkins, 2006). Ratledge, in 2103, estimated a production of 6000 tons of ARA-SCO (Ratledge, 2013). In 2003, the total production of ARA was around 560 tons, which was mostly used in infant formulas, much higher than the production in the period of 1985–2002 (690 tons total). At that time, 80% was used in formulas and the rest was used in other dietary supplements. Another player in this market is Suntory Ltd., which sells only the ARA-rich oil named SUNTGA40S in Japan. Since 2010, Cargill Inc. has become a rival of these two companies.

In 2006, the global production of infant milk formula was 1.8 million tons, including about 400,000 tons of oil and fat ingredients containing ARA and DHA (Euromonitor, 2006). By 2008, sales of supplemented milk formulas increased considerably, reaching 98% of the sales – only 2% of the formulas were unsupplemented (Oliveira & Smallwood, 2011). According to the Global Arachidonic Acid Industry Report of 2015 (QY Research Center, 2015), the price of ARA has decreased by 0.89% since 2010. The global arachidonic acid market is projected to reach $1.8 billion by 2019, and the study predicts a big dynamic market growth in Asia (GostReports, 2016).

The demand of ARA might reach 1 million tons per year by 2025, based on the daily intake (reported by Forsyth et al., 2016), and the growth rate of each country. (United Nations, Department of Economic and Social Affairs – Population Division – World Population Prospects, 2017) (Fig. 2B). A considerably lower projection for production – 410 thousand tons in 2025 – can be made by extrapolating data from (QY Research Center, 2015), which estimated the global production of ARA as 284 thousand tons in 2020, with a growth rate of 10%.

The market demand or deficit can also be based on nutritional recommendations. The minimum daily intake recommendation for ARA is 150 mg/day per capita (Forsyth, Gautier, & Salem, 2017). Considering this requirement, a global deficit of 170 thousand tons of ARA will exist on top of the projected demand (Fig. 2A), totaling 1.17 million tons/year of ARA needed by 2025, between ARA food sources and supplementation. Therefore, the global production will not meet the demand of ARA for 2025 without an increment of 35% in global production.

The use of traditional sources of ARA has decreased since the adoption of biotechnology for production using microbial resources. It is expected that the future production of ARA and other PUFAs will remain microbial-based, due to the process feasibility and eco-friendliness.

Current and future developments

According to the World Intellectual Property Organization (WIPO), there are 277 patents under the *Mortierella* + arachidon* research key-words and more specifically 53 patents under the *M. alpina* + arachidon* + oil research key-words, the main topic of this review. This last group is divided mostly in the research of the fatty acids and formulation of food and beverage fields (Fig. 3), according to the IP code classification. The principal market players of this innovation are shown in the Figure. Due to the importance and potential of *Mortierella*, many studies have been elaborated since their first laboratory tests in 1978, approximately 6993 studies are found when a research in the Derwent database is performed using *Mortierella* as keyword, in which 77% are patents and 23% are research articles among others.

The production of patents until today (Fig. 4) showed a non-uniform pattern in the last 30 years and it is a fact that the production remains a lucrative business.

Thus, further studies and/or improvements – besides genetic and molecular techniques – can be performed at different steps of the production pipeline, i.e., in the fermentation step, the improvement of media culture and conditions using cheap alternative substrates and the development of suitable morphology and oxygen transfer technologies. In the biomass production step, carbon sources, operational modes, and oxygen transfer can be improved. Although *M. alpina* prefers glucose, more complex carbon sources such as molasses could be used after a pretreatment of hydrolysis. The morphology of biomass in the reactor is linked to agitation, inoculation rate and type of inoculum, and further studies must be done to ensure that small pellets are produced, enhancing oxygen transfer to the cells.

In the extraction step, the use of solvents is accepted by industry, but there is space for enhancing the yield. Mechanical pre-treatment of the biomass could boost the extraction efficiency, requiring less solvent, and resulting in an eco-friendly process. There are plenty of

Figure 2 Global projection of ARA demand by 2025 expressed in tons of ARA per country, per year. (A) In red-orange scale, ARA-deficient countries[a] and (B) In blue scale, demand of ARA per country.[a] Projection based on the minimum daily recommendation (150 mg/day per capita).

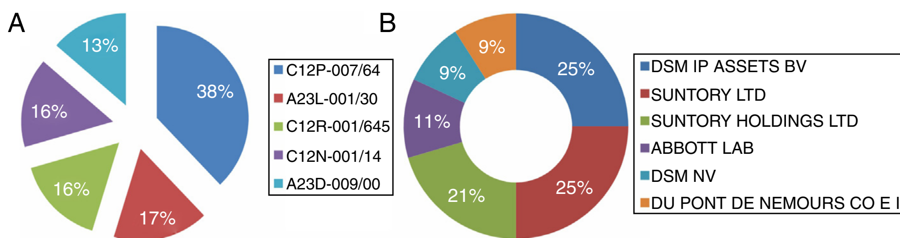

Figure 3 Chart of published patents. (A) codified patents according to the IP classification and (B) principal market players of innovation.

