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Review

Single Cell Protein Production Using Different Fruit Waste: A Review

Punniamoorthy Thiviya¹, Ashoka Gamage², Ranganathan Kapilan³, Othmane Merah^{4,5,*}
and Terrence Madhujith^{6,*}

¹ Postgraduate Institute of Agriculture, University of Peradeniya, Peradeniya 20400, Sri Lanka; thiviya904@gmail.com

² Chemical and Process Engineering, Faculty of Engineering, University of Peradeniya, Peradeniya 20400, Sri Lanka; ashogamage@gmail.com

³ Department of Botany, Faculty of Science, University of Jaffna, Jaffna 40000, Sri Lanka; ranganat@ualberta.ca

⁴ Laboratoire de Chimie Agro-Industrielle (LCA), Institut National de la Recherche Agronomique, Université de Toulouse, CEDEX 4, 31030 Toulouse, France

⁵ Département Génie Biologique, IUT Paul Sabatier, Université Paul Sabatier, 32000 Auch, France

⁶ Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya, Peradeniya 20400, Sri Lanka

* Correspondence: othmane.merah@ensiacet.fr (O.M.); tmadhujith@agri.pdn.ac.lk (T.M.); Tel.: +3353-432-3523 (O.M.); Tel.: +9471-341-2171 (T.M.)

Abstract: The single cell protein (SCP) technique has become a popular technology in recent days, which addresses two major issues: increasing world protein deficiency with increasing world population and the generation of substantial industrial wastes with an increased production rate. Global fruit production has increased over the decades. The non-edible parts of fruits are discarded as wastes into the environment, which may result in severe environmental issues. These fruit wastes are rich in fermentable sugars and other essential nutrients, which can be effectively utilized by microorganisms as an energy source to produce microbial protein. Taking this into consideration, this review explores the use of fruit wastes as a substrate for SCP production. Many studies reported that the wastes from various fruits such as orange, sweet orange, mango, banana, pomegranate, pineapple, grapes, watermelon, papaya, and many others are potential substrates for SCP production. These SCPs can be used as a protein supplement in human foods or animal feeds. This paper discusses various aspects in regard to the potential of fruit wastes as a substrate for SCP production.

Keywords: bioconversion; fermentation; fruit wastes; microbial protein; single cell protein



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1. Introduction

Single cell proteins (SCPs) or microbial proteins from fruit wastes have gained increased attention in the recent past as a relatively cheap and safe protein source because of the worldwide protein scarcity. The idea of using microorganisms as a food source is not new, as in the past century, microorganisms in the form of fermented food such as bread, wine, alcoholic drinks, beer, sake, cheese, yogurt, and soya sauce have been consumed as biomass or SCPs [1–3].

SCP refers to the dead, dried microbial cells or total protein extracted from the pure microbial culture of algae, bacteria, filamentous fungi, unicellular algae, and cyanobacteria cultivated on different carbon sources that are used as a protein supplement in human foods or animal feeds [4–7]. In addition to high protein content, SCP also contains fats, carbohydrates, nucleic acids, vitamins, and minerals and is rich in certain essential amino acids, including lysine, threonine, and methionine, limited in most plant and animal feeds [8,9]. Microbial protein has become popular for its various benefits, such as high efficiency in substrate conversion, high productivity due to the fast growth rate of microorganisms, and is neither seasonal nor climate dependent. Furthermore, SCP production is possible

using a wide variety of raw materials [10]. Microorganisms utilize cheap and abundant agro-waste as a carbon and energy source for growth and to produce biomass, which can help to reduce the environmental impact caused by improper waste disposal [11].

Carbohydrate is the most widely used substrate for SCP production [12]. Various substrates of agricultural origin commonly used for the production of SCP include sugarcane bagasse, paper mill waste, rice husk, wheat straw residue, cassava waste, sugar beet pulp, coconut waste, grape waste, orange peel residue, sweet orange residue and mango waste, whey, and many others [11,13]. Among these, fruit wastes are rich in fermentable sugars and other essential nutrients that support microbial growth. Thus, fruit wastes are suitable substrates for the production of microbial proteins [10,14,15]. The majority of these fruit wastes are often improperly disposed into the environment and constitute huge environmental problems [16]. In some fruits, peels are the primary by-product representing almost 30% of the total weight [17]. In this regard, this review aimed to explore the use of different types of fruit wastes as a substrate to produce SCPs.

Various microorganisms have been used for the production of SCPs; bacteria (*Cellulomonas*, *Alcaligenes*, *Brevibacterium*, *Lactobacillus* spp., and *Rhodopseudomonas*), algae (*Spirulina* and *Chlorella*), fungus (*Aspergillus*, *Trichoderma*, *Fusarium*, and *Rhizopus*) and yeast (*Candida*, *Saccharomyces*, *Rhodotorula*, and *Rhodospiridium*) [9,10,18,19]. Bacteria possess a high protein content (50–65%, Table 1) and a short generation time. However, the use of bacteria in SCP production is limited because of the poor public acceptance, difficulty in harvesting, and high nucleic acid content compared to yeast and molds [4,20].

Table 1. Nutritional compositions of microorganisms (% dry weight) (Kalaichelvan and Arulpandi, 2019).

