



# Recovery and valorization of agri-food wastes and by-products using the non-conventional yeast *Yarrowia lipolytica*

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## ABSTRACT

**Background:** *Yarrowia lipolytica* is one of the most studied “non-conventional” yeasts. Due to its intrinsic biological characteristics and variability, the potential applications of wild type isolates in the agri-food sector are broad (production of biomasses, enzymes, and metabolites). By-products generated by the food industry are rising environmental and economic concerns worldwide. These side streams have different compositions and stability. However, they can still be applied as substrates for microbial growth.

**Scope and approach:** The fitness of wild type strains to adverse and different environments can be exploited not only for food production but also for recovery and valorization of agri-food wastes and by-products. This review brings together a selection of the most relevant and recent data about the physiology, nutritional requirements, and metabolites produced by wild type isolates of *Y. lipolytica*. Moreover, the principal agri-food side streams, their specific productions and valorization using wild type strains have been critically discussed.

**Key finding and conclusions:** Critical aspects of side streams and by-products can be solved (e.g. reduction of COD and pollutants before discharging), novel ingredients can be generated (e.g. lipases, single cell oils and citric acid) and, eventually, yeast biomasses can be produced for further applications (food adjuncts or supplements). Selecting and characterizing wild type isolates able to consume or convert or valorize the different waste/by-product components into added value products is extremely important in the view of a sustainable process and sustainable economy.

## 1. Introduction

*Yarrowia lipolytica* is one of the most extensively studied yeast species after *Saccharomyces cerevisiae*. It is classified as Generally Regarded As Safe (GRAS) for industrial productions by the American Food and Drug Administration (FDA) and it is assumed to be safe for feed and food applications by the European Food and Safety Authority (EFSA) (EFSA BIOHAZ Panel, 2018; Groenewald et al., 2014; Zieniuk & Fabiszewska, 2019). This yeast is widespread in nature and since it can accumulate lipids is classified as an oleaginous microorganism. Fundamental for the ripening of some traditional chesses and dry fermented sausages, in some cases *Y. lipolytica* is considered a spoilage yeast and, therefore, undesired (Zinjarde, 2014). It has been isolated from food matrixes, such as yoghurt, kefir, soy sauce, rancid margarine, shrimp salads, sourdough and different environments like soil, oil-polluted soil, rivers and sea

water (Hassanshahian, Tebyanian, & Cappello, 2012; Paramithiotis et al., 2000; Sinigaglia, Lanciotti, & Guerzoni, 1994). To grow in such different systems, this yeast developed a unique broad spectrum of biological features, covering a wide range of substrates (both hydrophilic and hydrophobic), physico-chemical conditions, pH (from 2.5 to 8) and temperatures (from 2°C to 32°C) (Egermeier, Russmayer, Sauer, & Marx, 2017; Sinigaglia et al., 1994; Zinjarde, 2014). Moreover, it tolerates well metal ions (such as cadmium, nickel, cobalt, zinc and copper sulfate) and salt solutions (up to 12% (v/v)) (Carsanba, Papanikolaou, Fickers, & Agirman, 2019; H. H. Liu, Ji, & Huang, 2015). This natural capability to fit in different, alternative and, in some cases, extreme environments, with limited source of nutrients makes *Y. lipolytica* an important ally to tackle the issue of food industry waste.

About 1.3 billion tons of wastes and by-products are annually produced by the food industry (FAO, 2019). About 38% of food wastes

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occur during food processing. The sources of these wastes can derive from animal, seafood, dairy and vegetable processing industries. The high concentration of some components (e.g. NaCl and lactose in cheese whey; polyphenols and organic acids in olive mill wastewaters) makes them polluting and dangerous for water, soil fertility and environment. Therefore, an efficient use of waste and by-products could greatly influence both economy and environment. For instance, food wastes and side-streams could be potentially transformed and valorized into added value products by sustainable biotechnological processes based on safe and tailored microorganisms, such as *Y. lipolytica*.

A search in Scopus of the key words “*Yarrowia lipolytica* and waste”, showed that in the last 10 years there has been an exponential interest in studying the applications of this yeast in waste and by-product recovery. Although *Y. lipolytica* isolates can fit well in adverse environments, several authors proposed to create engineered strains in order to boost their activities (Ledesma-Amaro & Nicaud, 2016; Spagnuolo, Hussain, Gambill, & Blenner, 2018). For instance, it has been reported that wild-type strains could produce around tenths g/L of citric acid (CA), whereas more than 100 g/L can be obtained with mutants (Carsanba, Papanikolaou, Fickers, & Agirman, 2019). However, engineered genotypes tend to be more unstable and, at the moment, few tools used for engineering *Y. lipolytica* seem predictable and reliable (Larroude, Traubelsi, Nicaud, & Rossignol, 2020).

The purpose of this review is to bring together a selection of the most relevant and recent data about the physiology, nutritional requirements, metabolites produced and enzymatic machinery of wild type isolates of *Y. lipolytica*, and how they have been used to valorize or improve the characteristics of food waste and by-products up to now.

## 2. Physiology, nutritional requirements, and enzymatic machinery

*Y. lipolytica* is a dimorphic microorganism that changes between yeast cells, pseudohyphae and septate hyphae as a defense mechanism against adverse conditions, such as variation in oxygen, pH, carbon and nitrogen substrates (Coelho, Amaral, & Belo, 2010, pp. 930–944; Liu, Lv, Zhang, & Deng, 2015). Testing various parameters, Ruiz-Herrera and Sentandreu (2002) reported that mycelium formation was maximal at neutral pH, while almost only cell form was present at pH 3, and in presence of citrate. On the contrary, Bellou, Makri, Triantaphyllidou, Papanikolaou, and Aggelis (2014) observed that dissolved oxygen concentration (and not carbon or nitrogen sources) was the major parameter affecting the dimorphism of the yeast. *Y. lipolytica* grows as a strict aerobe on a wide variety of carbon sources. Among the sugars, it can degrade several hexoses, such as glucose, fructose, mannose and galactose (Ledesma-Amaro & Nicaud, 2016). While glucose is the favorite substrate for all the isolates, efficiency in catabolizing fructose is strain dependent (Lazar, Dulermo, Neuvéglise, Crutz-Le Coq, & Nicaud, 2014). Sucrose and lactose cannot be utilized by wild type strains (Kurtzman, Fell, & Boekhout, 2011) but one isolate from soil (*Y. lipolytica* B9) that was lactose-positive (Taskin, Saghafian, Aydogan, & Arslan, 2015). This is not surprising given the high variability in phenotypes among different strains. Even galactose can be consumed by *Y. lipolytica* W29 only in presence of glucose at concentrations higher than 0.4% (Lazar, Gamboa-Meléndez, Le Coq, Neuvéglise, & Nicaud, 2015). The yeast possesses also the complete genetic pathway for arabinose and xylose utilization. However, not consistent results on their real utilization have been published (Spagnuolo et al., 2018). Another favorite substrate for *Y. lipolytica* is glycerol (Rywińska et al., 2013). When glycerol or glucose are not present, the yeast can use ethanol, as well as acetic, propionic, butyric, malic, succinic, citric and lactic acids (Coelho et al., 2010, pp. 930–944; Gao, Li, Zhou, Cheng, & Zheng, 2017; Rodrigues & Pais, 2000). The latter can also be used as substrate if no other nitrogen sources (free amino acids) are present in the medium (Mansour, Beck-erich, & Bonnarne, 2008).