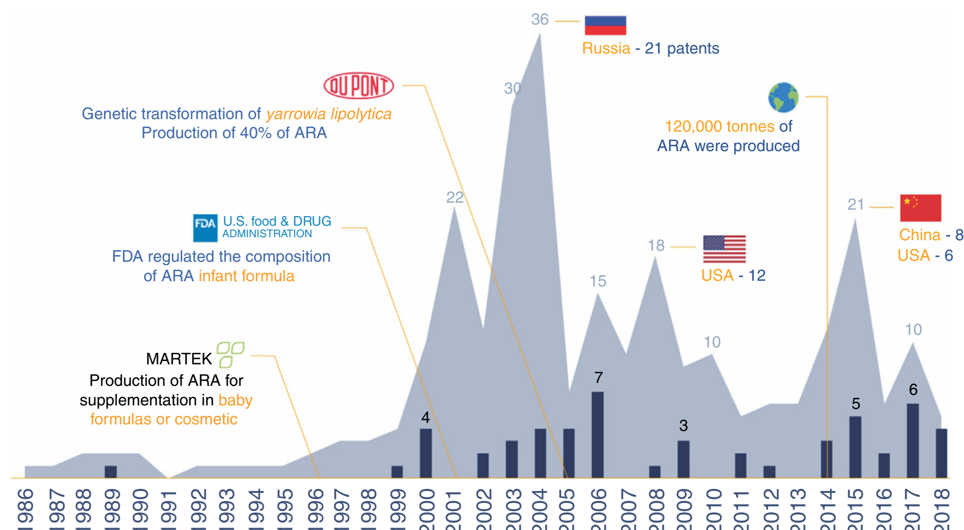

Figure 4 Time evolution in patent publications. In Bars, a number of patents found at the Derwent database under the search terms ''*Mortierella alpina* + *arachidon** + *oil*''; in area, a number of patents found at the Derwent database under the search terms ''*Mortierella* + *arachidon**''.

mechanical disruption pre-treatment options of the biomass, such as ultrasound, electric pulses, electroporation, sonication, among others, although some may not yet be cost-effective. As for the use of solvents, supercritical fluid extraction (SFE) and humid extraction can prove promising. SFE using solvents such as CO_2 is efficient and eco-friendly because the solvent is non-toxic. Despite SFE being uncommon for large-scale oil extraction, the price and quality of the resulting oil may justify its use of SFE. Humid extraction, on the other side, uses common solvents but dispenses the biomass drying step. It is a more complex extraction because three phases (water, solids, and solvent) may form, and additional studies must be performed to check the extraction yield and stability of PUFAs in this extraction. In case of incomplete disruption, additional alternatives could be applied such as the use of enzymes (e.g. lysozymes, snailase, proteases) to achieve a successful extraction.

In the oil refining step, the use of antioxidants and modified atmospheres is advised right after the fermentation step, to prevent the oxidation of PUFA. However, the oxidation and cleavage products of PUFAs may give undesirable organoleptic characteristics to the oil. This can be reduced through distillation and adsorption treatments and be avoided by careful formulation. New salts or microencapsulated forms of ARA-rich oil can have promising uses in the food and nutraceutical markets. Even the raw biomass, if properly formulated, may find use in human nutrition. Finally, the residual biomass from the extraction process can be used for animal feed, due to its nutritional content of proteins and remaining fatty acids — another possibility of product to be investigated.

Conflicts of interest

The authors declare that they have no competing interests.

Acknowledgments

This research was partly funded by CNPq, the Brazilian National Council for Scientific and Technological Development, grant number 407543/2013-0, and CAPES, the Coordination of Improvement of Higher Education Personnel - PROEX program.

References

Akhtar, M. W., Mirza, A. Q., Nawazish, M. N., & Chughtai, M. I. D. (1983). Effect of triglycerides on the production of lipids and lipase by *Mucor hiemalis*. *Canadian Journal of Microbiology*, *29*(6), 664–669.

Akimoto, K., Kawashima, H., & Shimizu, S. (2011). Microorganisms that extracellularly secrete lipids and methods of producing lipid and lipid vesicles encapsulating lipids using said microorganisms. U.S. Patent No. 7,972,855. 05 July 2011.

Amano, H., Shinmen, Y., Akimoto, K., Kawashima, H., Amachi, T., Shimizu, S., & Yamada, H. (1992). Chemotaxonomic significance of fatty acid composition in the genus *Mortierella*. *Mycotaxon*, *45*, 257–265.