Microorganisms	Protein	Fat	Ash	Nucleic Acid
Fungi	30–45	2–8	9–14	7–10
Algae	40–60	7–20	8–10	3–8
Yeast	45–55	2–6	5–10	6–12
Bacteria	50–65	1–3	3–7	8–12

The filamentous nature of fungi facilitates the harvesting process, however, they have limitations, such as lower protein content (30–45%), lower growth rate, and poor acceptability [10]. Some fungal species, such as *Aspergillus*, *Fusarium*, and *Penicillium*, produce mycotoxins that are harmful to human health [21]. Therefore, such fungi must be avoided, or toxicological evaluations should be done before being recommended for SCP production. *Arthrospira platensis*, also known as *Spirulina*, is a widely used algae and has many benefits, including higher digestibility, as they lack cell walls and are rich in vitamins (A and B) [9,22]. Yeast is suitable for SCP production because of its superior nutritional quality, high lysine content, larger size making them easier to harvest, a considerable amount of lysine and tryptophan content, ability to grow on low pH, and lower nucleic acid content [10]. In addition, yeast and fungi are the most accepted and highly utilized microorganisms for SCP production because of their long history of use in traditional fermentation [3]. Further, cyanobacteria including *Spirulina* spp., *Arthrospira platensis*, and *Aphanizomenon flos-aquae*; algae including *Chlorella luteoviridis*, *Chlorella pyrenoidosa*, *Chlorella vulgaris*, *Odontella aurita*, and *Tetraselmis chuii*; yeasts, including *Saccharomyces cerevisiae*, *Fusarium venenatum*, *Yarrowia lipolytica*; and bacteria, *Clostridium butyricum*, are the accepted microorganisms for food use in the EU [6].

SCPs have a wide application in animal feed and human food supplements. SCPs are used in animal feed for fattening calves, poultry, pigs, and fish breeding, while in the food industry, they are widely used as meat substitutes, texture-providing agents, flavor enhancers, vitamin carriers, emulsifiers, and to enhance the nutritive value of baked products, soups, ready-to-serve meals, and many other food products [9,23]. Moreover, SCP is being manufactured under different commercial names such as Quorn[®], AlgaVia[®], Marmite[®], Vitam-R[®], Pruteen[®], Brovile[®], FermentIQ[™], among others [12,24].

Though SCP has been successfully commercialized in Japan, Russia, France, Finland, and England for decades [25], the study of optimal fermentation conditions, various potential substrates, and a broad range of microorganisms is still being carried out by many researchers. The search for cheap and abundant carbon sources is the domain concept as the future development of SCP production mainly depends on the type of substrate. This review mainly discussed the potential of fruit wastes as substrates in SCP production.

2. SCP

The increasing protein deficiency in an increasing world population urges us to focus on new, alternate, and unconventional protein production in order to meet the nutritional demand. Alternative proteins include SCPs, plant-based novel proteins, cultured meat, seaweed or macroalgae, and insects [6]. SCP techniques have become a more popular technology in recent days. SCP refers to the dead, dried microbial cells or total protein extracted from the pure microbial culture of algae, bacteria, filamentous fungi, unicellular algae, and cyanobacteria cultivated on different carbon sources that are used as a protein supplement in human foods or animal feeds [4,5,7,9]. Algae as a source of SCP refers to the true algae and prokaryotic cyanobacteria [1]. Despite the fact that the name suggests one single cell, biomass produced from fungi and some algae has been considered in the term SCP [26]. Carol L Wilson coined the term SCP to designate microbial biomass products in 1966 [9]. In Germany, consumption of *Saccharomyces cerevisiae* as a requirement for food increased rapidly during the First World War, and during the Second World War, aerobic yeasts, such as *Candida utilis* were produced as a food supplement mainly incorporated into soups and sausages. Since then, the rapid growth of SCPs has taken place on a large scale [8,27].

Although animal proteins are considered high-quality proteins, SCP can be a replacement for the expensive conventional plant and animal protein sources in human, animal, and fish diets. SCP or microbial protein has complied with the essential amino acid requirements and scoring patterns for adults recommended by the FAO/WHO [28–30]. In addition to the high protein content (60–82% of dry cell weight), SCP contains fats, carbohydrates, nucleic acids, vitamins, minerals, and higher essential amino acids, including lysine and methionine, which are limiting in many plant and animal proteins [8,31,32].

Potential feedstocks for SCP production are industrial wastewater, agricultural wastes, petroleum residues including fuel oil and n-paraffins, methane, heptane, methanol, biogas, CO₂, ethanol, methanol, methane, molasses, brewery residues, cellulosic biomass, and many other industrial and agricultural residues [33,34]. Microbial protein grown on agricultural wastes is emerging as one of the important protein supplements because of its higher protein content and short growth cycle of microorganisms, which lead to rapid biomass production [35]. In addition, microbes can grow on cheap nutrient sources, which result in economically beneficial protein supplements for balanced nutrition [36].

3. SCP Production Methods

The production of SCPs involves the growth of cells in a fermenter and includes processes such as washing to separate the unused medium, pre-concentration to a suitable level, final drying, and packaging [37]. After fermentation, the yeast biomass is harvested and may be subjected to downstream processing steps such as washing, cell disruption, protein extraction, and purification [35].

Solid, semi-solid, and submerged fermentation methods are the three techniques widely used to cultivate microorganisms for SCP production [4]. In solid-state fermentation, microorganisms are grown on solid substrates (rice or wheat bran, rice bran, straw, fruit, and vegetable waste) in the absence of free-flowing water. Furthermore, solid-state fermentation has been extensively studied for the production of various value-added products such as SCP, feeds, enzymes, ethanol, organic acids, biologically active secondary metabolites, B complex vitamins, pigments, and flavors, amongst others [38–40]. Semi-solid fermentation is a type of solid-state fermentation in which the free liquid content is

increased to facilitate nutrient availability and control fermentation [39]. In submerged or liquid state fermentation, substrates containing the nutrients needed for microbial growth are always used in a liquid state. Soluble sugars, molasses, liquid media, and fruit and vegetable juices are a few common substrates used in submerged fermentation [9,41]. Though the purification of the products is easier in submerged fermentation methods, it requires huge capital investment and has high operating costs [4].

