Trends in Food Science & Technology 91 (2019) 139–146

Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: www.elsevier.com/locate/tifs

Review

Fructooligosaccharides production from agro-wastes as alternative low-cost source☆,☆☆

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ARTICLE INFO

Keywords:
Agro-wastes
Enzymes
Fructooligosaccharides
Prebiotics

ABSTRACT

Background: The prebiotic properties of fructooligosaccharides (FOS) are well documented. The high demand of functional food by the food, pharmaceutical and biotechnology industries have lead researchers to explore new and more feasible processes to produce FOS. Not only economical substrates are being exploited to reduce costs, but also, seeking to attend a global problem, the excessive generation of agro-industrial wastes that are polluting the earth, which are not being completely exploited, have been a concern.

Scope and approach: The purpose of this review is to present a concise (but wide-ranging) appraisal on the latest advances in fructooligosaccharides production from agro-wastes, as alternative low-cost source. Emphasis is placed on the examination, analysis and discussion of the prospects for using different agro-industrial waste bioresources for the production of FOS and FOS-producing enzymes.

Key findings and conclusions: The food, agro-industrial and forestry industries generate large volumes of waste, that are mainly composed of complex carbohydrates and crude proteins, that can be useful as nutrients for microbial growth, and enzymes or other metabolites production. Agro-industrial wastes are discarded, and its accumulation generates a severe environmental impact. The development of value-added processes using agro-industrial wastes is very attractive and becomes an environmentally friendly waste management method.

1. Introduction

There is a growing interest to improve human health through good nutrition. Nutraceutical and functional food industry have attracted special attention, by improving and developing new products during the last decades (Bitzios, Fraser, & Haddock-fraser, 2011). Prebiotics are ingredients used in functional foods that cannot be hydrolyzed by the gastrointestinal enzymes (Nobre et al., 2018c). They have a positive influence on the host by selectively stimulating the growth and/or activity of bacteria or a limited number of bacterial species in the colon; prebiotics are used as substrates by the probiotic bacteria for growth, impacting in the composition of bacterial communities as well as in the microbial metabolic activities. This provides a better balance in the microbiota of the host and improves health (Bindels, Delzenne, Cani, & Walter, 2015; Hutkins et al., 2016). Among the prebiotics, fructooligosaccharides (FOS) fully comply with all the described requirements. Thus, due to their beneficial attributes and promising economical potential for the sugar industry, they are attracting more and more attention (Verspreet et al., 2016) (see Table 1).

Health benefits and applications of FOS have been well documented, including the activation of the immune system and resistance to infections. FOS provide low caloric content because they are rarely hydrolyzed by digestive enzymes and are not used as a source of energy in the body. Therefore, FOS are safe to be included in products for diabetics. Also, since FOS are non-cariogenic they can be used in chewing gums and dental products. Moreover, FOS play also a very important role in reducing the levels of cholesterol, triglycerides and phospholipids, as well as helping to improve the absorption of minerals

☆ 1. Grandviewresearch.com. (2016). *Prebiotics Market Size, Share | Global Industry Report, 2024*. [online] Available at: http://www.grandviewresearch.com/industry-analysis/prebiotics-market.

☆☆ 2. Transparencymarketresearch.com. (2016). *Prebiotic Ingredients Market (FOS, GOS, MOS, Inulin) for Food & Beverage, Dietary Supplements & Animal Feed - Global Industry Analysis, Market Size, Share, Trends, and Forecast 2012–2018*. [online] Available at: http://www.transparencymarketresearch.com/prebiotics-market.html.

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https://doi.org/10.1016/j.tifs.2019.06.013
Received 25 March 2019; Received in revised form 11 June 2019; Accepted 23 June 2019
Available online 02 July 2019
0924-2244/

Table 1
Alternative substrate sources and microorganisms used to produce fructooligosaccharides (FOS) and FOS producing enzymes.