Bajpai, P., & Bajpai, P. K. (1992). Arachidonic acid production by microorganisms. *Biotechnology and Applied Biochemistry*, *15*(1), 1–10.

Bajpai, P., Bajpai, P. K., & Ward, O. P. (1991). Eicosapentaenoic acid (EPA) production by *Mortierella alpina* ATCC 32222. *Applied Biochemistry and Biotechnology*, *31*, 267–272.

Bijl, H. L., & Wolf, J. H. (2004). Purifying crude PUFA oils. U.S Patent No. 13/295,869. 17 May 2004.

Brooks, D. D., Berbesi, R., & Hodgson, A. S. (2011). *Optimization of bleaching process*.. http://lipidlibrary.aocs.org/OilsFats/content.cfm?ItemNumber=40321 28.03.16

Certik, M., & Shimizu, S. (1999). Biosynthesis and regulation of microbial polyunsaturated fatty acid production. *Journal of Bioscience and Bioengineering*, *87*(1), 1–14.

Certik, M., Megova, J., & Horenitzky, R. (1999). Effect of nitrogen sources on the activities of lipogenic enzymes in oleaginous fungus *Cunninghamella echinulata*. *The*

Journal of General and Applied Microbiology, *45*(6), 289–293.

Cohen, Z., Vonshak, A., & Richmond, A. (1987). Fatty acid composition of Spirulina strains grown under various environmental conditions. *Phytochemistry*, *26*(8), 2255–2258.

Davies, R. J., Holdsworth, J. E., & Reader, S. L. (1990). *The effect of low oxygen uptake rate on the fatty acid profile of the oleaginous yeast Apiotrichum curvatum*.

De Greyt, W. (2013). Edible oil refining: Current and future technologies. In W. Hamm, R. J. Hamilton, & G. Calliauw (Eds.), *Edible oil processing* (2nd Ed., pp. 127–152). Chichester, UK: John Wiley & Sons, Ltd.

Dongping, E., Kai, C., Tao, C., & Hua, T. (2014). *Mortierella alpina* mutant strain capable of producing high-yield arachidonic acid, and fermentation method and application thereof. Patent Application CN103484385 A. 01 January 2014.

Dueppen, D. G., Zeller, S. G., Diltz, S. I., & Driver, R. H. (2005). Extraction and winterization of lipids from oilseed and microbial sources. Patent application U.S. Patent 7,695,626. 01 August 2005.

Eroshin, V. K., Satroutdinov, A. D., Dedyukhina, E. G., & Chistyakova, T. I. (2000). Arachidonic acid production by *Mortierella alpina* with growth-coupled lipid synthesis. *Process Biochemistry*, *35*(10), 1171–1175.

Euromonitor, a market research provider. (2006). *Infant milk formula industry*.. http://www.euromonitor.com/global-baby-food-overview-key-categories-countries-and-trends/report 24.02.16

Feofilova, E. P. (2010). The fungal cell Wall: modern concepts of its composition. *Microbiology*, *79*(6), 711–720.

Fischer, L., Stressler, T., Eisele, T., Rost, J., Haunschild, E., & Kuhn, A. (2013). Production of polyunsaturated fatty acids by *Mortierella alpina* using submerse and solid state fermentation. *Chemie Ingenieur Technik*, *85*(3), 318–322.

Forsyth, J. S., Willatts, P., Agostoni, C., Bissenden, J., Casaer, P., & Boehm, G. (2003). Long chain polyunsaturated fatty acid supplementation in infant formula and blood pressure in later childhood: follow up of a randomised controlled trial. *British Medical Journal*, *326*(7396), 953.

Forsyth, S., Gautier, S., & Salem, N., Jr. (2016). Global estimates of dietary intake of docosahexaenoic acid and arachidonic acid in developing and developed countries. *Annals of Nutrition and Metabolism*, *68*(4), 258–267.

Forsyth, S., Gautier, S., & Salem, N., Jr. (2017). Dietary intakes of arachidonic acid and docosahexaenoic acid in early life – with a special focus on complementary feeding in developing countries. *Annals of Nutrition and Metabolism*, *70*(3), 217–227.

Fukui, S. (1995). Selection and breeding of microorganisms secreting and producing fuel lipids. *Bio Industry*, *12*(3), 36–46.

GostReports. (2016). *Global arachidonic acid market research report 2016*.. http://www.gosreports.com/global-arachidonic-acid-market-worth-1-18-billion-by-2019/ 05.09.16

Gunstone, F. D. (2001). Oilseed crops with modified fatty acid composition. *Journal of Oleo Science*, *50*(5), 269–279.

