Fungi as an alternative to produce essential fatty acids

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Abstract

Fungi have the ability to produce essential fatty acids of the omega-3 family, what is of great scientific interest, since these microorganisms are capable of developing under varied conditions, making their production a viable alternative. Fatty acids are of great importance in human food due to their beneficial effects to the organism, such as reduction in the development of cardiovascular problems and other chronic-degenerative diseases. Currently, the main food source of omega-3 fatty acids is saltwater fish, but there are concerns about potential health risks, including environmental contaminants, such as some metals, as well as ecological issues related to the extinction of some species. Therefore, interest in the biotechnology area has been increasing with the aim of extracting bioactive compounds from certain fungi species, besides using these species to produce biomass, food, and energy, among others.

Additional keywords: biomass; biotechnology; industrial application; polyunsaturated fatty acids.

Introduction

Fatty acids are the compounds that give lipids their main nutritional properties. However, there are differences regarding their physiological effect (Lehninger & Cox, 2014). The essential term is applied to fatty acids that need to be ingested through food. However, the designation is only given to two polyunsaturated fatty acids classes, which are omega-3 (ω-3) and omega-6 (ω-6).

The compounds aforementioned are usually found in tissues and biological fluids, and are used in vital organism processes maintenance (Perini et al., 2010). They promote cell proper functioning and perform important functions for the organism, such as reducing the risks of cardiovascular and immunological disorders. In addition, omega-3 and omega-6 also promote blood pressure regulation, neuroprotection, mood stabilization, and inflammatory states reduction. They also help transmitting nerve impulses, among other important organism functions (Nuria et al., 2010; Perini et al., 2010).

The main omega-3 fatty acids sources for human consumption are cold-water fish, such as salmon, mackerel, sardines, and herring, among others. However, increased fish intake in some countries has resulted in environmental impacts, with the possibility of extinction of such species (Lenihan-Geels et al., 2013).

In addition to environmental risks, some contaminant derivatives are found in fish (Domingo et al., 2007). Fish are also sensitive to lipid oxidation, temperature variations, seasons, and location, being more susceptible to contamination by metals, pesticides and/or packaging on ships. In addition, difficulty inserting fish in diets for a long time period should be considered, since their flavor makes is considered of low acceptability (Martins et al., 2008).
Thus, lipids extracted from fungi may contribute to supply the demand for essential polyunsaturated fatty acids (Papanikolau & Aggelis, 2002). Some industrial sectors use microorganism cultivation, mainly of filamentous fungi, in solid and submerged bioprocesses to produce biomass that have compounds with economically high and diversified yields, such as fatty acids (Zen et al., 2014).

Agroindustrial wastes, for example, can be used as an alternative in culture media to develop fungi and produce lipids in biomass. The extracted lipids can be incorporated into animal feed and biodiesel production, among others (Zen et al., 2014).

The present paper aims to present a brief review on essential fatty acids production by filamentous fungi and perspectives on their use in industries in general.

**Omega-3 fatty acids nutritional importance**

Transition in Brazil’s nutritional habit is due to several factors associated with changes in the individuals’ diet and body profile resulting from social, economic, demographic, technological and cultural changes that directly affect the health profile of a population. The population has been increasingly ingesting foods high in saturated fats and decreasing fiber consumption, which, associated with the lack of physical activities, contributes to the emergence of some diseases (Santos et al., 2013).

Thus, the importance of ingesting polyunsaturated fatty acids of the omega-3 family has been widely discussed and publicized due to its beneficial effects on human health (Tonial et al., 2010; Garneau et al., 2012). Among the several benefits of these compounds, prevention of cardiovascular and inflammatory diseases, infections and immunological changes currently stands out. Polyunsaturated fatty acids are also responsible for neuroprotection and fetal neurodevelopment (Cortes et al., 2013).