The main characteristic of *Y. lipolytica* is the capability to grow and

consume alkanes, fatty acids and triacylglycerols. Therefore, vegetable oils, fatty esters, soap-stocks, pure free-fatty acids, animal fats, vegetable oils and crude fish oils can be easily used as substrates (X. Liu, Lv, et al., 2015; Papanikolaou & Aggelis, 2011). Nitrogen sources are also fundamental and their presence and availability on the medium can affect the metabolic pathways and, consequently, the metabolites produced (Mansour et al., 2008). For instance, nitrogen-limited conditions are considered optimal for citric acid production or lipid accumulation when glucose or glycerol are present in the medium. However, some strains produced lipids when nitrogen was still present (or barely after its deprivation), while they consumed these stored lipids later on during incubation when nitrogen-limited conditions were reached (Filippoussi et al., 2019). The ability of growing in different environments requires specific enzymatic activities. Several enzymes of *Y. lipolytica* have been reported. Lipases and proteases are the most studied and characterized ones. Their production and application for fats and proteins valorization have been described (Brígida, Amaral, Coelho, & Gonçalves, 2014; Fickers, Marty, & Nicaud, 2011; Lanciotti, Gianotti, et al., 2005; van den Tempel & Jakobsen, 2000; Young et al., 1996; Zinjarde, 2014). However, the yeast possesses other minor enzymes, some of them not completely exploited yet, such as chitinase, phosphatases, and inulinases (Park, Han, Lee, Cheon, & Kang, 2014; Patrignani et al., 2020; Zinjarde, 2014).

## 3. Industrial applications of *Y. lipolytica*

### 3.1. Production of added value compounds

#### 3.1.1. Single cell protein (SCP) and biomass

For many years yeast biomass has been used as a valuable component of animal feedstuff (Petkov, Rymowicz, Musiał, Kinal, & Biel, 2002). Traditionally, the most widespread biotechnological application of *Y. lipolytica* was focused on the production of SCP or microbial biomass (Papanikolaou & Aggelis, 2010). One of the most important factors influencing the nutritional value of yeast biomass is their protein content. In fact, the de-fatted biomass of *Y. lipolytica* can contain approximately 30–40% w/w of proteins, with all the essential amino acids in significant quantities. In particular, the lysine content was estimated to be like the one present in whole egg protein. For this reason, biomasses or SCP, obtained as a stand-alone process or as by-products of other biotechnological productions (microbial lipids, citric acid, etc) have been proposed as environment-friendly protein source for animal feed (Patsios, Dedousi, Sossidou, & Zdragas, 2020). Moreover, in 2019, EFSA declared *Y. lipolytica* biomass a Novel Food safe for use pursuant to Regulation (EU) 2015/2283 on dietary supplements (EFSA NDA Panel, 2019). Eventually, live biomasses of *Y. lipolytica* have also been applied as an alternative co-starters or adjuncts in food productions (Lanciotti, Vannini, Chaves Lopez, Gobetti, & Guerzoni, 2005).

#### 3.1.2. Microbial lipid or single cell oil (SCO)

As an oleaginous yeast, *Y. lipolytica* can accumulate lipids, referred also as microbial lipids or SCO. However, among the species belonging to this group, this is not the most efficient one. In fact, wild type strains were reported to reach only 4–20% of SCO in cell dry weight (CDW) when grown on sugars, or higher percentages if grown on hydrophobic substrates (Darvishi, Salmani, & Hosseini, 2019; Papanikolaou et al., 2006). On this regard, depending on the substrate, lipid production could happen in two ways: *de novo* and *ex novo*. The so-called *de novo* production of lipids occurs when *Y. lipolytica* is grown on sugars or similarly metabolized compounds (e.g. glycerol). In this case, lipids are synthesized during a secondary metabolism performed usually when the medium has a high carbon/nitrogen (C/N) ratio or nitrogen limitation (M. Lopes, Gomes, Silva, & Belo, 2018; Papanikolaou & Aggelis, 2010). Unbalanced N triggers a cascade of biochemical events with the consequent accumulation and release of citrate from the mitochondria into the cytosol. Here it is cleaved by the ATP-citrate lyase into acetyl-CoA

that will be used to synthesize fatty acids (Papanikolaou & Aggelis, 2010). Bellou, Triantaphyllidou, Mizerakis, and Aggelis (2016) reported that a limitation of both nitrogen and magnesium favored higher lipid accumulation compared with the presence of only one of the two (from 13.6 to 38.5%). However, neither nitrogen limitation nor high C/N ratio seem always sufficient to guarantee high lipid production. In fact, in some studies, lipids were produced already during the first growth step, despite the presence of nitrogen and crude glycerol as sole carbon source (Diamantopoulou et al., 2020; Filippousi et al., 2019). Kuttiraja, Dhouha, and Tyagi (2018) suggested that controlling the pH of the system may also enhance lipid accumulation. When hydrophobic materials (fats, oils) are the main carbon and energy source, lipids are produced *ex novo*. In this case, their production represents the primary anabolic process of the cell. *Ex-novo* production is independent from nitrogen limitations and lipid-free materials are generated together with SCO. Usually, *Y. lipolytica* rapidly incorporates unsaturated fatty acids (i.e. oleic acid) for growth needs and organic acid production, while saturated fatty acids (i.e. stearic acid) are slowly incorporated and used for both growth needs and SCO production (Papanikolaou & Aggelis, 2010). The two routes of lipid production do not exclude one another. In fact, Papanikolaou et al. (2006) observed *de novo* fatty acids biosynthesis when both glucose and waste hydrophobic substrates were simultaneously applied. Compared with other oleaginous yeasts (such as *Rhodospiridium toruloides*, *Lipomyces starkeyi*, *Cryptococcus curvatus*), *Y. lipolytica* tends to rapidly consume lipids (turnover) after reaching a maximum value without any apparent cause, even if high substrate amounts are still present. This is usually associated with citric acid and polyols production (Makri, Fakas, & Aggelis, 2010). However, Sarantou, Stoforos, Kalantzi, and Papanikolaou (2021) demonstrated recently that extraction procedure can also affect the final SCO recovery.

SCO accumulated in *Y. lipolytica* are mainly composed of triglycerides (TAGs) and in lower extent of free fatty acids, neutral lipids, sterols and polar fractions. The most representative fatty acids accumulated are usually oleic, palmitic and linoleic acids (H. H. Liu, Lv, et al., 2015; Patsios et al., 2020; Rakicka, Lazar, Dulermo, Fickers, & Nicaud, 2015), however, tailor-made SCO can be obtained modulating growth parameters or substrates (M. Lopes et al., 2018; Papanikolaou & Aggelis, 2010). For instance, cells growing on different oils or oil industry residues generate SCO rich in oleic acid, while saturated fatty acids are more abundant when grown on stearin. Further, lipids with a composition similar to cocoa butter were obtained using a mix of hydrolyzed rapeseed oil and stearin (50:50) as substrate (Papanikolaou & Aggelis, 2011).

### 3.1.3. Citric acid (CA) - isocitric acid (ICA)

CA is one of the most interesting metabolites due to its applications as flavoring agent, acidifier, antioxidant and preservative (Fickers, Cheng, Sze, & Lin, 2020). The 70% of this acid are applied in food and 18% in pharma sector (Carsanba, Papanikolaou, Fickers, & Erten, 2019). CA production is mainly dependent on carbon source, nitrogen content, C/N ratio, and bioreactor conditions (i.e., oxygen transfer, pH, and temperature). Low nitrogen content (i.e. 0.1–0.4 g/L) (Gonçalves, Colen, & Takahashi, 2014) has been considered fundamental for CA production, while other authors highlighted the importance of a high C/N molar ratio (in the range of 150–391) (Cavallo, Charreau, Cerrutti, & Foresti, 2017; Cavallo, Nobile, Cerrutti, & Foresti, 2020; Ferreira, Lopes, Mota, & Belo, 2016; Levinson, Kurtzman, & Kuo, 2007; Papanikolaou, Muniglia, Chevalot, Aggelis, & Marc, 2002). Since these parameters are similar to the ones required for SCO production, some strains can produce both compounds at the same time (Dobrowolski, Mituła, Rymowicz, & Mirończuk, 2016), while, in others, CA is produced during the turnover of SCO (Makri et al., 2010). Also low sulfur and phosphorus concentrations (Arslan, Aydogan, & Taskin, 2016; Cavallo et al., 2017) or higher amount of potassium and iron (the last one only up to 2.5 mg/g) have a positive effect on CA production (Gonçalves et al., 2014; Kumar, Yellapu, Yan, Tyagi, & Drogui, 2020). Other than substrates