Furthermore, fermenters are also classified based on the mode of operation; batch fermentation, fed-batch fermentation, and continuous fermentation. Microbial culture is inoculated to a fixed volume of media in a fermenter, and the broth is removed at the process end in the batch fermenter, while feeding rates control the nutrients supply in the fed-batch fermenter. Continuous fermentation is perfect for biomass production, where the fresh medium is continuously added, and the used medium and cells are harvested simultaneously [42]. Fermenters are equipped with aerators to supply oxygen for the aerobic process, a stirrer for mixing the medium, a thermostat for temperature control, a pH detector, and other control devices to keep different parameters required for the constant growth [4].

After fermentation, the biomass is washed, dried, and mixed up with animal feed or directly used. Generally, fermentation products contain only 1–5% solids. Thus, pre-concentration is required to facilitate the dehydration process. Pre-concentration can be done in several ways, including centrifugation followed by heating, filtration, and evaporation. The final product should be in a dry powder form which facilitates subsequent handling and decreases transportation costs. From an economic standpoint, drum drying and spray drying are the cheapest methods for water removal [4,37]. The final product should be light in color, highly soluble, high in nutritional value, and free of viable cells for human feeding purposes. In addition, the breakdown of cell walls and nucleic acid reduction would increase the digestibility and palatability [4,43]. Finally, the dried biomass is packed under a vacuum or nitrogen atmosphere, and the packaging method varies with manufacturers and the product type [35]. The basic operation in SCP production is shown in Figure 1 and shows the basic operations of SCP production.

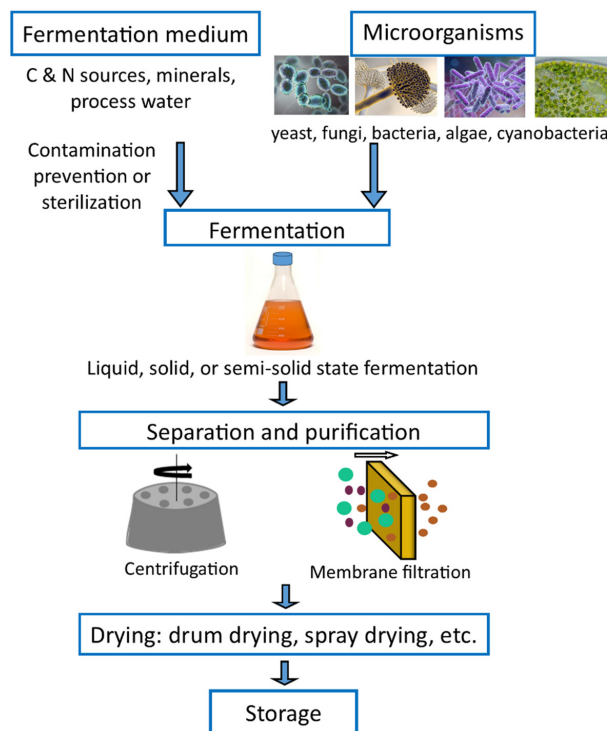


Figure 1. Schematic diagram depicting the SCP production.

4. Factors Affecting the SCP Production

According to the literature, yield and productivity of SCP production depend on various factors such as microorganism type, inoculum size, inoculum age, culture medium composition such as carbon and nitrogen sources, substrate concentration, incubation period, shake rate, and environmental conditions such as incubation temperature, medium pH, the moisture content of solid cultures, dissolved oxygen, and aeration rate [7,11,23].

Microbial strains with a low generation time, high nutritional content, low nucleic acid content, high digestibility, and pH tolerance are invariably considered for SCP production. In addition, they should be nonpathogenic and non-toxin producers or recognized as GRAS (generally recognized as safe) and have the ability to grow on different complex substrates. Other characteristics that make microorganisms suitable for SCP production include suitability in downstream processing, tolerance to high cell density, ease in handling, stability in growth rate during continuous cultivation, and good organoleptic properties [44].

Many studies focused on the effect of process parameters, including pH, temperature, substrate concentration, and fermentation time, on SCP production using various microorganisms [45,46]. Fungi usually require a lower pH than bacteria for their growth [30]. Filamentous fungi have optimum pH in the range of 3.8 to 6.0 and can grow in a wide range of pH, 2.0–9.0. Yeasts can grow at a pH of 2.5 to 8.5 with an optimal range of 4.0–5.0 [47]. Generally, the fermentation processes are carried out with mesophilic strains (temperature up to 50 °C) [47,48].

Limonene (91–95%) is a terpene compound predominantly found in citrus essential oils. Limonene and other bioactive compounds present in fruit peels exhibit antimicrobial activity in nature and inhibit the fermentation process [49]. Autoclave sterilization can remove a huge amount (62%) of limonene content [50]. Moreover, after the extraction of bioactive compounds, fruit peels can be used in the fermentation process [49].

Lignocellulosic materials contain 70% carbohydrates, mainly cellulose and hemicellulose, which require pre-treatment in order to convert carbohydrates to fermentable sugars. These pre-treatments generate numerous by-products that are toxic to yeast cells, such as phenolic compounds including vanillin and 4-hydroxybenzoic acid (PHBA), furan derivatives, like 5-hydroxymethyl-2-furaldehyde (5-HMF) and furfural and weak acids (acetic acid and formic acid) [51].