Source	Microorganism	Enzyme	Reference
Molasses	*Aspergillus niger* PSSF21	β-fructofuranosidase	Reddy et al., 2010
	Saccharomyces cerevisiae	Fructofuranosidase	Ali. 2011
	Aspergillus japonicus-FCL 119T and *Aspergillus niger* ATCC 20611	β-Fructosyltransferase	Dorta & Cruz, 2006
Sugar cane bagasse	*Aspergillus flavus* NFCCI 2364	Fructosyltransferase	Ganaie et al., 2017
	Aspergillus ochraceus	β-D-fructofuranosidase	Guimar & Terenzi, 2007
Banana peel-leaf	*A. flavus* NFCCI 2364	Fructosyltransferase	Ganaie et al., 2017
	Chrysonilia sitophila	β -fructofuranosidase	Patil, Reddy, & Sulochana, 2011
	Saccharomyces cerevisiae GVT263	β-D-fructofuranosidase	Gnaneshwar Goud et al., 2013
Agave mead	*Aspergillus oryzae* DIA-MF	Fructosyltransferase	Muñiz-Márquez et al., 2016
Coffee by-products	*Aspergillus japonicus*	β-fructofuranosidase	Mussatto & Teixeira, 2010
	Aspergillus japonicus	β-fructofuranosidase	Mussatto et al., 2013
Cassava wastes	*Rhizopus stolonifer* LAU 07	Fructosyltransferase	Lateef & Gueguim Kana, 2012
Apple pomace	*Aspergillus versicolor*	β-fructofuranosidase	Arfelli et al., 2016
Wheat bran	*Fusarium graminearum Aspergillus awamori* GHRTS	β -D-fructofuranosidase	Gonçalves et al., 2016
		Fructosyltransferase	Sathish & Prakasham, 2013
Spent osmotic solutions	*Aspergillus oryzae* N74	Fructosyltransferase	Ruiz et al., 2014
Date by-products	*Aspergillus awamori* NBRC 4033	β-D-fructofuranosidase	Smaali et al., 2012
Cashew apple	*Lactobacillus acidophilus*	n.d.	Kaprasob, Kerdchoechuen, Laohakunjit, & Somboonpanyakul, 2018

such as calcium and magnesium in the intestine (Yun, 1996; Tanriseven & Aslan 2005; Mutanda et al., 2014).

The market for prebiotics is gaining strength and is growing greatly in recent years since the consumers demands are changing and are highly influenced by the increasing consumption of healthier products (Markets & Markets, 2015). Transparency Market Research (TMR) (2013) forecasted that the global prebiotic ingredients market would be increased. It stated that the global prebiotic ingredients market was expected to rise up to USD$4.5 billion by the end of 2018. The Grand View Research Inc. (2014) projected that the market would reach USD 5.75 billion by 2020. In terms of volume, the global prebiotics market reached 581.0 kilo tons in 2013 and it is expected to turn up to 1,084.7 kilo tons by 2020 (Grand View Research Inc. 2014).

One reason for the expected growth is the development of symbiotic solutions adding probiotic strains to the FOS mixtures. These symbiotic mixtures are further incorporated into new products such as yogurts, infant formulas, low fat creams, chocolates and bakery products that is going to be fundamental for the industry. FOS are expected to substitute a diverse amount of sweeteners in the food & beverage industry including aspartame, sucralose and xylitol due to their better properties and cost effectiveness.