Hansson, L., & Dostálek, M. (1988). Effect of culture conditions on mycelial growth and production of γ-linolenic acid by the fungus *Mortierella ramanniana*. *Applied Microbiology and Biotechnology*, *28*(3), 240–246.

Higashiyama, K. (2005). Method for polyunsaturated fatty acid production using novel cell preservation technique Patent application WO2006016702 A1). 16 February 2005.

Higashiyama, K., Yaguchi, T., Akimoto, K., Fujikawa, S., & Shimizu, S. (1998). Enhancement of arachidonic acid production by *Mortierella alpina* 1S-4. *Journal of the American Oil Chemists' Society*, *75*(11), 1501–1505.

Higashiyama, K., Yaguchi, T., Akimoto, K., Fujikawaa, S., & Shimizu, S. (1998). Effects of mineral addition on the growth morphology of and arachidonic acid production by *Mortierella alpina* 1S-4. *Journal of the American Oil Chemists' Society*, *75*(12), 1815–1819.

Higashiyama, K., Fujikawa, S., Park, E. Y., & Shimizu, S. (2002). Production of arachidonic acid by *Mortierella* fungi. *Biotechnology and Bioprocess Engineering*, *7*(5), 252–262.

Higashiyama, K., Yaguchi, T., Akimoto, K., & Shimizu, S. (2005). Media for culturing microorganisms and process for producing unsaturated fatty acids or lipids containing the same Patent application U.S. Patent No. 6,746,857. 08 June 2005.

Ho, S.-Y., & Chen, F. (2008). Lipid characterization of *Mortierella alpina* grown at different NaCl concentrations. *Journal of Agricultural and Food Chemistry*, *56*(17), 7903–7909.

Ho, S.-Y., Jiang, Y., & Chen, F. (2007). Polyunsaturated fatty acids (PUFAs) content of the fungus *Mortierella alpina* isolated from soil. *Journal of Agricultural and Food Chemistry*, *55*, 3960–3966.

Hoffman, D. R., Birch, E. E., Birch, D. G., Uauy, R., Castañeda, Y. S., Lapus, M. G., & Wheaton, D. H. (2000). Impact of early dietary intake and blood lipid composition of long-chain polyunsaturated fatty acids on later visual development. *Journal of Pediatric Gastroenterology and Nutrition*, *31*(5.).

Hunter, K., & Rose, A. H. (1972). Lipid composition of *Saccharomyces cerevisiae* as influenced by growth temperature. *Biochimica et Biophysica Acta (BBA) Lipids and Lipid Metabolism*, *260*(4), 639–653.

Huong, L. M., Slugen, D., & Sajbidor, J. (1998). Production of dihomo-y-linolenic acid by *Mortierella alpina*. *Biologia Bratislava*, *53*(6), 801–806.

Jang, H.-D., Lin, Y.-Y., & Yang, S.-S. (2005). Effect of culture media and conditions on polyunsaturated fatty acids production by *Mortierella alpina*. *Bioresource Technology*, *96*(15), 1633–1644.

Jareonkitmongkol, S., Kawashima, H., Shimizu, S., & Yamada, H. (1992). Production of 5,8,11-cis-Eicosatrienoic acid by a △12-desaturase defective mutant of *Mortierella alpina* 1S-4. *Journal of the American Oil Chemists' Society*, *69*(9), 939–944.

Jareonkitmongkol, S., Kawashima, H., & Shimizu, S. (1993). Inhibitory effects of lignan compounds on the formation of arachidonic acid in a delta-5-desaturase-defective mutant of *Mortierella alpina* 1s-4. *Journal of Fermentation and Bioengineering*, *76*(5), 406–407.

Jareonkitmongkol, S., Sakuradani, E., & Shimizu, S. (1994). Isolation and characterization of an omega-3-desaturation-defective mutant of an arachidonic acid-producing fungus *Mortierella alpina* 1s-4. *Archives of Microbiology*, *161*(4), 316–319.

Ji, X.-J., Zhang, A.-H., Nie, Z.-K., Wu, W.-J., Ren, L.-J., & Huang, H. (2014). Efficient arachidonic acid-rich oil production by *Mortierella alpina* through a repeated fed-batch fermentation strategy. *Bioresource Technology*, *170*(5), 356–360.

Jin, M.-J., Huang, H., Xiao, A.-H., Zhang, K., Liu, X., Li, S., & Peng, C. (2008). A novel two-step fermentation process for improved arachidonic acid production by *Mortierella alpina*. *Biotechnology Letters*, *30*(6), 1087–1091.