5. Substrates for the Production of SCP

The degree of SCP production depends on the substrate types and culture medium composition, which have a considerable effect on cell growth rate [7,20,23]. Substrates used for SCP production should be nontoxic, abundant, regenerable, nonexotic, inexpensive, have a carbohydrate and nutrient content, and capable of supporting rapid growth [7].

Several carbon sources are used as energy sources by microorganisms for producing SCP. Conventional materials such as starch, molasses, lignocellulosic biomass, fruit and vegetable wastes, and brewery residues, and unconventional substrates such as petroleum by-products, natural gas, ethanol, methane, and methanol have been used as the substrates for SCP production [7,9,33]. Agricultural wastes are low-cost, naturally abundant, nontoxic, and renewable resources and have increased attention in multiapplication [52].

Several agricultural and agro-industrial waste products have been used for the production of SCP and other metabolites, including whey, sugarcane bagasse, rice husk, wheat straw residue, cassava waste, sugar beet pulp, coconut waste, grape waste, orange peel residue, sweet orange residue, and mango waste [9,11]. Furthermore, important factors that influence microbial growth are the energy and carbon sources and the ability to utilize carbon sources. Microorganisms such as bacteria, fungi, yeast, and algae utilize inexpensive raw materials such as starch, lignocelluloses, and organic wastes as carbon and energy sources for cell growth. In some cases, raw materials require pre-treatment or hydrolysis before use [8]. Waste sources of carbon are customary and cheap to use. This paper mainly discusses the potential of fruit wastes as the substrate for SCP production.

6. Fruit Production and Waste Generation

Global production of fruits has been growing steadily over the past decade, and the estimated global fruit production was 883.4 metric tons (MT) in 2019, and Asia produced 512.6 MT of fruits which contributed to 58.0% of the world production. China is the first major producer of fruits globally, followed by India, Brazil, the United States, and Mexico. In 2019, the most produced fruit in the world was bananas (116.8 MT), followed by watermelons (100.4 MT), oranges (78.7 MT), mangoes, mangosteens, and guavas (55.9 MT), pineapple (28.2 MT), citrus fruits (14.5 MT), and papaya (13.7 MT) [53].

A recently published WHO/FAO report recommends a minimum of 400 g of fruit and vegetables per day (excluding potatoes, cassava, and other starchy tubers) to improve health and for the prevention of non-communicable diseases including heart diseases, cancer, diabetes, and obesity, as well as for the prevention of several micronutrient deficiencies [54]. Increasing concern for health has led to an increase in fresh fruit consumption over the past few years [55]. Increasing fresh fruit consumption leads to the accumulation of fruit skins, rinds, and the residue left over at the point of consuming fruits.

Further, fruits are generally consumed directly as food or dessert. As most fruits are seasonal and have a low shelf-life, fruits are processed into various products to extend their availability all over the year. Fruits are generally processed into bottled fruits, juices, jams, marmalades, jellies, bars, pickles, canned, frozen, concentrates, dehydrated products, alcoholic beverages, and other minimally processed products [17].

In the recent past, intensive fruit production has caused a massive generation of fruit wastes, and the improper management of these wastes can constitute a public health risk and severe environmental problems. The main solid waste in the fruit processing industry is fruit peels [56]. In general, the non-edible portion of fruits and vegetables, such as peels, pods, seeds, and skins, are discarded during processing, and it accounts for about 10–60% of the total weight of the fresh produce [57]. Peels are the primary by-product representing almost 30% of the total weight [17], and can be very high in some fruits (e.g., banana 30–40%, papaya 10–20%, pineapple 29–40%, mango 25–40%, orange 30–50%) [58–60].

Traditionally fruit wastes are used as animal feed, source of fuel, fertilizers, and various other value-added novel products, including pectin, biodiesel, bioethanol, biogas, biohydrogen, bio-oil, organic acids, enzymes, polysaccharides, flavors, coloring agents, bioactive functional phytonutrients, probiotics, edible coatings, green nanoparticles, biodegradable plastics, biochar, biosorbent, SCP, single cell oil [49,56,61–65].

The fruit processing industry generates massive waste, and the proper disposal increases processing costs. Generally, to reduce the production costs, these fruit wastes are discarded into the environment. Though the fruit wastes are biodegradable, if not processed further, these fruit wastes become spoiled rapidly and cause objectionable odor and give rise to immense environmental and health problems. Decaying fruit wastes are harbourage for microorganisms and attract pests, including flies which can cause infectious diseases and other serious health issues [13,66,67].

Agro-industrial wastes contain phenolic compounds and other toxic compounds, which may cause deterioration of the environment when the waste is discharged into the environment [68]. Fruit waste dumped in the landfills gradually rotten on landfills and releases methane, a potent greenhouse gas that traps 21 times more heat in the atmosphere than carbon dioxide [67]. Therefore, recycling or reusing fruit peel is a timely requirement. Using agro-wastes in SCP production can minimize environmental pollution associated with waste disposal and fulfil the world protein demand.

7. Physico-Chemical Properties of Fruit Waste

Physico-chemical composition gives an idea about the potential of fruit wastes in SCP production. The lignocellulosic fruit peel wastes contain a large number of soluble sugars, starch, fiber (cellulose, hemicelluloses, lignin, and pectin), ash, fat, protein, and other micronutrients. Liquid peel waste contains mainly simple sugars such as sucrose, glucose, and fructose and a significant amount of minerals and nitrogen content [58].