Conventionally FOS have been produced using sucrose as substrate, applying enzymes such as β-fructofuranosidase (FFase; 3.2.1.26) and fructosyltransferase (FTase; 2.4.1.9) to catalyze sucrose bioconversion into FOS. Synthesis of FOS is a two-stage process in which enzyme is produced in the first step and subsequently used for bio-transformation process to yield FOS under controlled conditions (Ganaie, Lateef, & Gupta, 2014). For the production of FOS, a high concentration of initial sucrose is required (Hidaka, Hirayama, & Sumi, 1988; Dominguez, Rodrigues, Lima, & Teixeira, 2013). Several strains have been reported as producers of these FOS producing enzymes, between them fungi are the most reported strains: *Aspergillus japonicus* (Mussatto, Aguilar, Rodrigues, & Teixeira, 2009, 2013; Mussatto & Teixeira, 2010; Sheu, Chang, Wang, Wu, & Huang, 2013), *Aspergillus ibericus* (Nobre et al., 2018a; 2018b), *Aspergillus oryzae* (Muñiz-Márquez et al., 2019, 2016), *Aspergillus niger* (Lateef, Oloke, Gueguim-Kana, & Raimi, 2012), *Aureobasidium pullulans* (Castro, Nobre, Duprez, De Weireld, & Hantson, 2017, 2019; Dominguez et al., 2012; Lateef, Oloke, & Prapulla, 2007), *Rhizopus stolonifer* (Lateef et al., 2008; Lateef & Gueguim Kana, 2012) and *Penicillium citreonigrum* (Nobre et al., 2019; Nascimento, Nobre, Cavalcanti, Teixeira, & Porto, 2016). At industrial level, when enzymes are produced, components of the growth media represent around 30–40% of the production cost, mainly contributed by carbon and nitrogen sources (Laxman et al., 2005). Nowadays, more profitable processes are needed to meet global demand. That has led to the search of new production processes with alternative low-cost sources. One alternative for sucrose-based FOS production is the use of agro-wastes and cheap by-products, which could be employed either for FOS production or to produce FOS producing enzymes. Some agro-wastes contain inducer molecules than can be used to stimulate the biosynthesis of such enzymes resulting in the production of FOS.

2. Agro-industrial by-products as bioresource for biotechnological production of FOS

Agro-industrial wastes/by-products are principally composed of complex carbohydrates and crude proteins that can be useful as nutrients for microbial growth and enzyme production. Among the agro-industrial wastes that could be exploited for FOS production and FOS producing enzymes are: sucrose rich solutions (sugar cane molasses, beet molasses, agave syrups, spent osmotic solutions and date-fruit by-products) fruit peels (like mango peel, banana peel, pineapple peel, orange peel. etc.), some bagasse (such as sugar cane bagasse, agave bagasse, corn bagasse, coconut bagasse, cassava bagasse, etc.), leaves (banana leaf, corn leaf, sugar cane leaf, etc.), pomaces (like apple pomace and grape pomace) and coffee processing by-products (coffee pulp, coffee husk and coffee spent grain) (Ali, 2011, pp. 48–54; Arfelli et al., 2016; Ghazi et al., 2006; Gnaneshwar Goud, Chaitanya, & Reddy, 2013; Mehta & Duhan, 2014; Muñiz-Márquez et al., 2016, 2019; Mussatto et al., 2013; Ruiz et al., 2014; Smaali et al., 2012).

3. Sugar industry by-products as bioresource for FOS production

Sugarcane is one of the main agricultural crops cultivated in tropical regions. Annual global sugarcane production was evaluated in 1600 million tons (Chandel, Silva, & Singh, 2012). As a result, millions of tons of by-products are generated every year, which have great potential to be exploited, such as sugarcane bagasse (SCB) and molasses (Galbe, 2002). These by-products can be reused and revalued giving rise to a variety of products of commercial interest (Maitan-alfenas & Visser, 2015).

Biotechnology at industrial level, offers opportunities for valorization of these residues. Over the last decades, SCB and molasses have been explored about bioconversion.

3.1. Molasses

Molasses are defined as the final viscous liquids left after the extraction of sugar from sugarcane juice (Abe et al., 2016). Molasses have a sugar content of > 43% in weight (Wu et al., 2017). Molasses are mainly produced from sugar cane, sugar beet and citrus fruits (Abe et al., 2016; Jain & Venkatasubramanian, 2017).