Kamada, N., Kawashima, H., Sakuradani, E., Akimoto, K., Ogawa, U., & Shimizu, S. (1999). Production of 8 11-cis-eicosadienoic acid by a delta 5 and delta 12 desaturase-defective mutant derived from the arachidonic acid-producing fungus *Mortierella alpina* 1S-4. *Journal of the American Oil Chemists Society*, *76*(11), 1269–1274.

Katano, K., & Kawashima, H. (2015). Methods for producing polyunsaturated fatty acid and lipid containing polyunsaturated fatty acid. Patent application US20150064749 A1. 05 March 2015.

Kawashima, H., Sakuradani, E., Kamada, N., Akimoto, K., Konishi, K., Ogawa, J., & Shimizu, S. (1998). Production of 8 11,14,17-cis-eicosatetraenoic acid (20:4ω-3) by a △5 and △12 desaturase-defective mutant of an arachidonic acid-producing fungus *Mortierella alpina* 1S-4. *Journal of the American Oil Chemists' Society*, *75*(11), 1495–1500.

Kikukawa, H., Sakuradani, E., Ando, A., Okuda, T., Shimizu, S., & Ogawa, J. (2016). Microbial production of dihomo-γ-linolenic

acid by △5-desaturase gene-disruptants of *Mortierella alpina* 1S-4. *Journal of Bioscience and Bioengineering*, *122*(1), 22–26.

Kikukawa, H., Sakuradani, E., Ando, A., Shimizu, S., & Ogawa, J. (2018). Arachidonic acid production by the oleaginous fungus *Mortierella alpina* 1S-4: A review. *Journal of Advanced Research*, *11*, 15–22.

Koike, Y., Jie Cai, H., Higashiyama, K., Fujikawa, S., & Park, E. Y. (2001). Effect of consumed carbon to nitrogen ratio of mycelial morphology and arachidonic acid production in cultures of *Mortierella alpina*. *Journal of Bioscience and Bioengineering*, *91*(4), 382–389.

Koletzko, B., Mrotzek, M., & Bremer, H. J. (1988). Fatty acid composition of mature human milk in Germany. *The American Journal of Clinical Nutrition*, *47*(6), 954–959.

Kyle, D. J. (1997). Arachidonic acid and methods for the production and use thereof. Patent application No. 5658767. 19 August 1997.

Kyle, D. J. (1997). Plant extract; vegetable oil; infant's formula. Patent application U.S. Patent 5,658,767. 19 August 1997.

Kyle, D. J. (2001). The large-scale production and use of a single-cell oil highly enriched in docosahexaenoic acid. In F. Shahidi, & J. W. Finley (Eds.), *Omega-3 fatty acids* (pp. 92–107). Washington, DC: ACS symposium series.

Li, Z.-Y., Lu, Y., Yadwad, V. B., & Ward, O. P. (1995). Process for production of arachidonic acid concentrate by a strain of *Mortierella alpina*. *The Canadian Journal of Chemical Engineering*, *73*(1), 135–139.

Lindberg, A. M., & Molin, G. (1993). Effect of temperature and glucose supply on the production of polyunsaturated fatty acids by the fungus *Mortierella alpina* CBS 343.66 in fermentor cultures. *Applied Microbiology and Biotechnology*, *39*(4–5), 450–455.

Ling X, Liu X, Yao C, Chen C, P. X. (2017). New *Mortierella alpina* LU166 for producing arachidonic acid, is preserver in General Microbiology Center of China Microbial Culture Collection Committee. Patent application CN106244468-A. 21 December 2017.

Lounds, C., Eagles, J., Carter, A. T., MacKenzie, D. A., & Archer, D. B. (2007). Spore germination in *Mortierella alpina* is associated with a transient depletion of arachidonic acid and induction of fatty acid desaturase gene expression. *Archives of Microbiology*, *188*(4), 299–305.

Lu, J., Peng, C., Ji, X., You, J., & Cong, L. (2011). Fermentation characteristics of *Mortierella alpina* in response to different nitrogen sources. *Applied Biochemistry*, (5), 979–990.

Michaelson, L. V., Lazarus, C. M., Griffiths, G., Napier, J. A., & Stobart, A. K. (1998). Isolation of a ⌬ 5-fatty acid desaturase gene from *Mortierella alpina*. *Journal of Biological Chemistry*, *273*(30), 19055–19059.

Morris, G. J., Smith, D., & Coulson, G. E. (1988). A comparative study of the changes in the morphology of hyphae during freezing and viability upon thawing for twenty species of fungi. *Microbiology*, *134*(11), 2897–2906.

Nagamuna, T., Uzuka, Y., & Tanaka, K. (1985). Physiological factors affecting total cell number and lipid content of the yeast *Lipomyces starkeyi*. *The Journal of General and Applied Microbiology*, *31*(1), 29–37.