The solid fruit peel waste contains simple sugars (reducing and non-reducing sugars) and complex carbohydrates, such as cellulose, hemicellulose, and lignin, which can be metabolized by microorganisms [16,69]. The physico-chemical composition of fruit peel varies with fruit, types of cultivars, maturity level, geographic locations of cultivations, seasonal variations, and processing conditions (e.g., drying method, drying temperature, particle size) [17,46,70].

Carbohydrates are an abundant component in many fruit peels (above 50% of fruits' dry weight) [17,71]. Dias et al., 2020 reported that pineapple contains 83% carbohydrates, while a lower value was reported with other peels such as yellow passion fruit (59%), orange (59%), and avocado peels (8%) on a dry weight basis. Dias et al., 2020 also stated that the selected fruit peels contained a significant amount of fat and ash, and the values vary with the fruit peel varieties [72]. Ripe banana peel contains 13.8% soluble sugar, 8% crude protein, 6.2% ether extract, and 4.8% total phenolic compounds [73]. Rivas et al., 2008 stated that the orange peel contains 16.9% soluble sugars, 3.8% starch, fibre (9.2% cellulose, 10.5% hemicelluloses, 42.5% pectin and 0.8% lignin), 3.5% ash, 2.0% fats and 6.5% proteins in dry weight [74]. Orozco et al., 2014 reported that orange peel contains 14.5% hemicellulose, cellulose 11.9%, and a small amount of lignin 2.2% [75]. Many studies reported a low value for lignin which makes the fruit peels amenable to the hydrolysis process [74–76].

Furthermore, the use of fruit peel for the production of SCP is determined by its availability and low cost, composition, and absence of toxic substances and fermentation inhibitors [35]. For instance, citrus peels, such as orange peels, are rich in essential oils and limonene, a predominant component with antimicrobial property, which hinders the digestion process of microbes or fermentation process, thus resulting in less biomass production. Therefore, prior to hydrolysis, limonene is removed from the citrus bio-waste in the pre-treatment steps [20,49].

8. Fruit Waste as Substrate for SCP Production

Fruit waste is rich in carbohydrates and other essential nutrients that could support microbial growth. Thus, fruit processing waste is a potential substrate for value-added products such as organic acids, methane/biodiesel, ethanol, enzyme, secondary metabolites, organic acids, and SCP [10,14,15]. SCP production has gained more attention in recent decades, and a wide variety of fruit wastes have been used as substrates. The cost and the economic viability of SCP production largely depend on substrate cost [10]. Hence, waste from various fruits can be a suitable substrate for SCP production. Fruit peel waste is lignocellulosic wastes [77] containing simple and complex sugars that can be metabolized by microorganisms [16]. The proximate analysis also revealed that the fruit waste contained variable amounts of carbohydrates, protein, lipid, and moisture content essential for microbial growth in SCP production [20].

Many studies recently aimed at producing CP from various fruit peels by using solid-state, semi-solid, and liquid-state fermentation. Fruit peels such as beles fruit, watermelon, banana, papaya, mango, sweet orange, apple, pineapple, plantain, pomegranate rind, cactus pear, and virgin grape marc are some potential substrates used for microbial growth and SCP production [18,36,78–80]. Table 2 shows the various microorganisms and fruit wastes used for SCP production.

Table 2. SCP production using various microorganisms and fruit wastes as a substrate.

Microorganism	Substrate (Fruit Waste)	Type of Fermentation Medium	Reference
Yeast			
<i>Yarrowia lipolytica</i> (formerly <i>Candida lipolytica</i> , or <i>Saccharomyces lipolytica</i>)	Olive fruits wastes	SF/LSF	[81]
<i>Candida utilis</i>	Pineapple cannery effluent	SF/LSF	[82]
	Pineapple waste	SF/LSF	[83]
	Mixture of the banana and orange waste	SF/LSF	[84]
	Orange peel	SF/LSF	[85]
	Mango wastes	SSF	[86]
<i>Cyberlindnera</i> spp.	Banana peel hydrolysate	SF/LSF	[87]
<i>Geotrichum candidum</i>	Orange peel	SF/LSF	[88]
<i>Saccharomyces cerevisiae</i>	Watermelon, mixture of fruit wastes	SF/LSF	[89]
	Watermelon, pineapple	SF/LSF	[90]
	Yam peel	SF/LSF	[91]
	Apple, orange peel	SF/LSF	[36]
	Cucumber peel, orange peel	SF/LSF	[20]
	Pineapple waste	SF/LSF	[11,46,92,93]
	Papaya waste	SF/LSF	[94]
	Apple, papaya, banana	SF/LSF	[77]
	Guava peels and cashew bagasse	SSF	[95]
	Rind of pomegranate, mango, banana, apple, sweet orange peel	SSF	[79]
	Orange peels	SSF	[96,97]
<i>Pichia pinus</i>	Mango waste	SF/LSF	[98]
Fungi			
<i>Aspergillus niger</i>	Banana peel, orange peel, cucumber peel, pineapple peel, watermelon peel	SF/LSF	[29]
	Banana peel	SF/LSF	[99]
	Banana peel	SF/LSF	[45]
	Banana, papaya, orange	SF/LSF	[100]
	Lemon peel, orange peel, apple pomace	SSF	[101]
<i>Aspergillus niger</i> <i>Rhizopus oryzae</i>	Orange peels	SSF	[97]
<i>Aspergillus niger</i> <i>Saccharomyces cerevisiae</i>	Orange peel	SSF	[96]
<i>Aspergillus terreus</i>	Banana peel	SSF	[102]
<i>Penicillium roqueforti</i> , <i>Penicillium camemberti</i>	Bergamot fruit (citrus fruit) peel	SSF	[103]

Table 2. Cont.