availability, CA synthesis is affected by external pH. Values above 4.5 are usually considered sufficient for CA production. However, *Y. lipolytica* ACA-YC 5029 produced mainly mannitol and erythritol when grown on glycerol at pH above 4.8 in flask. CA increased when the strain was grown in a batch-bioreactor, using similar substrate composition. This means that controlled pH, aeration and agitation have also a positive impact (Papanikolaou et al., 2017). An improvement of CA concentration (130% increase), moving from flask to a controlled bioreactor, was also reported by Cavallo et al. (2020) using high fructose syrup and corn steep liquor as C and N source, respectively. The most optimal conditions for high CA production on raw glycerol requires ranges of 50%–80% dissolved oxygen saturation (Morgunov, Kamzolova, & Lunina, 2013). It was also reported that dissolved oxygen tension promotes CA synthesis when the yeast is grown on glucose (Sabra, Bommareddy, Maheshwari, Papanikolaou, & Zeng, 2017). Usually, in wild type strains CA is co-produced with ICA, a molecule having potential applications in pharm and medical sector but also as a standard for food quality control. However, their co-production is not always desired since it is difficult to separate them and get pure compounds. For this reason, several authors tried to modulate CA/ICA ratio either by adapting growth parameters (i.e. substrates, micro nutrients or pH) or creating mutants (Fu et al., 2016; Kamzolova, Samoilenko, Lunina, & Morgunov, 2020; Morgunov, Kamzolova, & Samoilenko, 2013; Rzechonek, Dobrowolski, Rymowicz, & Mirończuk, 2018; Yuzbasheva et al., 2021).

### 3.1.4. Organic acids and lactones

$\alpha$ -ketoglutaric acid (KGA) and pyruvic acid (PA) are important keto acids in food, pharmaceutical, animal feed, and other industries (Li, Chen, & Lun, 2001; Otto, Yovkova, & Barth, 2011). The market price of both KGA and PA (12–15 \$/kg) is significantly higher than that of citric (0.5 \$-kg/1), fumaric (1.5 \$-kg/1) and succinic acid (2.5 \$-kg/1). Thiamine deficiency, low pH, and substrate degraded via glycolysis contribute to the secretion of both KGA and PA by impairing the Krebs' cycle. On the contrary, substrates that follow the  $\beta$ -oxidation (i.e. rapeseed oil) do not allow the accumulation of PA (Rywińska, Tomaszewska-Hetman, Rakicka-Pustulka, Juszczak, & Rymowicz, 2020). The possibility to specifically obtain KGA has been studied in the last years, either by improving the separation of the two acids (Lei, Zeng, Zhou, & Du, 2019) or avoiding PA production. In the last case, screening of wild types strains or changing growth conditions were assessed (Cybulski et al., 2018; Morgunov, Kamzolova, & Samoilenko, 2013). Eventually, *Y. lipolytica* has also been studied to produce compounds with “fruity” aroma, such as lactones. Among them, the most interesting one is  $\gamma$ -decalactone, produced from ricinoleic acid (Braga & Belo, 2016).

### 3.1.5. Polyols

Erythritol and mannitol are two important polyols used as food additives for their flavor enhancer, sweetener and humectant properties (Grembecka, 2015). In general, sugar alcohols, are produced by plants, fungi, yeasts, and bacteria to counteract stress conditions, such as the presence of osmotic stress. In *Y. lipolytica*, high C/N ratio, high sugars or similarly metabolized compounds, low pH (3–3.5) and low oxygen are key parameters that have been associated with polyols production (Papanikolaou et al., 2017). However, as already discussed for CA production, yeasts response is strain specific. Recently, Egermeier et al. (2017) showed that at pH 3.5 and after 48h of cultivation, 15 out of 20 strains tested on glycerol produced mainly mannitol (max 30 g/L), erythritol and arabitol. The other five strains, other than the polyols, produced important amounts of CA (28.9 g/L). As expected, increasing the pH to 5.5 for 72h, determined a shift in the metabolism towards CA production, that in some cases reached values above 40 g/L. However, the same 15 strains mentioned above, other than slightly higher amount of CA, continued to produce polyols. These 15 strains were all dairy isolates while among the remaining ones there were primary lab strains,

W29, NRRLYB-423 and H222 isolated from sewage, maize-processing plant and soil, respectively. Therefore, culture conditions affect the production of metabolites but also the origin of the strains has an impact. Concerning the proportion of polyols, erythritol is usually the most abundant (Tomaszewska, Rywińska, & Gladkowski, 2012) while, depending on the strain, mannitol could be dominant (around 80–88%) (André et al., 2009; Filippousi et al., 2019). With the mutant MK1, Mirończuk, Dobrowolski, Rakicka, Rywińska, and Rymowicz (2015) reported an erythritol production up to 225 g/L from glycerol. Comparing with mutants, wild types produce less polyols (X. Liu et al., 2020; Mirończuk et al., 2015; Rymowicz, Rywińska, & Marcinkiewicz, 2009). For instance, starting from raw glycerol, the mutant *Y. lipolytica* Wratislavia K1 produced more erythritol than the wild type strain A-15 (80 and 65 g/L, respectively) (Tomaszewska et al., 2012). However, the wild type isolate simultaneously produced higher concentrations of mannitol compared to the recombinant strain (14 vs 4 g/L, respectively) when NaCl concentration was modified in the medium. This shows the specificity and higher efficiency of engineered strains but lower flexibility to adapt to environmental changes. As discussed for CA, also the setup used for the process impacts the final yield of polyol (Mirończuk, Furgała, Rakicka, & Rymowicz, 2014; Papanikolaou et al., 2017; Rakicka, Biegalska, Rymowicz, Dobrowolski, & Mirończuk, 2017). Eventually, it was reported that polyols can be completely re-consumed by the microorganism after exhaustion of glycerol exclusively for maintenance energy requirements (André et al., 2009). This is an important aspect to be considered in order to define the best working parameters.

### 3.1.6. Enzymes

*Y. lipolytica* has a very efficient secretory pathway that allows to release a huge amount of proteins and enzymes. Lipases and proteases, released to retrieve nutrients necessary to sustain its growth, are the most studied ones for their industrial applications. The recent genome survey revealed 25 putative lipases and among them some extracellular (Lip2), intracellular (Lip1, Lip3 and Lip6) and cell-bounded enzymes (Lip7, Lip8) were isolated and characterized (Brígida et al., 2014; Kumari & Gupta, 2012). One of the characteristics of these enzymes is their specificity. It was reported that Lip2 has a high specificity towards saturated triglyceride–tricaprylin (C8:0) and triglycerides containing the mono-unsaturated fatty acid trioleine (C18:1), but it can act also on C12, C14 and C16 methyl esters. Instead, Lip7 and Lip8 prefer *p*-nitrophenyl C8–C12 and *p*-nitrophenyl C8–10 esters, respectively (Fickers et al., 2011). Another important feature of these lipases is their activity in a wide spectrum of temperatures ranging from 4 to 55 °C, depending on the strain (Brígida et al., 2014; Fickers et al., 2011; Parfene et al., 2011). A specific production of lipases was obtained mainly using substrates containing fats or oils, while the proteolytic activity is something that may have a stronger impact in substrates rich in proteins, such as cheese whey and soybean solid waste (Vong, Yang, & Liu, 2016; Yalcin, Bozdemir, & Ozbas, 2009). The two main proteases of *Y. lipolytica* are the acidic extracellular protease (AXP) and the alkaline extracellular protease (AEP) (Lopes, Farias, Belo, & Coelho, 2016). Even in this case, the enzymes have been reported to be active in a wide range of temperatures, even the lower ones (6 °C) (Gottardi, 2013). Among the proteins produced, 1–2% belongs to AEP; hence, over 1 g/L of AEP could be produced at high cell densities (Matoba, Fukayama, Wing, & Ogrzydziak, 1988). Production of these enzymes starting from low value and low-cost substrates represents an advantage from an economical and sustainable point of view. If for lipases these studies are still ongoing, for proteases the topic is not well explored.