Nisha, A., & Venkateswaran, G. (2011). Effect of culture variables on mycelial arachidonic acid production by *Mortierella alpina*. *Food and Bioprocess Technology*, *4*(2), 232–240.

Nisha, A., Rastogi, N. K., & Venkateswaran, G. (2011). Optimization of media components for enhanced arachidonic acid production by *Mortierella alpina* under submerged cultivation. *Biotechnology and Bioprocess Engineering*, *16*(2), 229–237.Nishizaki, T., Nomura, T., Matsuoka, T., & Tsujishita, Y. (1999). Arachidonic acid as a messenger for the expression of long-term potentiation. *Biochemical and Biophysical Research Communications*, *254*(2), 446–449.

Okuda, T., Ando, A., Negoro, H., Kikukawa, H., Sakamoto, T., Sakuradani, E., . . ., & Ogawa, J. (2015). Omega-3 eicosatetraenoic acid production by molecular breeding of the mutant strain S14 derived from *Mortierella alpina* 1S-4. *Journal of Bioscience and Bioengineering*, *120*(3), 299–304.

Oliveira, V., & Smallwood, D. (2011). *The infant formula market: consequences of a change in the WIC contract brand (No. ERR-124)*.

Ono, K., Aki, T., & Higashiyama, K. (2011a). Culturing *Mortierella* in nutrient broth containing saccharified starch and recovering gamma-linolenic, dihomo-gamma-linolenic, arachidonic, eicosapentaenoic, mead, 6, 9-octadecadienoic and 8, 11-eicosadienoic acids. Patent application U.S. Patent No. 7,863,024. 01 April 2011.

Ono, K., Aki, T., & Higashiyama, K. (2011b). Process for producing highly unsaturated fatty acid-containing lipid. Patent application EP1498488 B1. 23 November 2011.

Oregon State University. (2014). *Essential fatty acids*.. http://lpi.oregonstate.edu/mic/other-nutrients/essential-fatty-acids 03.03.16

Packter, N. M. (1981). Lipid biochemistry of fungi and other organisms. *Biochemical Education*, *9*(4), 156.

Papanikolaou, S., & Aggelis, G. (2011). Lipids of oleaginous yeasts Part I: Biochemistry of single cell oil production. *European Journal of Lipid Science and Technology*, *113*(8), 1031–1051.

Papanikolaou, S., Fakas, S., Fick, M., Chevalot, I., Galiotou-Panayotou, M., Komaitis, M., . . ., & Aggelis, G. (2008). Biotechnological valorisation of raw glycerol discharged after bio-diesel (fatty acid methyl esters) manufacturing process: Production of 1,3-propanediol, citric acid and single cell oil. *Biomass and Bioenergy*, *32*(1), 60–71.

Park, Y., Tamura, S., Koike, Y., Toriyama, M., & Okabe, M. (1997). Mycelial pellet intrastructure visualization and viability prediction in a culture of *Streptomyces fradiae* using confocal scanning laser microscopy. *Journal of Fermentation and Bioengineering*, *84*(5), 483–486.

Park, E. Y., Koike, Y., Higashiyama, K., Fujikawa, S., & Okabe, M. (1999). Effect of nitrogen source on mycelial morphology and arachidonic acid production in cultures of *Mortierella alpina*. *Journal of Bioscience and Bioengineering*, *88*(1), 61–67.

Peng, C., Huang, H., Ji, X., Liu, X., You, J., Lu, J., . . ., & Ouyang, P. (2010). A temperature-shift strategy for efficient arachidonic acid fermentation by *Mortierella alpina* in batch culture. *Biochemical Engineering Journal*, *53*(1), 92–96.

QY Research Center. (2015). *Global arachidonic acid industry report 2015*.. http://www.qyresearch.com/english/goods.php?id=45013

Ratledge, C. (2001). Microorganisms as sources of polyunsaturated fatty acids. *Structured and Modified Lipids*, 351–399.

Ratledge, C. (2004). Fatty acid biosynthesis in microorganisms being used for single cell oil production. *Biochimie*, *86*, 807–815.

Ratledge, C. (2013). Microbial oils: An introductory overview of current status and future prospects. *OCL*, *20*(6), D602.

Ratledge, C., & Hopkins, S. (2006). Lipids from microbial sources. In F. D. Gunstone (Ed.), *Modyfing lipids for use in food* (pp. 80–113). University of Hull, UK: Department of Biological Sciences.

Ratledge, C., & Wynn, J. P. (2002). The biochemistry and molecular biology of lipids accumulation in oleaginous microorganisms. *Advances in Applied Microbiology*, *51*, 1–52.

Rocky-salimi, K., Hamidi-esfahani, Z., & Abbasi, S. (2011). Statistical optimization of arachidonic acid production by *Mortierella*

alpina CBS 754 68 in submerged fermentation. *Iranian Journal of Biotechnology*, *9*(2), 87–93.