Microorganism	Substrate (Fruit Waste)	Type of Fermentation Medium	Reference
<i>Phanerochaete chrysosporium</i> , <i>Panus tigrinus</i>	Banana peel, pineapple peel, papaya peel	SF/LSF	[16]
<i>Phanerochaete chrysosporium</i>	Banana peels, pineapple peels, and papaya peels	SF/LSF	[16]
<i>Rhizopus oligosporus</i>	Papaya waste, cucumber peelings, pomegranate fruit rind, pineapple fruit skin, and watermelon skin.	SSF	[104]
<i>Trichoderma viride</i> , <i>Trichoderma reesei</i>	Orange peel	SSF	[105]
Bacteria			
<i>Rhodococcus opacus</i>	Orange wastes, lemon wastes	SF/LSF	[106]
Other natural sources/mixed cultures			
Natural microorganisms in Palmyrah toddy	Papaya, watermelon, and banana peel	SF/LSF	[80]
<i>Lactobacillus</i> culture isolated from curd	Mix fruit wastes such as pineapple peel residue, pomegranate waste, apple waste, and pear waste	SF/LSF	[107]

SF/LSF, Submerged or liquid state fermentation; SSF, Solid state fermentation.

These agro-wastes used as a substrate for the selected microorganisms are composed of sugar, starch, and other cellulose materials that are metabolizable by microorganisms through the secretion of extracellular enzymes [16]. Lignocellulosic wastes such as agricultural residues and fruit peels are mainly composed of cellulose, hemicellulose, and lignin. Cellulose is converted into sugars, generally by the action of acids or cellulolytic enzymes. Starch materials such as wastes from corn, cassava, potatoes, and root crops are hydrolyzed to fermentable sugars by enzymes from malt or moulds. Cane, molasses, and fruit waste extract, like pineapple waste extract, contain valuable components, mainly sucrose, glucose, fructose, and other nutrients [108].

9. Types of Fruit Waste

9.1. Fruit Wastes Rich in Simple Sugars

SCP production depends on the type of substrate used and the composition of the culture medium. In a liquid state fermentation system, a fruit waste extract medium is used. Fruit waste extract consists of various components with a significant amount of carbohydrates, a small amount of protein, lipid, and ash [17,72,80], and they are rich in valuable components, mainly sucrose, glucose, fructose, and other nutrients [108]. Most microorganisms readily utilize simple sugars such as carbon and energy sources, and amino acids are used as nitrogen sources [109,110].

9.2. Fruit Waste Rich in Fibers

Fruit processing waste mainly consists of outer and inner shells, peels, and seeds. These fruit wastes contain fiber, and hence the waste can be categorized as structural polysaccharides-rich sources [66]. Large amounts of agro-industrial wastes such as bagasse, straw, stem, stalk, cobs, husk, and fruit peel are mainly composed of cellulose (35–50%), hemicellulose (25–30%), and lignin (25–30%), also being called “lignocellulosic materials” [111,112]. Typically, cellulose forms a skeleton surrounded by hemicellulose and lignin in lignocellulosic materials and acts as a protective barrier to cell destruction by bacteria and fungi (Figure 2).

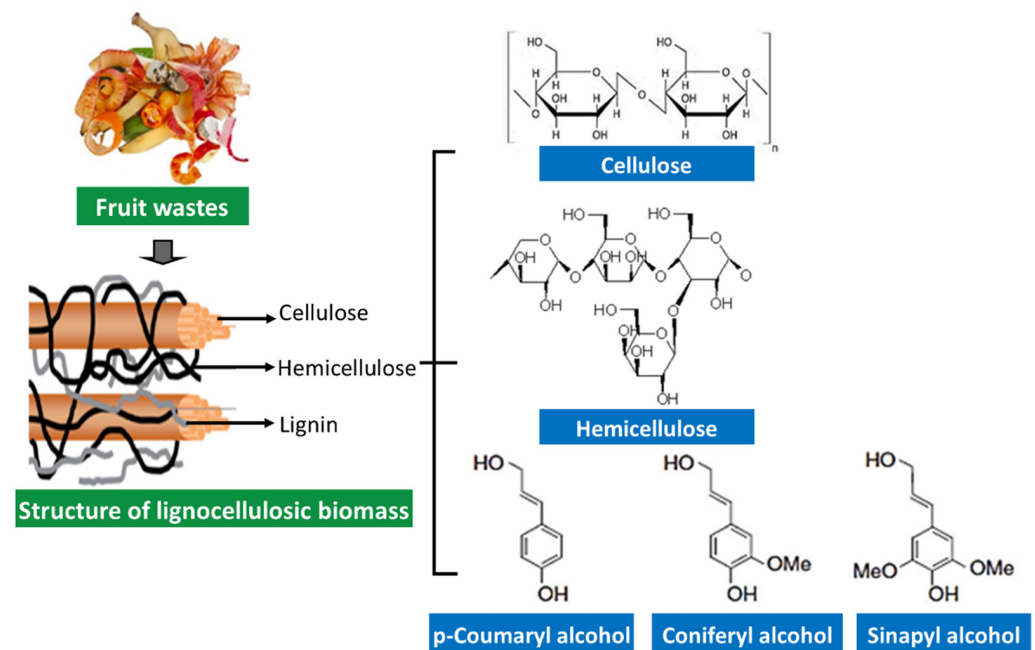


Figure 2. Structural components of lignocellulosic biomass.