Omega-3 family fatty acids of nutritional relevance are the alpha-linolenic acid (ALA-C18:3) and its derivatives, eicosapentaenoic (EPA-C20:5) and docosahexaenoic acids (DHA-C22:6) (Novello et al., 2008).

A diet rich in these compounds alters the synthesis of eicosanoids from arachidonic acid (AA-ω-6), favoring the formation of anti-inflammatory eicosanoids (Surette, 2008).

Chronic diseases include cardiovascular diseases, which are the primarily responsible for deaths of young adults in developed and developing countries. Although there has been a decrease in mortality related to these diseases in the course of the years, cardiovascular diseases were the most common cause of death in Brazil in 2011 (Vilela et al., 2014; Farias, 2014). According to the Brazilian Society of Cardiology (SBC), cardiovascular diseases in Brazil account for 29% of deaths. Although the final data are not finalized yet, estimated deaths in 2015 already reach 346,696. In the first days of 2016, more than 10,000 people died in the country due to cardiovascular diseases, with a death prospect during the course of the year of approximately 350,000 Brazilians (SBC, 2016). Deaths may be particularly related to saturated fats, which raise blood cholesterol levels. However, polyunsaturated fats reduce blood cholesterol (Novello et al., 2008).

It is believed that organic concentrations of omega-3 family fatty acids depend on the ingested amount of omega-6 family fatty acids or the ratio between them. Some studies consider that the omega-6/omega-3 ratio is not fixed, since there is no consensus on what ratio would be ideal (Vilela & Bazotte, 2013).

Omega-3 fatty acids, especially DHA, are responsible for the functioning and development of the brain and the central nervous system. An unbalanced diet, rich in omega-6 fatty acids and deficient in omega-3, may lead to neurological and behavioral disorders (Jones et al., 2013).

According to the National Sanitary Surveillance Agency (ANVISA), products with functional properties regarding omega-3 should offer at least 0.1 g of EPA and/or DHA in the portion or in 100 g or 100 mL of the product ready for consumption, if the portion is greater than 100 g or 100 mL (Brasil, 2015).

Recently, one study evaluated the impact of fish oil-based omega-3 (docosahexaenoic-DHA) supplementation on pregnant and breastfeeding women. The authors observed that daily supplementation influenced lipid composition and fatty acid profile in human milk (Bortolozo et al., 2013). According to them, need for higher docosahexaenoic fatty acid (DHA) concentrations occur in the last trimester of pregnancy and in the first months of life, that is, if mothers ingest high fatty acid amounts, the bioavailability of this nutrient is increased to infants through the placenta, or through milk in the first months of the child’s life.

Thus, through industrial technology advances, fungi and other microorganisms are being increasingly studied as viable alternatives in the production of bioactive compounds for use by food industries or in food enrichment, through nutritional supplements (Dyal & Narine, 2005; Esposito & Azevedo, 2010).

**Fungi and other microorganisms as fatty acids producers**

Different microorganism types are able to synthesize long-chain polyunsaturated fatty acids, among which there are bacteria, yeasts, filamentous fungi and microalgae, according to table 1 (Gupta et al., 2012).

However, filamentous fungi of Mucorales order, especially Mortierella species (Xian et al., 2003; Zhu et al., 2006), are highlighted. Some species of this fungus are already being studied due to their capacity to produce docosahexaenoic (DHA) and eicosapentaenoic (EPA) polyunsaturated fatty acids belonging to the omega-3 family. Compared to vegetable oils, for example, oils extracted from microorgan-
isms have some advantages, such as shorter life cycle and abundant and cheap raw material, easy to produce on a large scale (Liang & Jiang, 2013). Currently, few microorganism species are used commercially for EPA and/or DHA production. However, the microalga Crypthecodinium cohnii ee and the protist Scizochytrium sp can be mentioned. DHA produced by these microorganisms is already a viable alternative to the industry (Gong et al., 2014).

Table 1- Main fatty acids production by microorganisms.