Saccardo, P. A., Traverso, G. B., & Trotter, A. (n.d.). *Sylloge fungorum omnium hucusque cognitorum. Digessit P. A. Saccardo*. (Vol. v.1). Patavii,sumptibus auctoris. http://www.biodiversitylibrary.org/item/102968.

Sajbidor, J., Kozelouhova, D., & Certik, M. (1992). Influence of some metal-ions on the lipid-content and arachidonic-acid production by *Mortierella* sp. *Folia Microbiologica*, *37*(6), 404–406.

Šajbidor, J., Dobroňová, S., & Čert'ik, M. (1990). Arachidonic acid production by *Mortierella* sp S-17 influence of C/N ratio. *Biotechnology Letters*, *12*(5), 455–456.

Sakamoto, T., Sakuradani, E., Okuda, T., Kikukawa, H., Ando, A., Kishino, S., ..., & Ogawa, J. (2017). Metabolic engineering of oleaginous fungus *Mortierella alpina* for high production of oleic and linoleic acids. *Bioresource Technology*, *245*, 1610–1615.

Sakuradani, E., Kamada, N., Hirano, Y., Nishihara, M., Kawashima, H., Akimoto, K., ..., & Shimizu, S. (2002). Production of 5,8,11-eicosatrienoic acid by a $\Delta5$ and $\Delta6$ desaturation activity-enhanced mutant derived from a $\Delta12$ desaturation activity-defective mutant of *Mortierella alpina* 1S-4. *Applied Microbiology and Biotechnology*, *60*(3), 281–287.

Sakuradani, E., Hirano, Y., Kamada, N., Nojiri, M., Ogawa, J., & Shimizu, S. (2004). Improvement of arachidonic acid production by mutants with lower $n-3$ desaturation activity derived from *Mortierella alpina* 1S-4. *Applied Microbiology and Biotechnology*, *66*(3), 243–248.

Sakuradani, E., Ando, A., Shimizu, S., & Ogawa, J. (2013). Metabolic engineering for the production of polyunsaturated fatty acids by oleaginous fungus *Mortierella alpina* 1S-4. *Journal of Bioscience and Bioengineering*, *116*(4), 417–422.

Shimada, Y., Sugihara, A., Nakano, H., & Kuramoto, T. (1997). Purification of docosahexaenoic acid by selective esterification of fatty acids from Tuna oil with *Rhizopus delemar* lipase. *Journal of the American Oil Chemists Society*, *74*(2), 97–101.

Shimizu, S., & Jareonkitmongkol, S. (1995). *Mortierella* species (fungi): Production of C20 polyunsaturated fatty acids. In Y. P. S. Bajaj (Ed.), *Medicinal and aromatic plants* (pp. 308–325). Berlin: Springer.

Shimizu, S., Kawashima, H., Shinmen, Y., Akimoto, K., & Yamada, H. (1988). Production of eicosapentaenoic acid by *Mortierella* fungi. *Journal of the American Oil Chemists' Society*, *65*(9), 1455–1459.

Shinmen, Y., & Shimizu, S. (1989). Production of arachidonic acid by *Mortierella* fungi. *Applied Microbiology*, *31*(1), 11–16.

Shu, C.-H., & Lung, M.-Y. (2004). Effect of pH on the production and molecular weight distribution of exopolysaccharide by *Antrodia camphorata* in batch cultures. *Process Biochemistry*, *39*(8), 931–937.

Sijtsma, L., & Anderson, A. J. (2010). Alternative carbon sources for heterotrophic production of docosahexaenoic acid by the marine alga *Crypthecodinium cohnii*. In Z. Cohen, & C. Ratledge (Eds.), *Single cell oils* (2nd Ed., pp. 131–149). Illinois: Elsevier.

Singh, A., & Ward, P. O. (1997). Production of high yields of arachidonic acid in a fed-batch system by *Mortierella alpina* ATCC 32222. *Applied Microbiology and Biotechnology*, *48*(1), 1–5.

Srigley, C. T., & Rader, J. I. (2014). Content and composition of fatty acids in marine oil omega-3 supplements. *Journal of Agricultural and Food Chemistry*, *62*(29), 7268–7278.

Stredanská, S., & Šajbidor, J. (1993). Influence of carbon and nitrogen sources on the lipid accumulation and arachidonic acid production by *Mortierella alpina*. *Acta Biotechnologica*, *13*(2), 185–191.

Totani, N., Watanabe, A., & Oba, K. (1987). An improved method of arachidonic acid production by *Mortierella alpina*. *Yukagaku*, *36*(5), 328–331.