Among molasses that are produced during raw sugar production, another classification is given based on the sugar processing, which is important because different molasses can have different composition. The different parameters that determine the chemical composition of molasses are: plant type, cultivation area conditions, plant maturity, and juice processing level (Olbrich, 2006). Molasses commonly contain sugar, minerals, vitamins, ash and polyphenols (Manthey & Grohmann, 2001; Takara, Ushijima, Wada, Iwasaki, & Yamashita, 2007; Jain & Venkatasubramanian, 2017). Due to all its nutrients, molasses have been exploited for enzyme production. Several reports have been evaluating different agro-industrial by-products as carbon sources for the production of invertase, such as sugar cane molasses, among other wastes (Ohara et al., 2015; Arfelli et al., 2016). Reddy, Reddy, and Sulochana (2010) also reported the use of sugarcane molasses for FFase production with *A. niger* PSSF21. The best results were obtained with the basal medium containing molasses (2%) with 0.5% soybean meal supplementation, achieving after 96 h of cultivation a maximum enzyme activity of 30.84 ± 0.477 U/mL and producing 6-kestose and nystose as the major products.

Molasses were evaluated among other substrates as carbon source for the production of FFase with an improved mutant *S. cerevisiae* strain in a semi commercial scale (Ali, 2011, pp. 48–54). Fermentation parameters, such as carbon and nitrogen (sources and concentrations) air flow rate (0.2 vvm), agitation (300 rpm) and additives (NaF) were optimized. The productivity was improved > 2.0-fold in comparison to the parental strain on medium based on black strap molasses, for extracellular and intracellular FFase production, under semi commercial alcohol production conditions. Bulk production of FFase can be exploited using molasses, an inexpensive and abundant by-product with mutant *S. cerevisiae* strain.

Besides FOS production from molasses, other bioactive compounds have been produced using molasses as fermentation media, such as: isomaltulose (using un-pretreated sugar cane molasses) (Wu et al., 2017) and levan type fructans (using sugar beet molasses, starch molasses, sugar cane molasses and sugar cane syrup) (Küçüka, Kazak, & Güney, 2011; Roberto, Oliveira, Rui, Antonia, & Colabone, 2007). Sharma et al. (2016) evaluated a bioprocess using cane molasses as substrate with the strain *L. mesenteroides* to produce oligosaccharides (OS), in which successfully produced OS of DP3-DP6, approximately 124 g/kg of fresh molasses. Also, a functional monosaccharide D-psicose was produced by epimerization of D-Fructose.

Different strains have been isolated from molasses (Muñoz, Mosquera, Alméciga-Díaz, Melendez, & Sánchez, 2012; Zambelli et al., 2014). In a research conducted by Zambelli et al. (2014) filamentous fungi were isolated from molasses and jams (kiwi and fig) and their ability to produce FOS was evaluated. Two strains were selected based on FOS yield and kestose/nystose ratio, the strains were identified as *Penicilium* sizovae (CK1) and *Cladosporium cladosporioides* (CF215). Carbohydrates produced were identified by NMR analysis. *C. cladosporioides* synthesized mainly 1-kestose (158 g/L), nystose (97 g/L), 1F-fructosylnystose (19 g/L), 6-kestose (12 g/L), neokestose (10 g/L) and blastose [Fru-β(2 → 6)-Glc] (34 g/L). *P. sizovae* produced mainly 1-kestose, low amount of neoFOS and traces of levan-type FOS.

Enzymatic synthesis of FOS was performed using beet sugar syrup and beet molasses with a sucrose concentration of 620 and 570 mg/mL, respectively. A commercial pectinase preparation (Pectinex Ultra SP-L) containing transfructosylating activity from *Aspergillus aculeatus* was used. FOS maximum concentration of 388 mg/mL was achieved after 30 h reaction with syrup, and 235 mg/mL at 65 h with molasses, using 10 U transfructosylating activity per g sucrose. Therefore showing the suitability of syrup and molasses in FOS enzymatic synthesis applying Pectinex Ultra SP-L for an efficient FOS synthesis (Ghazi et al., 2006).