<table>
<thead>
<tr>
<th>Microorganisms type</th>
<th>Microorganisms species</th>
<th>Fatty Acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungus</td>
<td>Pythium debaryanum</td>
<td>Gamma-linolenic acid (10.4)</td>
</tr>
<tr>
<td>Fungus</td>
<td>Mortierella alpina</td>
<td>Arachidonic acid (68.5-78.8)</td>
</tr>
<tr>
<td>Fungus</td>
<td>Mortierella alpinaperyon</td>
<td>Arachidonic acid (11)</td>
</tr>
<tr>
<td>Protozoa</td>
<td>Saprolegnia parasitica</td>
<td>AA (19) / EPA (18)</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Vibrio sp</td>
<td>DHA and EPA</td>
</tr>
<tr>
<td>Fungi</td>
<td>Pythium irregulare</td>
<td>EPA (25.2)</td>
</tr>
<tr>
<td>Fungi</td>
<td>Candida guillermondii</td>
<td>DHA (6.7) and EPA (2.8)</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Colwellia sp</td>
<td>DHA (0.7-8)</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Shwenella sp</td>
<td>EPA (2-22)</td>
</tr>
<tr>
<td>Yeast</td>
<td>Pichia methanolica</td>
<td>DHA (32)</td>
</tr>
<tr>
<td>Fungi</td>
<td>Mortierella sp.</td>
<td>AA (25.9-53.8)</td>
</tr>
<tr>
<td>Fungi</td>
<td>Mortierella alliacea</td>
<td>GLA (4.3-4.7)</td>
</tr>
<tr>
<td>Fungi</td>
<td>Rhodotorula mucilaginosa</td>
<td>EPA (1,3-13)</td>
</tr>
</tbody>
</table>

EPA-Eicosapentaenoic; DHA-Docosahexaenoic; AA-Arachidonic; GLA-Gamma-linolenic; ALA- alpha-Linolenic acid; DGLA- Dihomo-γ-linolenic acid. Source: Gupta et al., 2012.

Srianta et al. (2010) observed polyunsaturated fatty acids production of the fungi Rhizomucor miehei and Rhizopus microsporus by submerged fermentation in a medium based on sugarcane molasses and wheat bran. Among the different concentrations studied, the authors observed that 15% was more adequate, yielding 0.82% of DHA and 0.86% of EPA by the fungus Rhizomucor miehei. The species mentioned above obtained the highest production of these compounds when compared to Rhizopus microsporus. Omega-3 ingestion is available in the market through fish oils containing 20-30% of these fatty acids (Gayathri et al., 2010).

Oil production from a single fungal cell containing polyunsaturated fatty acids is attractive for biotechnological production and market interest has been increasing, as microorganisms such as Yarrowia lipolytica, Mucor circinelloides, Mortierella alpina and the microalgae Schizochytrium and Crypthecodinium cohnii are already being used for this purpose (Salunke et al., 2014).

Although some microorganism strains are omega-3 fatty acid producers, further studies are needed to improve yield. With the emergence of metabolic engineering, it is possible to significantly maximize polyunsaturated fatty acids production from native microorganisms, although it is necessary to know the factors that may affect their development (Gong et al., 2014).

Fatty acids profile in fungal biomass

Biomass can be produced by both micro and macroscopic fungi, and may be of great economic interest, since they contribute to increase substrates nutritional value, even adding value to agroindustrial waste (Silveira & Furlong, 2007).

Fatty acids produced by fungi have some advantages, since they are capable of developing under varied conditions, resulting in lipids production in different substrates (Abu et al., 2000).

However, regardless of substrate used for fungi growth, most of the lipids produced in the fungal mass are mainly represented by palmitic (C16:0), oleic (C18:1), and linoleic (C18:2) fatty acids, besides docosahexaenoic acid (DHA-C22:6), which is a fatty acid relevant for health, as it is highly nutritional (Silveira et al., 2010).