Cellulose is a homopolysaccharide composed of β -D-glucopyranose units joined via β -1,4 glycosidic linkage. The long chain cellulose polymers are linked together by hydrogen and Van der Waals bonds and packed into microfibrils [68,113]. Hemicelluloses are heterogeneous polymers that comprise five main sugars (L-arabinose, D-galactose, D-glucose, D-mannose, and D-xylose) and some organic acids (acetic and glucuronic acids). Hemicellulose has different classifications based on the main sugar in the backbone: xylans, glucans, mannans, arabinans, xyloglucans, arabinoxylans, gluconoxylans, glucomannans, galactomannans, galactoglucomannans, and β -glucans. In contrast, lignin is not formed by sugar units but formed by a complex three-dimensional structure of phenylpropane units. Three phenyl propionic alcohols are primary monomers of lignin; *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol [68,114].

10. Bioconversion of Lignocellulosic Fruit Waste

The preparation and pre-treatment of lignocellulosic wastes into a suitable form for SCP production include size reduction by grinding and chopping, physical, chemical, or enzymatic hydrolysis of polymers to increase substrate availability, supplementation with nutrients (phosphorus, nitrogen, salts), and setting the pH and moisture content, heat treatment for macromolecular structure pre-degradation and elimination of major contaminants [113].

Liquid extraction of fruit wastes mainly contains soluble sugars and minerals required for microbial growth. Microorganisms can utilize these simple sugars as carbon and energy source. Therefore, the liquid extraction of fruit wastes does not require pre-treatment such as hydrolysis. However, in solid-state or semi-solid state fermenters, the substrate is used in the form of a solid. Basic macromolecular structure (cellulose, starch, lignocellulose, pectin, fiber) is a common feature in all solid agro-industrial wastes, which gives the substrate the properties of solids [113]. Microorganisms cannot utilize these structural polysaccharides, and the biomass production on lignocellulosic wastes implies a high economic cost; hence conversion (Figure 3) of these structural polysaccharides into fermentable sugars such as glucose and xylose is necessary [115].

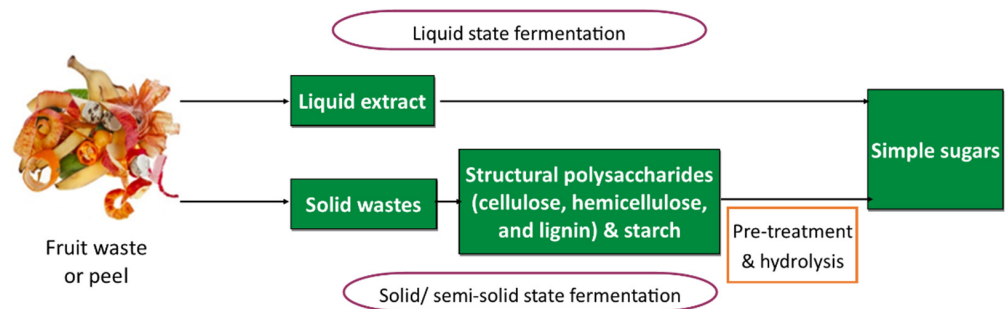


Figure 3. Bioconversion of fruit waste.

Hydrolyzing cellulose is difficult because of its large molecular structure, which imparts crystallinity and poor solubility. Further, lignin presence around the cellulose fiber prevents direct contact between cellulose and hydrolyzing solvents [116]. However, fruit wastes mainly contain free sugars besides hemicelluloses, cellulose, and lesser amounts of lignin [117]. The bioconversion of lignocellulosic materials to fermentable sugars involves a combination of pre-treatment (biological, chemical, mechanical, or a combination of three) and hydrolysis/saccharification (chemical or enzymatic) [118].

Pre-treatment is required to break down the crystalline structure of the cellulose, remove hemicellulose, and break down the lignin barrier to increase the accessibility of cellulose for hydrolysis [112,118,119]. The mechanical pre-treatment aims to reduce the overall size of the material and reduce cellulose crystallinity by means of mechanical comminution such as grinding, milling, and chipping [120]. Dilute-acid (0.5–2.0% H_2SO_4) with a high-pressure steam explosion method is the commonly used pre-treatment technique [121]. Of the three components, lignin is more resistant to hydrolysis or degradation than cellulose and hemicellulose because of its highly ordered crystalline structure.

Alkaline and acid hydrolysis methods are used to degrade lignocellulose under high temperatures; lignin is liberated, hemicellulose is readily hydrolyzed into monomeric sugars and a complex mixture of compounds that tend to inhibit the fermentation of sugars, while cellulose is essentially inert. Many other technologies such as acid, alkaline treatment, steam explosion, enzymatic hydrolysis, and subcritical and supercritical water treatments have been developed for the pre-treatment and hydrolysis of lignocelluloses [116,122].