### 3.2. Reduction of pollutants

Agri-food wastewaters have extremely high costs for discharges if not treated. Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD) are used to quantify the amount of oxidizable pollutants

found in wastewaters. Usually, to be disposed in sewerage and waterways, these products should have values of COD and BOD below 125 and 25 mg/L, respectively, according to the European Wastewater Plant Effluent Standards (EU 91/271/EEC). There is an important piece of literature regarding the application of *Y. lipolytica* for COD reduction (from 40 to 80%, and above) in agri-food wastewaters or side streams (Louhasakul, Cheirsilp, & Prasertsan, 2016; Louhasakul, Cheirsilp, Treu, Kougias, & Angelidaki, 2019; Oswal, Sarma, Zinjarde, & Pant, 2002; De Felice, Pontecorvo, & Carfagna, 1997; C.; Gonçalves, Lopes, Ferreira, & Belo, 2009; Lanciotti, Gianotti, et al., 2005; M.; Lopes et al., 2009; Papanikolaou, Galiotou-Panayotou, Fakas, Komaitis, & Aggelis, 2008; Sarris, Rapti, Papafotis, Koutinas, & Papanikolaou, 2019; Darvishi et al., 2019; Wu, Ge, & Wan, 2009; Lopes, Miranda, Alves, Pereira, & Belo, 2019; Tarón Dunoyer, González Cuello, & Perez Salinas, 2020). Vegetable oil side streams also contains phenolic fractions that are responsible for several biological implications, such as antibiosis and phytotoxicity. The capability of detoxifying phenol and polyphenol compounds has been reported and it seems to be strain-specific (Bankar, Kumar, & Zinjarde, 2009; Lanciotti, Vannini, et al., 2005; Lopes et al., 2009). Biosurfactants are amphiphilic organic compounds produced by microorganisms that act as detergents, wetting agents, emulsifiers, foaming agents, and dispersants. Due to their properties, they can accelerate oil-polluted chemical bioavailability and removal. Yansan, Rufisan and BS-1 are the most studied biosurfactants produced by *Y. lipolytica* using agri-food by-products (Yilmaz, Ergene, Yalcin, & Tan, 2009; Zinjarde, Apte, Mohite, & Kumar, 2014).

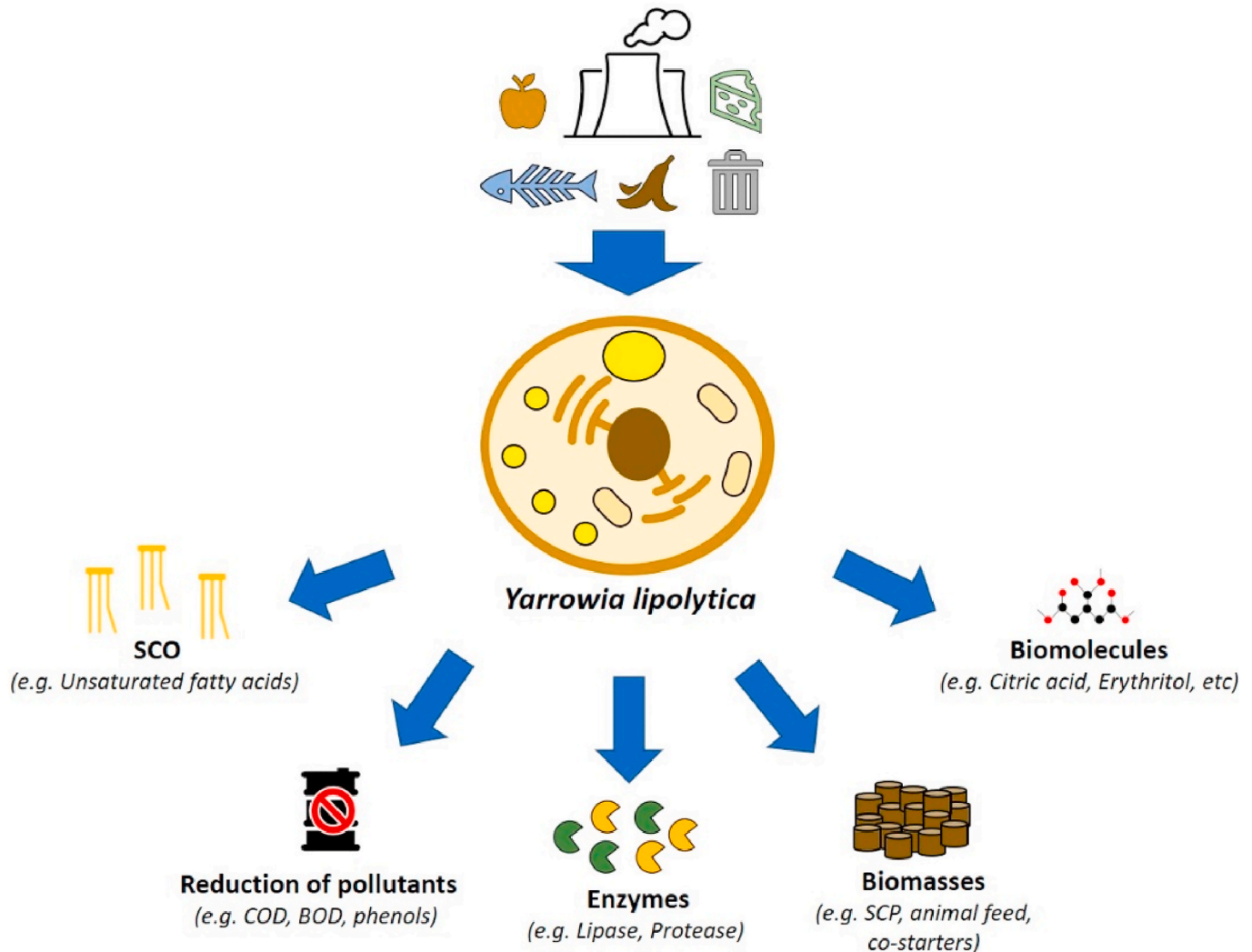
## 4. Application of wild type strains of *Y. lipolytica* in agri-food wastes and by-products

The use of low-cost agro-industrial wastes and by-products as substrates for *Y. lipolytica* increased in the last years. Studies were mainly focused in exploiting hydrophobic waste streams (vegetable oils and animal fat) or hydrophilic wastes as substrates (fruit, vegetables, dairy and others). Their valorization and recovery could be performed adding higher, or removing lower, value compounds (Fig. 1). The principal industrial wastes and by-products utilized as substrates with wild type strains of *Y. lipolytica* are reported below and summarized in Tables 1–6.

### 4.1. Vegetable oil industry

Global vegetable oil production should expand from 189.9 million tons in 2017 to 219.8 million tons by 2026, according to the last report of the Organization for Economic Co-operation and Development (OECD) and the Food and Agriculture Organization (FAO) of the United Nations (Darvishi et al., 2019). Of these, 37.6% are provided by palm and palm kernel, 30% by soybean, and the remaining 32.5% by canola, sunflower, peanut, cotton seed and olive oils (USDA 2017). Palm oil is an edible vegetable oil obtained from the mesocarp of the fruit of the oil palms (Colombo, Chorfi Berton, Diaz, & Ferrari, 2018). Palm Oil Mill Effluent (POME) represents a possible source of inland water pollution. Currently, 0.65 tons of POME are generated from the processing of every ton of palm oil (Louhasakul et al., 2016). It contains mainly lignocellulosic wastes with a mixture of carbohydrates and oil. The COD and BOD values varies a lot according to the production period, but they can range between 70–87 and 32–85 g/L, respectively (Poh, Wu, Lam & Poon, 2020). The marine isolate *Y. lipolytica* NCIM 3589 was applied on POME, without addition of nutrients or dilutions (Oswal et al., 2002) and, after 48h, it reduced COD and BOD of about 95 and 77%, respectively. Louhasakul et al. (2016) tested four different strains of *Y. lipolytica* (Table 1) on centrifuged, autoclaved, and ammonium sulfate supplemented POME. In this case, after 72h incubation, COD was reduced between 45.8 and 72.9%, and the strain TISTR 5151 produced at the same time 28.8% lipids and 3353 U/L of cell-bound lipase. Since working with minimal treading by-products could be important for a sustainable and cost-effective process, the same authors (Louhasakul

## Agri-food wastes and by-products



**Fig. 1.** Schematic representation of the possible targets reached using *Y. lipolytica* for boosting the recovery and valorization of agri-food by-products. SCO: single cell oil; COD: chemical oxygen demand; BOD: biochemical oxygen demand; SCP: single cell protein.

et al., 2019) reported the ability of *Y. lipolytica* to outcompete microbial contaminants and persist in not sterile POME. The use of untreated vegetable oil refinery wastewater (VORW) was also studied by Darvishi et al. (2019). In this case COD was reduced by 80%, but the strain tested produced also 60% of lipids. This higher increase in SCO was achieved diluting VORW (30 mL/L) and supplementing it with nitrogen sources. As expected, starting from oil as the main substrate, SCOs were rich in unsaturated fatty acids (C18:2, 36.42%; C18:1, 16.49%; C16:1, 10.53%).