Emergence of molecular biology techniques have provided new ways of using filamentous fungi and yeasts, which are beginning to be used in the production of microbial cells and metabolites such as pigments, antibiotics and certain fatty acids groups. However, in order to use them in the food industry, it is important to evaluate fungal groups according to their production yields, as well as to regulatory standards, such as using fungi recognized by the Food and Drug Administration (FDA) as GRAS (Generally Recognized as Safe) (Adrio & Demain, 2003).
According to Adrio & Demain (2003), Mortierella isabelina and Mucor circinelloides can accumulate up to 5 g of conjugated linoleic acid (CLA) when grown in medium containing molasses or glucose, while Mortierella alpina, when cultivated at low temperatures, is able to accumulate Eicosapentaenoic acid (EPA – C20:5, ω-3).

Fakas et al. (2008) observed palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and gamma-linolenic (C18:3) fatty acids in the biomass produced by the fungus Cunninghamella echinulata when it was grown in Potato Dextrose Agar (PDA) medium containing glucose, whey concentrate, and hydrolyzed tomato residue, besides commercial corn gluten, corn infusion and yeast extract as nitrogen sources.

Gayathri et al. (2010) investigated economically important fatty acids produced by fungi. In order to perform the experiment, two fungi species isolated from the soil, Trichoderma sp. and Aspergillus niger, were cultivated in PDA medium. The authors observed that Trichoderma sp. produced high omega-3, EPA – C20:5 and DHA-C22:6 polysaturated fatty acids concentrations, with 0.298 mg/g and 7.47 mg/g respectively. As for Aspergillus niger, the authors only reported the production of 0.136 mg/g DHA.

Silveira et al. (2010), used rice and wheat bran as substrates for fungal fermentation, in order to alter their profile in essential fatty acids as an alternative to add value to these products. During the experiment, PDA medium enriched with rice and wheat bran was used for fungus Aspergillus oryzae growth, where 0.2% linolenic acid (C18:3 - ω3) production was observed, among other fatty acids.

A study evaluated the safety of omega-6 fatty acids (gamma-linolenic-GLA) present in Mucor rouxii (CFR-G15) biomass. The researchers provided ration for rats containing Mucor rouxii dry biomass for 13 weeks. After this period, they verified that the animals did not show significant alterations, organs were intact and triglyceride and cholesterol levels were reduced. Therefore, fungus Mucor rouxii (CFR-G15) biomass could be incorporated into rations and administered to animals to provide essential fatty acids for the purpose of controlling cholesterol and triglyceride levels (Mamatha et al., 2012).

According to Vadivelan and Venkateswaran (2014), the physiology of fungi belonging to the Mortierella alpina species can be altered by mutation induction, chemical inhibitors, and stress conditions, among other factors, in order to increase omega-3 fatty acids (EPA and DHA) production.

Thus, Mortierella alpina species was cultivated under stress conditions (2% starch, 0.5% yeast extract and 12 °C temperature for 4 days) to increase omega-3 polyunsaturated fatty acids production. Under these conditions, this fungus was able to increase EPA fatty acids from 2.4% to 4.7% and DHA from 1.84% to 3.8%, which belong to the omega-3 family, in cell dry weight.

Furlan et al. (2014) assessed polyunsaturated fatty acids production synthesized by the fungus Thraustochytrium sp. The authors observed high production of omega-3 family polyunsaturated fatty acids, with 69 to 73% of DHA, followed by docosapentaenoic acid (DPA-C22: 5), with 21 to 15%, regardless of culture type and time. For the oleaginous group, this fungus was noted for being able to accumulate 50% of its weight as lipids, being more than 25% of DHA.

According to Salunke et al. (2014) the fungus Pythium acanthium was able to accumulate high docosapentaenoic acid (DPA-C22:5, ω-3) concentrations when cultured in media containing linseed oil. Still, these authors observed that the fungus Mucor sp., when grown with 10% linseeds, yielded 6.01 mg/g DHA and 19.78 mg/g EPA. On the other hand, the fungus Rhizopus oligosporus cultivated with 3% of linseed seeds produced 14.32 mg/g DHA and 13.21 mg/g EPA, respectively.