Lignocellulose can be hydrolyzed into simple sugars either enzymatically by cellulolytic enzymes or chemically by sulfuric or other acids [123]. The enzymatic hydrolysis process of agro-industrial lignocellulose is in two steps: pre-treatment of lignocellulosic material to destroy its complicated structure and enzymatic conversion of material into fermentable sugars [119]. Lignocellulosic waste requires physical/chemical pre-treatment to liberate cellulose from lignin since cellulose in the lignin-hemicellulose-cellulose complex network is not accessible to enzymatic hydrolysis. Enzymes such as cellulases, β -glucosidases, and pectinase are highly used in the enzymatic hydrolysis of fruit peel [124]. Jahid et al., 2018 also stated that the enzymatic hydrolysis of fruit peel using cellulase and xylanase enzymes gives good yields of total reducing sugars and pentose sugars [117].

For the complete hydrolysis of cellulose, the synergistic action of four cellulase enzymes is necessary; endoglucanases, exoglucanases, β -glucosidases, and endoglucanases. Hydrolysis of the hemicellulose fraction requires a more complex group of enzymes, “hemicellulases”. Xylan is the major polymer found in hemicelluloses which require endo- β -1,4-xylanase, β -xylosidase, α -L-arabinofuranosidase, α -glucuronidase, α -galactosidase, acetylxyylan esterase and ferulic acid esterase [123].

Therefore, the use of mixed cultures of *Saccharomyces cerevisiae* and cellulolytic microorganisms such as *Aspergillus niger* can be one of the solutions to enhance the hydrolysis of fruit peels. *Aspergillus niger* is known to produce cellulase, amylase, and pectinase enzymes which can hydrolyze carbohydrates [99]. *Clostridium* spp., *Thermomonospora* spp., *Cellulomonas* spp., *Trichoderma* spp., and *Aspergillus* spp. are known to be cellulase producers. Commercially, xylanases are produced from *Trichoderma reesei*, *Aspergillus niger*,

Humicola insolens, and *Bacillus* spp. [125,126]. Celluclast® 1.5 L, Pectinex® Ultra SP-L, Novozyme 188, Cellic® CTec2, and Biogazyme 2x are some of the commercial enzymes used in lignocellulose hydrolysis [124,127].

Maximizing the bioavailability of fermentable substrate components is a key challenge in biomass pre-treatment due to the loss of sugars during conventional pre-treatment approaches. The formation of inhibitory compounds such as hydroxymethylfurfural (HMF) and luvilinic acid should be prevented during pre-treatment [124]. Among the methods used in the hydrolysis of lignocellulose wastes to release fermentable sugars, cellulolytic enzymes are the most promising method for large-scale applications (DeMartini et al., 2013). The enzymatic method is preferred than the acid or alkaline processes since they are specific biocatalysts, can operate under much milder reaction conditions (pH 4.5–5.0 and 40–50 °C), and are environmentally friendly, while some fermentation inhibitor products are generated [123,128]. However, this enzymatic method requires expensive equipment, and the price of enzymes has a high impact on SCP production from lignocellulosic wastes. Figure 4 illustrates the generalized process stages of lignocellulose bioconversion into SCP.

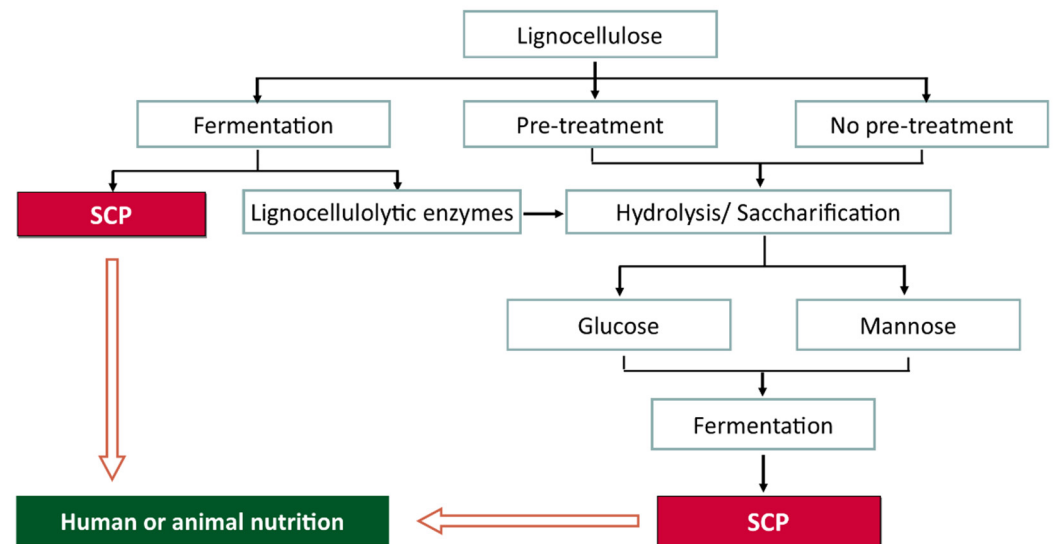


Figure 4. Generalized process stages of lignocellulose bioconversion into SCP; extracted from [129].

11. Conclusions

In conclusion, a large quantity of fruit waste is generated with an increased production rate. Fruit waste contains a significant amount of nutrients that can be utilized by microorganisms and thus can be a good source for SCP production. SCP can be produced by liquid, solid or semi-solid state fermentation system. Fruit waste extract rich in simple sugar is used in a liquid state fermentation system. Fruit waste used in solid or semi-solid state fermentation systems are rich in structural polysaccharides; cellulose, hemicellulose, lignin, and pectin. This lignocellulosic fruit waste cannot support microbial growth. Hence, the conversion of lignocellulosic wastes into simple sugars is required to increase SCP yield. The utilization of fruit wastes in SCP production not only helps to control pollution but also solves malnutrition problems by providing protein supplements at an affordable price.

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