Another waste, very important for the Mediterranean area, comes from the olive oil mills. In fact, they generate annually 30 million m<sup>3</sup> of wastewater (D'Annibale, Sermanni, Federici, & Petruccioli, 2006). Olive oil mill wastewater (OMW) composition depends on the type of olives, the cultivation system, and the production process (Lopes et al., 2009). Usually, it contains olive "vegetation waters," waters from processing, olive pulp, and oil, plus additional components such as sugars, polyphenols, polyalcohols, phosphate, pectins and metals. OMWs have high BOD and COD values, ranging between 12–63 and 80–200 g/L, respectively (Al-Malah, Azzam, & Abu-Lail, 2000). Scioli and De Felice (1993) were the first reporting the capability of *Y. lipolytica* to reduce COD value of OMW (146 g/L) by 80% within 24 h. However, in this study, OMW was supplemented with vitamins and yeast extract. On the other

hand, Lanciotti, Vannini, et al. (2005), using untreated and undiluted OMW, observed a high variability in response to the 62 isolates tested. In this case, the maximum COD reduction reached only 43% with the strain PO1, associated with a reduction of polyphenols (18%), and production of CA and lipases (4.2 g/L and 925 U, respectively). The lower efficiency observed compared with other studies highlights that using oil effluent as such can be sometimes a limiting factor. This explain why most of the works applied supplements such as glucose (Papanikolaou, Galiotou-Panayotou et al., 2008) or ammonium sulfate, alone or with Tween 80 (M. Lopes et al., 2009).

Eventually, waste cooking oils (WCOs), generated in household, hotels, restaurants, and catering after frying, have a production in Europe of about 1 million tons per year. Their potential value as a substrate for microorganisms was already reviewed (M. Lopes, Miranda, & Belo, 2020). Culturing conditions or supplementation of carbon and nitrogen sources could be implemented to tailor metabolite production (Domínguez, Deive, Angeles Sanromán, & Longo, 2010; M.; Lopes et al., 2019). For instance, Lopes et al. (2019) used *Y. lipolytica* W29 to treat WCO. In particular, diluted WCO supplemented with arabic gum, YNB without amino acids, ammonium sulfate and Tween 80, allowed the production of lipases (12000 U/L) and lipids, mainly based on linoleic (71%) and oleic (21%) acids. Other than supplementing pure

**Table 1**

An overview of the main vegetable oil industry wastes and by-products valorized with *Y. lipolytica*. The wild type strains applied and the final achievements obtained are also reported.

Waste or by-product	Strain	Outputs	Reference
OMW	ATCC 20255	Reduction of COD (80%)	De Felice et al. (1997)
OMW	W29 and IMUFRJ 50682	Production of lipase (70 U/L) - reduction of COD (80%) and phenols (70%)	Lopes et al. (2009)
OMW	62 wild type strains (best performing: PO1, Y17, B16, C11 and Y9)	Reduction of COD (1.47–43%), polyphenol (up to 22%) - Production of CA (up to 5.2 g/L) and lipases (35–2315 U)	Lanciotti, Vannini, et al. (2005)
OMW	CBS 2073, W29 and IMUFRJ 50682	Reduction of COD (29–52%) and production of lipase (320–1041 U/L)	Gonçalves et al. (2009)
OMW	W29 (ATCC 20 460), ACA-YC 5028, ACA-YC 5033	Production of CA (0.9–18.9 g/L), lipid (3–51%)	Sarris, Galiotou-Panayotou, Koutinas, Komaitis, and Papanikolaou (2011)
OMW	ACA-YC 5033	Production of CA (0.4–51.9 g/L), SCO (5–45%) - reduction of phenols (~51%) and colors (~58%)	Sarris et al. (2017)
OMW	ACA-DC 50109	Production of CA (28.9 g/L) and reduction of phenolic compounds (15%) and colour	Papanikolaou, Fakas, et al. (2008)
POME	TISTR 5054, TISTR 5212, TISTR 5621, TISTR 5151	Reduction of COD (45.8–93.4%) - production of lipases (61–4081 U/L), biomass (2.9–7.2 g/L) and SCO (20.2–28.8%), depending on the strain and pH	Louhasakul et al. (2016)
POME	TISTR 5151	Reduction of COD (53.7–63.5%) - production of biomass (6.9 g/L) and lipid (53.0%)	Louhasakul et al. (2019)
POME	NCIM 3589	Reduction of COD (95%) and BOD (77%)	Oswal et al. (2002)
Corn wet milling products	W29	Production of CA (35 g/L)	Cavallo et al. (2020)
Rapeseed oil processing products	20 isolates, among them A10*	* Production of KGA (72 g/L), PA (48.1 g/L), biomass (35.7 g/L) and SCO (13.2%)	Cybulski et al. (2018)
Rapeseed oil	VKM Y-2373* and a mutant	* Production of CA (~80 g/L), ICA (~70 g/L), lipase (~17 U/mg)	Kamzolova, Lunina, and Morgunov (2011)
Salad oil and grease from food wastewater	W29	Reduction of oil and COD (above 80%)	Wu et al. (2009)
Vegetable oil refinery wastewater	CBS 6303	Reduction of COD (80%) -production of SCO (60.1%) and biomass (18.3 g/L)	Darvishi et al. (2019)
WCO	W29	Production of lipase (12000 U/L), biomass (6.2 g/L) and SCO (48.3%)	Lopes et al. (2019)
WCO	NCIM 3229, NCIM 3450, NCIM 3472, NCIM 3589, NCIM 3590	Production of biomass (5.0–7.9 g/L) and SCO (22–45%)	Katre et al. (2012)
WCO	CECT 1240 (ATCC 18942)	Reduction of COD (~90%)	Domínguez et al. (2010)
Soybean oil refinery residue	UCP 0988	Production of biosurfactants	Rufino et al. (2011)

OMW, olive mill wastewater; POME, palm oil mill wastewater; WCO, waste cooking oil; CA, citric acid; KGA,  $\alpha$ -ketoglutaric acid; PA, piruvic acid; SCO, single cell oil (% w/w, dry weight biomass), COD, chemical oxygen demand; BOD, biochemical oxygen demand.

**Table 2**

An overview of the main and most recent dairy industries by-products valorized with *Y. lipolytica*. The wild type strains applied and the final achievements obtained are also reported.

Waste or by-product	Strain	Outputs	Reference
Deproteinized whey	B9	Production of Biomass (7.4 g/L) and SCO (57.9%)	Taskin et al. (2015)
Whey with fructose	59	Production of CA (49.2 g/L)	Yalcin et al. (2009)
Dairy waste	ATCC 9773	Reduction of fat (82.9%), COD (44.3%) and BOD (43.3%)	Tarón Dunoyer et al. (2020)
Partially deproteinized whey	B9	Production of CA (33.3 g/L)	Arslan et al. (2016)
Ricotta whey	NRRL YB-423, NRRL Y-1095, NRRL Y-7208	Production of biomass (1.1–1.6 g/L) and SCO (25–33%)	Carota et al. (2017)
Whey wastewaters	MFW5	Production of biosurfactants	Yilmaz et al. (2009)

CA, citric acid; COD, chemical oxygen demand; BOD, biochemical oxygen demand; SCO, single cell oil (% w/w, dry weight biomass).

compounds, oil wastewater or by-products can be valorized by creating blends of different side-streams, such as raw glycerol (Sarris, Rapti, et al., 2019). The most relevant publications regarding the use of *Y. lipolytica* with oil mill industry by-products are reported in Table 1.