Another study used different concentrations of sucrose (3–25% w/v) and peptone (2–5% w/v) and compared with cane molasses (3.5–17.5% w/v total sugar) and yeast powder (1.5–5% w/v) as alternative nutrients for the formulation of a cultivation media for of *A. japonicus*-FCL 119T and *A. niger* ATCC 20611. Cellular growth, β-fructosyltransferase and FOS production were analyzed. Results showed that cane molasses and yeast powder were as good as sucrose and peptone in the formulation of media for enzyme and FOS production (around 60% w/w) (Dorta & Cruz, 2006).

Shin et al. (2004) evaluated and compared three different strains of *A. pullulans* in term of their capability to produce FOS using molasses as sucrose equivalent. *A. pullulans* KCCM 12017 was selected as the best producer because of the highest enzyme activity and FOS production was realized with a synthesis of 166 g/L FOS from 360 g/L molasses after 24 h incubation, at 55 °C and pH 5.5.

Kumar and Kesavapillai (2012) obtained invertase utilizing two agro-industrial by-products from the industry, namely, sugarcane pressmud from sugar industry and spent yeast from ethanol distilleries. Sugarcane pressmud was cultured in solid state fermentation with a combination of spent and fresh yeast (7:3) at 50% w/w of the inoculum. Maximum enzyme activity of 430 U/mg was found after 72 h fermentation, at 40 °C and pH 5.0. Enzyme production through these bioprocess provides an alternative strategy to convert agro-wastes produced in large volume by the industry into useful high value products.

3.2. Sugar cane bagasse

SCB is one of the largest sources of cellulosic agro-industrial by-products. It is a fibrous residue of cane stalks, left after the extraction of the juice from sugarcane. SCB is mainly composed of cellulose, hemicellulose and lignin (Rezende, Lima, Maziero, & Ribeiro, 2011). Every year, nearly 100 million tons of dry SCB are produced (Cheng & Zhu, 2013) and for each ton of sugar produced, 280 kg of bagasse is generated (Cerqueira, Filho, & Meireles, 2007).

It is estimated that 80 sugarcane producing countries have the potential to exploit and get more profit from SCB (Botha & Blottnitz, 2006). SCB is commonly used as the prime energy source needed in sugar mills and ethanol distilleries, and for electricity generation. Nevertheless, a large part of the bagasse generated is still underutilized, which may be exploited for several purposes, such as production of ethanol, enzymes, organic acids, paper, boards, animal feed, furfural, among others (Mussatto, Dragone, & Rocha, 2006).

Several studies describe the production of FFase by *Aspergillus* strains using agro-industrial wastes and low-cost sources. *Aspergillus niveus* was reported to produce extracellular invertase (20 U/mL) utilizing SCB with sucrose and glucose supplementation as the carbon sources (Arfelli et al., 2016; Terenzi, Somera, & Guimara, 2009).

Sugar cane bagasse has always showed great potential to exploit in bioprocessing. Guimar and Terenzi (2007) worked in the production of a thermostable extracellular FFase supplementing Khanna liquid medium, with 1% agroindustrial residues (cassava flour, corncob, crushed corncob, oat meal, rice straw, sugar cane bagasse or wheat bran) or 2% of glucose or sucrose. The extracellular.

FFase was produced at high levels by *Aspergillus ochraceus* atfer 96 h of culture, at 40 °C, in Khanna medium that was supplemented with SCB as carbon source. Later the same research group conducted a similar study employing sugar cane bagasse as carbon source in Khanna medium to produce FFase by *Aspergillus niveus* (Terenzi et al., 2009).

More recently, the great qualities of SCB as substrate for growth and FTase production by *Aspergillus flavus* NFCCI 2364 were demonstrated (Ganaie et al., 2017). Among sixteen agro-industrial wastes evaluated, SCB was the most suitable substrate for FTase production, after 96 h of fermentation. As a nitrogen source, the yeast extract (0.2 g/gds (gram

per gram dry substrate)) was found as the best source. A FTase activity of 197.10 U/gds (unit per gram dry substrate) was determined, achieving 423.18 U/gds while using recuperated enzyme after optimization of the process variables. Thus, showing the feasibility of SCB as a suitable and low cost substrate for the industrial production of FTase.