Totani, N., Someya, K., & Oba, K. (1992). Industrial production of arachidonic acid by *Mortierella*. In D. J. Kyle, & C. Ratledge (Eds.), *Industrial applications of single cell oils* (pp. 52–60). Illinois: AOCS Publishing.

Totani, N., Suzaki, K., & Kudo, T. (1992). Process for the production of arachidonic acid-containing lipids. Patent application EP0223960 B1. 15 July 1992.

Totani, N., Hyodo, K., & Ueda, T. (2000). Minerals essential for growth of the filamentous fungus, *Mortierella alpina*. *Journal of Japan Oil Chemists' Society*, *49*(5), 487–493, 515

Uauy, R., Mena, P., & Rojas, C. (2000). Essential fatty acids in early life: Structural and functional role. *Proceedings of the Nutrition Society*, *59*, 3–15.

United Nations, Department of Economic and Social Affairs – Population Division – World Population Prospects. (2017). https://esa.un.org/unpd/wpp/Download/Standard/Population/ 23.08.18.

Vadivelan, G., & Venkateswaran, G. (2014). Production and enhancement of omega-3 fatty acid from *Mortierella alpina* CFR-GV15: its food and therapeutic application. *BioMed Research International*, *2014*, 657414.

Vali, S. R., Sheng, H. Y., & Ju, Y. (2003). An efficient method for the purification of arachidonic acid from fungal single-cell oil (ARASCO). *Journal of the American Oil Chemists Society*, *80*(7), 725–730.

Vandamme, E., & Revuelta, J. L. (2016). Vitamins, biopigments, antioxidants and related compounds: a historical physiological and (Bio) technological perspective. In E. Vandamme, & J. L. Revuelta (Eds.), *Industrial biotechnology of vitamins, biopigments, and antioxidants* (pp. 1–14). Weinheim, Germany: WILEY-VCH Verlag.

Wynn, J. P., & Ratledge, C. (2005). Microbial production of oils and fats. In K. Shetty, G. Paliyath, A. Pometto, & E. Levin (Eds.), *Food biotechnology* (2nd Ed., pp. 443–472). NY: CRC Press.

Wynn, J. P., Hamid, A. A., Li, Y., & Ratledge, C. (2001). Biochemical events leading to the diversion of carbon into storage lipids in the oleaginous fungi *Mucor circinelloides* and *Mortierella alpina*. *Microbiology*, *147*(10), 2857–2864.

Xian, M., Yan, J., Kang, Y., Liu, J., Bi, Y., & Zhen, K. (2001). Production of c-linolenic acid by *Mortierella isabellina* grown on hexadecanol. *Letters in Applied Microbiology*, *33*, 367–370.

Xian, M., Kang, Y., Yan, J., Liu, J., Bi, Y., & Zhen, K. (2002). Production of linolenic acid by *Mortierella isabellina* grown on octadecanol. *Current Microbiology*, *44*(2), 141–144.

Yamauchi, A., Nagao, T., & Watanabe, Y. (2005). Purification of arachidonic acid from *Mortierella* single-cell oil by selective esterification with Burkholderia cepacia lipase. *Journal of the American Oil Chemists Society*, *82*(11), 833–837.

Yan, Y., Li, X., Wang, G., Gui, X., Li, G., Su, F., ..., & Liu, T. (2014). Biotechnological preparation of biodiesel and its high-valued derivatives: A review. *Applied Energy*, *113*, 1614–1631.

Yokochi, T., Kamisaka, Y., Nakahara, T., & Suzuki, O. (1995). Production of γ-Linolenic acid containing phospholipids by *Mortierella ramanniana* in a fed-batch culture of decane. *Journal of Japan Oil Chemists' Society*, *44*(1), 9–15. http://dx.doi.org/10.5650/jos1956.44.9 Yu, L. J., Qin, W. M., Lan, W. Z., Zhou, P. P., & Zhu, M. (2003). Improved arachidonic acids production from the fungus *Mortierella alpina* by glutamate supplementation. *Bioresource Technology*, *88*(3), 265–268.

Yuan, C., Wang, X., & Yu, Z. (2007). Separation and preparative purification of arachidonic acid from microbial lipids by urea inclusion reaction and HPLC. *Preparative Biochemistry and Biotechnology*, *37*(2), 149–159.Zhiming, W., Huan, L., & Xu, J. (2014). Method for producing arachidonic acid grease by utilizing *Mortierella alpina* mutant strain, and arachidonic acid grease produced by method. Patent application CN 103571896 A. 12 February 2014.

Zhu, M., Yu, L. J., Liu, Z., & Xu, H. B. (2004). Isolating *Mortierella alpina* strains of high yield of arachidonic acid. *Letters in Applied Microbiology*, *39*(4), 332–335.