**Table 3**

An overview of the main fruit and vegetable wastes and by-products valorized with *Y. lipolytica*. The wild type strains applied and the final achievements obtained are also reported.

Waste or by-product	Strain	Outputs	Reference
Okara (soya by-product)	NCYC 2904	Production of succinate (33.7 g/kg), glutamate (3.3 g/kg), short-chain methyl ketones and antioxidant compounds	Vong et al. (2016)
Grape must	59 and NBRC 1658	Production of CA (32.1 g/L)	Yalcin et al. (2009)
Mango tegument with yeast extract	IMUFRJ 50682	Production of lipases (3500 U/L)	Pereira, Fontes-Sant'Ana, & Amaral (2019)
Orange and banana peel, orange pulp and peapod	NCIM 3229, NCIM 3450, NCIM 3472, NCIM 3589 and NCIM 3590	Production of SCO (2–9%)	Katre et al. (2012)
Pineapple waste	NCIM 3589	Production of CA (202.35 g/kg)	Imandi et al. (2008)
Barley bran, tritirated nut	CECT 1240	Production of lipase (21–23000 U/L)	Domínguez, Costas, Longo, and Sanromán (2003)

CA, citric acid; SCO, single cell oil (% w/w, dry weight biomass).

**Table 4**

An overview of the main fish/seafood and meat wastes and by-products valorized with *Y. lipolytica*. The wild type strains applied and the final achievements obtained are also reported (when several conditions were tested, the maximum values were reported).

Waste or by-product	Strain	Outputs	Reference
Fish waste oil	KKP 379	Production of SCO (22.7%)	Fabiszewska et al. (2021)
Fish waste	NBRC-10073	Reduction of lipids (46%)	Yano et al. (2008)
Prawn shell and fish waste	NCIM 3229, NCIM 3450, NCIM 3472, NCIM 3589 and NCIM 3590	Production of SCO (2–14%)	Katre et al. (2012)
Mutton fat	CICC1778	Production of biomass (14.1 g/L), lipids (32.6%)	Xiong et al. (2015)
Pork lard	W29	production of SCO (57.9%), lipases (560 U/L) and CA (9.2 g/L)	Lopes et al. (2018)
Chicken waste	NCIM 3229, NCIM 3450, NCIM 3472, NCIM 3589 and NCIM 3590	Production of SCO (3–6%)	Katre et al. (2012)
Pork fat	35 different isolates	Production of lipids	Patrignani et al. (2011a)
Pork fat	Y16A	Free fatty acids and volatile compounds	Patrignani et al. (2011b)
Chicken tallow	MTCC 9520	Production of biosurfactant	Radha et al. (2020)
Derivative of tallow	ACA-DC 50109 (LGAM S(7)1)	Production of biomass (~16.0 g/L), SCO (52%) and lipase (2.5 IU/mL) in flask – production of biomass (30.5 g/L), SCO (7–16%) and lipase (0.9 IU/mL) in reactor	Papanikolaou et al. (2007)

SCO, single cell oil (% w/w, dry weight biomass); CA, citric acid.

#### 4.2. Dairy industry

Worldwide production of cheese whey is estimated to be around 190 million tons/year (Ryan & Walsh, 2016) and only 50% of this amount is processed. The remaining is considered as wastewater. However, because of its high biological and chemical oxygen demand (BOD and COD, 27–60 g/L and 50–102 g/L, respectively), whey disposal represents a serious issue from both an economical and an environmental point of view (Yadav et al., 2015). At the same time, the need of pre-treatments to stabilize and store such a perishable side-stream prior to processing makes difficult and expensive its valorization at industrial scale. Nowadays, whey excess is sometimes released into fields, with the consequent negative effects due to the high NaCl content, or used in animal feeding. Therefore, recovery of whey components and/or use of whey may be advantageous not only for the environment but also for a sustainable economy. Whey contains more than half of the solids present in the original whole milk, constituted mainly of lactose, proteins, lipids and mineral salts plus other compounds low represented (lactic, citric and uric acid, urea and B group vitamins) (Ryan & Walsh, 2016). For this reason, cheese whey represents an inexpensive and nutritionally rich raw material from which valuable compounds can be recovered and from which novel products can be generated and pollutants can be reduced. Studies regarding the application of *Y. lipolytica* in dairy by-products are not many (Table 2), maybe due to the complexity and high variability in terms of substrate composition, which depends on cheese type and milk quality. Some type strains of *Y. lipolytica* were tested for biomass production using ricotta cheese whey (pH 5.5 and C/N ratio of 55). Although *Yarrowia* strains were not the most efficient species tested in that work, 1.1–1.6 g/L of biomass and 25–33% of lipids were produced within 72h (Carota et al., 2017). Instead, Yalcin et al.

**Table 5**

An overview of the most recent works regarding crude or raw glycerol valorized with *Y. lipolytica*. The wild type strains applied and the final achievements obtained are also reported (when several conditions were tested, the maximum values were reported).

Waste or by-product	Strain	Outputs	Reference
Crude glycerol	ACA-YC 5029	Production of biomass (11.80 g/L), SCO (10.6%), CA (30.8 g/L), Ery (15.6 g/L) using ~75 g/L Glol	Sarris, Sampani, Rapti, and Papanikolaou (2019)
Crude glycerol with OMW	ACA-DC 5029	Production of CA (37.4 g/L), Man (13.1 g/L), Ara (3.1 g/L), Ery (13.5 g/L) and SCO (16%) - reduction of phenolic compounds (10%) and colour (30%) using ~70 g/L Glol and different concentrations of OMW	Sarris, Rapti, Papafotis, Koutinas, and Papanikolaou (2019)
Crude glycerol	Several wild types (A-1, A-2-4, A-3, A-6, A-15*) and mutants	* Production of biomass (15.9 g/L), Ery (65 g/L), Arb (3.5 g/L), Man (14.9 g/L) using ~150 g/L Glol with different concentrations of NaCl	Tomaszewska et al. (2012)
Crude glycerol	LFMB 19, LFMB 20 and ACA-YC 5033*	* Production of CA (50.1 g/L) and SCO (30.7%) using ~70 g/L Glol. Man (6 g/L) was produced using ~30 g/L Glol by LFMB 20	André et al. (2009)
Raw glycerol	NCIM 3589	Production of CA (77.3 g/L) using ~54.4 g/L Glol production of CA (~22.2 g/L)	Imandi, Bandar, Somalanka, and Garapati (2007)
Pure and raw glycerol	27 isolates (best performing NRRL YB-423)	Production of CA (~22.2 g/L)	Levinson et al. (2007)
Waste glycerol-based media	ACA YC 5029, ACA YC 5030	Production of Man (28.9–32.1 g/L), Ery (33.6–35.5 g/L) in flask using ~100–120 g/L Glol, only CA (39.0 g/L) in bioreactor using ~100 g/L Glol	Papanikolaou et al. (2017)
Raw glycerol	ACA-DC 50109 (LGAM S(7)1)	Production of CA (62.5 g/L), SCO (6–14%) using ~164 g/L Glol	Papanikolaou, Fakas, et al. (2008)
Crude glycerol	ACA-DC 5033* and LFMB Y19	* Max production of dry biomass (7.0 g/L), CA (16 g/L), Man (11 g/L), Ery (7.4 g/L), Ara (2.4 g/L), and SCO (47.9%)	Sarantou et al. (2021)
Crude glycerol	SM7	Production of SCO (63%) using ~830 g/L Glol	Magdoui et al. (2020)
Raw glycerol with Crustacean waste	SM7	Production of SCO (35%) and lipase (25–38 U/L) using ~40 g/L Glol	Magdoui et al. (2017)
Purified crude glycerol	SKY7	Production of SCO (37%), CA (5.4 g/L) using ~25 g/L Glol	Kumar, Yellapu, Tyagi, and Drogui (2020)
Crude glycerol	ACA-DC 50109, LFMB Y-20, LMBF Y-21,	Production of CA (27.8 g/L), Man (12.9 g/L), SCO	Diamantopoulou et al. (2020)

(continued on next page)

Table 5 (continued)

Waste or by-product	Strain	Outputs	Reference
Crude glycerol	LMBF Y-46, LMBF Y-47 and ATCC 20460	(18.8%) using ~45 g/L Glol	Kuttiraja et al. (2018)
	SKY7	Production of biomass (18 g/L), SCO (45.5%) using ~20 g/L Glol	
Crude glycerol	A-101* and mutants	* Production Ery (6.1 g/L), Man (5.5 g/L), CA (66.8 g/L) using ~150 g/L Glol	Rywińska, Rymowicz, Zarowska, and Skrzypiński (2010)
Glycerol and fatty materials	A-101	Production of B vitamins for 100 g dry biomass (thiamine 1.3 mg, riboflavin 5.3 mg, pyridoxine 4.9 mg, biotin 20.0 mg, and folic acid 249 mg)	Jach et al. (2021)

VFAs, volatile fatty acids; CA, citric acid; SCO, single cell oil (% w/w, dry weight biomass), Man, mannitol; Ara, arabitol; Ery, erythritol; Glol, glycerol.