4. Alternative agro-industrial sources as novel substrates for FOS production

4.1. Aguamiel

Aguamiel is a potential fermentable by-product due to its richness in carbohydrates, minerals and proteins (Ortiz-Basurto et al., 2008). It is obtained from the mature Agave plant (8–10 years old) by cutting off the flower stalk. Aguamiel is the sap that accumulates in the central part of the agave, which is collected twice a day. An average agave plant produces about 1500 L of "aguamiel" for an approximate duration of 3–6 months (Santos-Zea, Leal-Diaz, Cortes-Ceballos, & Gutierrez-Uribe, 2012; Tovar, Olivos, & Gutierrez, 2008).

Several agave species have been documented for aguamiel production, including *A. salmiana, A. mapisaga, A. atrovirens, A. americana* and *A. ferox* (Escalante et al., 2008; Isabel Enríquez-Salazar et al., 2017). This particular substrate has high potential to be exploited due to its rich composition. A report on aguamiel extracted from *A. mapisaga* showed that it contains 11.5% wt % of dry matter, represented mainly from sugars (75 wt %) (fructose, glucose and sucrose and also 10 wt % of FOS. Minerals (3 wt%), protein (3 wt%), and amino acids (0.3 wt%) (with all essential amino acids except methionine) were also analyzed (Ortiz-Basurto et al., 2008). The most abundant mineral documented in aguamiel is phosphorous, at a range from 200 to 211 mg/mL (Escalante et al., 2008; Hernot et al., 2009). Aguamiel also contains calcium (100–400.8 mg/L), iron (1.1–21.5 mg/L), zinc (0.8–17.2 mg/L) and magnesium (16.2–100.0 mg/L) (Ortiz-Basurto et al., 2008; Santos-Zea et al., 2012; Silos-Espino et al., 2007; Tovar et al., 2008). Aguamiel is therefore a very rich source of nutrients, such as protein, amino acids and sucrose, which are essential for FOS production and FOS production enzymes.

FOS production by *A. oryzae* DIA-MF using aguamiel from *A. salmiana* was compared with Czapek Dox medium with sucrose supplementation (Muñiz-Márquez et al., 2019). The strain was able to produce FOS with both fermentation media after 24 h of culture. However, when using aguamiel as substrate, FOS yield increased two folds (20.30 g/L), with a productivity of 0.84 g_{FOS}/L.h. Enzyme production, using the same strain in aguamiel under SSF, was optimized in a previous work from the same authors (Muñiz-Márquez et al., 2016). Maximum FTase activity of 1347 U/L was obtained at 32 °C using a packing density of 0.7 g/cm^3. Inoculum rate and initial pH had no significant influence on FTase production. FOS production was also possible using concentrated Ftase and aguamiel, although with low yield of conversion (0.30 g/g) (Muñiz-Márquez et al., 2016).

4.2. Banana peels-leaves

Banana (*Musa* spp.), a global consumed fruit, is cultivated especially in tropical and subtropical areas, where it grows in a sustainable way, contributing significatively to the economy of the countries dedicated to its production (Bello et al., 2014; Gabhane et al., 2014; Zhang, Whistler, Bemiller, & Hamaker, 2005). A high amount of waste is generated from banana industry. The main residue is the banana peel, which corresponds to 30–40% of total weigh, and has been mainly used for composting, animal feeding, and protein, ethanol, methane, pectin and enzymes production (Bhatnagar, Sillanpää, & Witek-Krowiak, 2015; Happi Emaga, Andrianaivo, Wathelet, Tchango, & Paquot, 2007; Silva et al., 2013). Therefore, it is important to find applications for this waste, as it constitutes a large environmental problem (Zhang et al., 2005).

The environmental impact of the industrial production of banana (ripe peels) is based in the high content of nitrogen and phosphorus. Nevertheless, the high content of banana water makes it susceptible of modifications by microorganisms, which may be exploited to generate high value-added products (Palacios-Ponce et al., 2017).