However, fatty acids high production capacity, as well as higher cell concentration demonstrated that fungal growth, fatty acids yield and cultivation time depend on the total nitrogen source available for microorganism consumption. Therefore, it is important to create parameters, in order to optimize polyunsaturated fatty acids production (Furlan et al., 2014).

Fungal biomass products application

Currently, research has been developed to isolate and chemically identify products produced by microorganisms. Fungi in particular have been attracting great interest of several industrial sectors due to the production of high added value substances that are used in the pharmaceutical, chemical and food industries to produce beers, wines, cheeses, and vitamins, among others (Tai & Stephanopoulos, 2013).

Food industries have also been using biomaterials in food coatings for flavor retention and appearance enhancement, besides using it in film and fiber preparation, as emulsifying or thickener agents, among others. The residual biomass resulting from fermentative processes has been researched to isolate its constituents, such as lipids, since these compounds contain specific properties and high added value, and can be used in food supplementation (Borges et al. 2014).

Cereal-based raw materials, such as rice and wheat bran and oat flakes provide an adequate nutrient source for fungi development and lipid production (Certik & Adamechova, 2009).

In the solid state fermentation process, Mortierella alpina species fungi use cereal-based substrates which, when enriched with linseed oils (composed of around 57% alpha-linolenic acid), convert them to EPA, producing a product with 23.4g/kg product or 17.8% in oil after pre-fermentation. Therefore, filamentous fungi belonging to the Mucorales order are able to produce polyunsaturated fatty acids in different substrates by solid state fermentation, allowing cereals enrichment by fermentation method as an alternative to
provide beneficial compounds for human or animal consumption (Certik & Adamechova, 2009).

Bioconversion of alpha-linolenic acid (ALA-omega-3), present in linseeds and DHA and EPA long-chain fatty acids, are of great commercial interest as a valuable alternative source for human supplementation (Salunke et al., 2014).

Vadivelan & Venkateswaran (2014) believe that processes using microorganisms to produce polyunsaturated fatty acids are reliable and economically attractive by producing beneficial compounds. In addition, these processes can be efficient for large-scale production for food or therapeutic purposes.

Fatty acids production as raw material has also been studied as a renewable source for biofuels and biological chemicals production, due to the depletion of non-renewable petrochemical resources (Liu et al., 2014).

Therefore, biofuels and chemicals production from free fatty acids from different biomass types arise from the need to deal with issues such as high energy costs and environmental concerns. This was made possible thanks to metabolic engineering studies where free fatty acids can be obtained from renewable resources, such as lignocellulosic sugars and carbon dioxide, through conversion by microorganisms (Liu et al. 2014).

Although production costs of polyunsaturated fatty acids by fungi are still high, some advantages are offered for production, such as high growth rates, simple nutrient requirements, controllable cultivation conditions, simple fatty acids composition and easy genetic manipulation. The high costs of producing these compounds may be reduced in part by biorefineries, where microbial fermentation is normally conducted in a closed system and can be expanded to commercial scales (Gong et al., 2014).

Final Considerations

Fungal biomass products can be an economically viable alternative to produce compounds of interest to various industrial sectors. In the food industry, fatty acids of nutritional relevance produced by certain fungi species could contribute through supplementation or food enrichment, in order to minimize risk factors related, for example, to cardiovascular or degenerative diseases.

Therefore, research on fungi that can produce and accumulate lipids in their biomass has been improved, contributing to supply the fatty acids demand, beneficial to organism functioning. Thus, there is a growing interest for new fatty acid production sources capable of producing compounds with potential for wide use in biotechnological processes and food sciences, sharpening interest for several scientific knowledge areas. However, this subject still needs to be further explored by the scientific communities.

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