Table 6

An overview of the most recent works regarding other substrates valorized with *Y. lipolytica* to obtain valuable compounds. The wild type strains applied and the final achievements obtained are also reported.

Waste or by-product	Strain	Outputs	Reference
VFAs with glucose or glycerol	MUCL 28849 (ATTC 8662)	Production of lipid (7.6–16.5 g/L) and biomass (34.5–38.8%)	Fontanille, Kumar, Christophe, Nouaille, and Larroche (2012)
	CECT 1240 (CBS 6124)	Production of biomass (1.3–3.9 g/L)	Llamas, Tomás-Pejó, and González-Fernández (2020)
Food waste derived VFAs	CICC 31596	Production of SCO (18.2%)	Gao et al. (2017)
Processed wheat straw	W29	Production of biomass (7.2–7.8 g/L), lipid (4.4–4.6%)	Yu, Zheng, Dorgan, and Chen (2011)

VFAs, volatile fatty acids.

(2009) used sterile whey (pH 5.2), supplemented with glucose or fructose, as a substrate for CA production. The supplementation of fructose counteracted the low specificity of *Y. lipolytica* for lactose and the high levels of proteins, favoring in turns the increase of C/N ratio. In this way, 49.2 g/L of CA were obtained. In the context of the European project INGREEN, biomasses of selected strains of *Y. lipolytica* are being produced for food sector applications, exploiting Ricotta, Caciotta and Squacquerone whey as substrates (Bains, 2020; Siroli et al., 2020). For the production of lipids, Taskin et al. (2015) used deproteinized cheese whey as substrate and the cold-adapted, lactose-positive wild type strain B9 as microorganism. Under the best optimized culture conditions (pH 5.5, 120 h, 15 °C), 57.9% SCO were obtained, mainly composed of oleic acid (18:1), *cis*-10-heptadecenoic acid (C17:1), palmitoleic acid (16:1) and palmitic acid (16:0). The same strain produced also good level of CA (33.3 g/L) in a non-sterile partly deproteinized cheese whey supplemented with lactose (20 g/L) and sodium alginate (2%) at 20 °C, pH 5.5 for 120 h (Arslan et al., 2016). Eventually, enzymes of *Y. lipolytica* ATCC 9773 were exploited to reduce the fat contaminants present in dairy waste effluents (Tarón Dunoyer et al., 2020).

#### 4.3. Fruit and vegetable processing

A huge quantity of fruit and vegetable wastes (FVW) and by-products

are produced throughout the world. For example, fruit and vegetable collection, processing, packing, distribution, and consumption already cause a 30–40% product loss. This reaches 50% in countries with limited access to advanced industrial technologies. India, the Philippines, China and the United States of America generate a total of approximately 55 million tons of FVW (Wadhwa & Bakshi, 2013). In Europe, this is estimated to be around 88 million tons (Stenmarck et al., 2016). A large proportion of these wastes are dumped in landfills or rivers, causing environmental hazards. Alternatives to such disposal methods could be their recycling as feed resources and/or their processing to generate extracts and valuable added products (Wadhwa & Bakshi, 2013). Their potential application as substrates for *Y. lipolytica* has been investigated (Table 3). Orange and banana peel, orange pulp and peapod were applied as sole carbon and energy source to produce SCO using five strains of *Y. lipolytica* (Katre, Joshi, Khot, Zinjarde, & Ravikumar, 2012). However, even if good amounts of biomasses were obtained, the yields of lipid were relatively low (2–9%). This was due to the use of pure by-products as the sole carbon and energy source, without any supplement. Pereira et al. (2019), investigated lipase production by *Y. lipolytica* IMUFRJ 50682 using mango waste streams as substrate. Mango tegument (1 g/L) was supplemented with yeast extract (2 g/L) allowing the production of about 2500 U/L lipase after 17 h in a 4-L scale reactor. Addition of yeast extract (0.34%) was also important to produce citric acid using pineapple waste. At optimal conditions *Y. lipolytica* NCIM 3589 produced 202.3 g CA for each kg of dried pineapple waste, using solid state fermentation, (Imandi, Bandaru, Somalanka, Bandaru, & Garapati, 2008). CA production was also studied in grape must. Here, no supplements were added since it was already a reach substrate. In fact, the domestic strain 59 was able to produce not negligible amount of CA (32.1 g/L) compared with the citric acid producer NBRC 1658 (10.4 g/L) (Yalcin et al., 2009). Exploitation of *Y. lipolytica* went also beyond the production of single or a few value-added compounds. For instance, Vong et al. (2016) applied *Y. lipolytica* NCYC 2904 for a solid-state fermentation of okara, an insoluble by-product obtained during soy-milk and tofu production. A good growth was observed in okara matrix at 30 °C for 5 days and this led to a significant increase in free amino acids (especially glutamate), and short-chain methyl ketones, responsible for the final umami and cheese-like flavor detected. Moreover, they observed a higher antioxidant activity. In this way application of *Y. lipolytica* valorized a by-product turning it into a nutritious and more palatable food.

#### 4.4. Animal products and fats

With relatively low market value, valorization of waste animal fats, effluents of slaughterhouses and meat processing have substantial economic potential. One of the main applications of *Y. lipolytica* in these types of products is to “upgrade” the composition of fats into high-valued oils, including cocoa butter equivalent, and polyunsaturated fatty acids. A tailor-made lipid production can be obtained modifying substrates or culture conditions (Papanikolaou & Aggelis, 2010). For instance, the use of mutton fat, promoted the production of SCO reach in oleic acid (Xiong et al., 2015). Addition of methyl stearate as a co-substrate supported the production of cocoa butter equivalent. Lopes et al. (2018) also tested the effect of culture conditions on lipid, lipase and CA production in pork lard. Varying four parameters (pH, substrate concentration, arabic gum concentration and oxygen transfer rate) they obtained three different profiles of SCOs: 1) those with similar fraction of saturated and unsaturated fatty acids, mainly palmitic and oleic acids (48 and 42%, respectively); 2) those with more unsaturated fatty acids, mainly oleic and linoleic acid (50 and 22%, respectively); 3) those with more saturated fatty acids, mainly palmitic and stearic acid (35 and 21%, respectively). This last profile represented the first report of a cocoa butter equivalent obtained from pork lard. Other than variations in growth parameters and substrates, *Y. lipolytica* strains possess a high interindividual variability for what concerns metabolic efficiency and

molecules released. Patrignani, Vannini, Gardini, Guerzoni, and Lanciotti (2011a) reported that 35 different strains gave rise to characteristic fingerprintings, in terms of released fatty acids, when grown on pork fat. Inside each strain, physicochemical conditions (i.e. temperature, water activity and inoculation level) were pivotal to modulate the release of free fatty acids as well as their further transformation into aroma compounds (Patrignani, Vannini, Gardini, Guerzoni, & Lanciotti, 2011b). Eventually, the use of chicken tallow as the sole carbon source was also applied for synthesis of biosurfactants (Radha, Suhazsini, Prabhu, Jayakumar, & Kandasamy, 2020) (Table 4).

#### 4.5. Fish and seafood

At global level, 20 million tons of wastes, equivalent to 25% of the total production of marine fishery catch, are discarded every year (Caruso, 2016). In Japan, where seafood is consumed at a high per capita level, the amount of fish waste is yearly estimated to be approximately 2 million tons, while in Europe it is 5.2 million tons per year (Caruso, 2016; Fickers et al., 2011). Fish industrial waste mainly consists of viscera, heads, bones of fish and fish which are too small to be processed. Its use to prepare fish meal is limited by the high lipid and low protein content. Yano, Oikawa, and Satomi (2008) tried to increase the value of this by-product using *Y. lipolytica* for lipid reduction (Table 4). Among the strains tested, *Y. lipolytica* NBRC-10073 had the highest efficiency in reducing the lipids by 29 and 46% in a solid state or intermittent mixing process, respectively. During fermentation, 41.5 g of crude lipids in 1 kg of the minces were reduced to 22.4 g and lipid oxidation was relatively suppressed. Katre et al. (2012) tested five strains of *Y. lipolytica* to produce SCO using fish waste or prawn shell as sole carbon and energy source. Due to the composition of the substrates, a maximum of 4% SCO was produced on prawn shell, while 14% SCO was reached on fish waste.

#### 4.6. Glycerol

Raw glycerol is a by-product of the conversion of vegetable oil, cooking oil, waste grease, or other suitable lipid feedstocks into biodiesel. For each kg of biodiesel there is a production of 0.1 kg of crude glycerol as by-product (Rywińska et al., 2013). Moreover, high quantities of glycerol-containing water can also be generated by bioethanol and/or alcoholic beverage production units (Sarris & Papanikolaou, 2016). Together with glucose, glycerol has been studied as one of the main substrates of *Y. lipolytica* to produce CA, polyols and SCO (Table 5). This aspect was extensively reviewed by Rywińska et al. (2013). Most of the recent publications focused on the optimization and enhancement of the already known yeast potentials, by creating efficient mutant strains. Instead, Magdouli, Guedri, Rouissi, Brar, and Blais (2020) adopted a metabolic approach, based on the stimulation of rate-limiting enzymes and favoring lipid synthesis, to obtain the best performances of the natural strain SM7. These functions, achieved by adding biotin, leucine and citric acid on crude glycerol, allowed the accumulation of around 63% of lipids. Other recent studies regarding the use of crude glycerol focused on combining this by-product with other food industrial side streams (Sarris, Rapti, et al., 2019). For instance, crustacean waste was used as nitrogen source with raw glycerol to enhance lipase production from 25 to 38 U/L (Magdouli, Guedri, Tarek, Brar, & Blais, 2017). Blends of raw glycerol and oil wastewater were also tested for the production of CA (37.4 g/L), mannitol (13.1 g/L), arabitol (3.1 g/L) and lipids (16% on DCW) (Sarris, Rapti, et al., 2019). The main purpose of making blends was to dilute the potential inhibiting compounds present in raw glycerol. In the last years, in fact, several researchers studied the effects of these impurities on growth efficiency (Kumar, Yellapu, Yan, et al., 2020), biomass and lipid production (Kumar, Yellapu, Tyagi, & Drogui, 2020). For instance, higher biomass productivity (0.21 g/L/h and 0.54 g/L/h, respectively) and lipid yield (0.21 and 0.124 g/g glycerol, respectively) were obtained in

purified crude glycerol compared with the use of unpurified one. These are important achievements that could also benefit the further exploitation of raw glycerol derivatives in the feed and food sectors, nowadays still limited due to presence of hazardous compounds (H. H. Liu, Lv, et al., 2015; Yang, Hanna, & Sun, 2012).

#### 4.7. Other possible substrates to produce food-related products

Food-related products obtained from biobased substrates have been used to grow *Y. lipolytica* (Table 6). Volatile fatty acids (VFAs) are interesting raw materials produced from the fermentation of a variety of organic wastes and their costs is less than the 10% of the cost of glucose (Gao et al., 2017). They have been exploited for biomass and lipid production; however, additional work is required to reduce the potential inhibitors present inside food wastes.

### 5. Genetically modified *Y. lipolytica*

In the recent years, the most performing wild type strains have been also genetically modified to improve their functionalities and final yields, outweighing their natural limitations. From one side, strains were engineered to increase substrate utilization and/or enhance the production of metabolites (Rakicka et al., 2017; Spagnuolo et al., 2018). For instance, Celińska, Nicaud, and Białas (2021) reviewed the different genetic engineering strategies employed to obtain strains able to efficiently hydrolyze common waste stream components such as starch, cellulose, xylan, and inulin. Moreover, Mano, Liu, Hammond, Currie, and Stephanopoulos (2020) created a strain capable of secreting  $\beta$ -galactosidase to hydrolyze lactose in cheese whey. This modification, together with the production of omega-3 desaturase, and overexpression of Leloir pathway genes, led to a W29-derived strain with improved performance in terms of lipid titer, yield, and productivity, compared to the wild type strain B9, on untreated whey. On the other side, recombinant strains were developed to produce novel compounds, usually not produced by *Y. lipolytica* such as carotenoids (Bruder, Melcher, Zoll, Hackenschmidt, & Kabisch, 2020; Worland et al., 2020) and limonene (Pang et al., 2019). Other possible molecules obtained by engineered strains were polyketides and aromatic amino acid-derived metabolites (Miller & Alper, 2019; Palmer, Miller, Nguyen, & Alper, 2020). Some of the most recent tools used for engineering *Y. lipolytica* seems nowadays more promising than in the past (Larroude et al., 2020). However, despite these tremendous potentials, industrial applications of engineered strains can be more complicated, especially in the food sector. Other than being less robust and less flexible than wild type strains, genetically modified microorganisms (GMMs) require extra risk assessment data focused on the changes introduced (intended and unintended) during their development (EFSA Panel on Genetically Modified Organisms (GMO) (2011). This should be performed despite the “Qualified Presumption of Safety” status possessed by *Y. lipolytica* (EFSA BIOHAZ Panel, 2018). Moreover, no matter how effective the modification might be, GMMs marketing can be impacted by the low consumer acceptance (particularly in the EU) and the regulatory restrictions for their use (Plavec & Berlec, 2020).

### 6. Conclusions

Worldwide agri-food wastes and by-products are constantly produced in large amount. Sometimes their direct disposal is not legally allowed and in other cases is too expensive. At the same time these side streams contain still precious components that can be used as substrates for microbial growth. *Y. lipolytica* is a fascinating microorganism with many potential industrial uses, even for agri-food side streams treatment and valorization. As shown in this review, *Y. lipolytica* can grow on many different substrates, either hydrophilic or hydrophobic ones. Most of the work was performed on oil polluted substrates such as vegetable oils or animal fats. These low-cost and low value lipids can be used to produce

SCOs, reducing in turns the production cost of the industrial process. Use of untreated by-products represents another source of cost-reduction. In fact, as describe above, *Y. lipolytica* can persist and be active in stringent conditions, even in presence of other microbial contaminants. At the same time, exploitation of microbial teamwork could enhance substrates availability (Gao et al., 2017). Finally, this yeast can tolerate well broad ranges of pH, salts, and pollutants. When substrates contained limiting growth elements, dilutions were performed using other by-products, making the process even more sustainable and cost-efficient. Looking from this perspective, tailored *Y. lipolytica* could be applied to valorize almost all types of waste, if these are properly formulated (i.e. C/N ratio, minerals and nutrients) and process parameters (such as. pH, oxygen, reactor, flask) are defined according to the final result required. In fact, the wide variability and adaptability found within this species can help to identify the most performing isolate for the process desired. Different strains can have different morphologies and entirely different metabolisms. These features may depend on the origin of the strains, the substrate available and the culture conditions. Therefore, an extensive characterization of the different strains as well as the optimization of their performances in relation to process sustainability can be important to tap the full potential of *Y. lipolytica*. This will lead to the valorization of side stream organic matters into valuable compounds exploitable in different industrial sectors, including food and feed industries, due to their safety feature. As environmental and economic concerns are growing worldwide, *Y. lipolytica* represents a sustainable and safe alternative for waste bioprocessing, planetary resources preservation, and the achievement of the 17 ambitious goals of UNESCO Agenda 2030.

#### Declaration of competing interest

None.

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