Banana peel has been successfully employed in bioprocesses to generate enzymes. Banana peel was recently studied as substrate source for FTase production by *A. flavus* NFCCI 2364 (Ganaie et al., 2017). Enzymes with transfructosylating activity of 102.60 ± 2.19 (U/gds) were produced from banana peel and 7.26 ± 1.42% (w/w) of FOS were also synthesized. Results demonstrated that banana peel may be a good alternative nutrient source for FTase production.

Patil et al., in 2011 evaluated different agro-wastes as sole carbon source for FFase production by *Chrysonilia sitophila* PSSF84, namely sugarcane bagasse, molasses, wheat bran, sawdust, banana peel and banana leaf, and compared the results with the use of synthetic substrates, such as: sucrose, inulin, maltose, glucose, fructose, starch, galactose, dextrose and lactose. Banana leaf and wheat bran were the substrates that obtained the highest FFase atívities, 23.4 ± 0.43 U/mL and 21.67 ± 0.08 U/mL, respectively.

In a similar study, Gnaneshwar Goud et al. (2013) evaluated agro-industrial wastes, such as peels from different fruits, leaves and oil cakes from different sources, as carbon and nitrogen sources to formulate a low-cost media, effective for FFase production by *Saccharomyces cerevisiae* GVT263, as alternative to more expensive media based on sucrose, peptone, yeast extract and malt extract (the most common carbon and nitrogen substrates for FFase production). The best results were achieved using banana leaf powder (BL) as carbon source and groundnut oil cake (GOC) as nitrogen source. Also, $MnSO_4$, inoculum and incubation time were optimized. Maximum FFase production was obtained using 4% BL, 4% GOC, 0.06% MnSO4 and 0.5% of inoculum. After 48 h, FFase increased 9-folds, from 400 U/mL in basal media to 3587 U/mL, with the formulated media.

Lateef et al. (2012) proposed the biotechnological use of ripe plantain peel and kola nut pod as substrates to produce FTase. Substrates were evaluated via Submerged fermentation (SmF) and solid-state fermentation SSF with a newly isolated strain from honey identified as *Aspergillus niger*. It was found that this fungus was capable of utilizing this by-products producing extracellular FTase. The best enzyme activities were obtained by SSF, 20.77 and 27.77 U/g, using ripe plantain peel and kola nut pod, respectively. Enzymes produced were then used to prepare FOS. A mixture of kestose and nystose was obtained, with a FOS production yield of 33.24%. FOS produced were found to be safe for human consumption.

4.3. Coffee by-products

The use of agro-industrial residues including coffee wastes have been explored in several occasions (Mussatto et al., 2009, 2013, 2015; Mussatto & Teixeira, 2010). Mussatto et al. (2009) worked on FOS and FFase production using sucrose (200 g/L) with the strain *A. japonicus* ATCC 20236 immobilized on different materials: brewer's spent grain, wheat straw, corn cobs, coffee husks, cork oak, and loofa sponge. The FOS production was between 128.35 and 138.73 g/L and with a FFase activity from 26.83 to 44.81 U/mL, for the different carriers used to immobilize cells. Later in 2010, Mussatto & Teixeira conducted a similar study where agro-industrial wastes employed as corn cobs, coffee silverskin, and cork oak were used as nutrient source and support for FOS production by *A. japonicas* under SSF. In this study, FOS yield and productivity were optimized. Wastes were evaluated with and without nutrients supplementation. Among the wastes, the most interesting results were found for coffee silverskin, resulting in similar FOS production with or without nutrient supplementation. FOS were produced at 128.7 g/L and FFase activity was 71.3 U/mL. In 2013, the maximization of the production of FOS and FFase enzyme by SSF using coffee silverskin was studied (Mussatto et al., 2013). The best

moistures, inoculum concentration and temperature were stablished to maximize FOS and FFase production. Further, an economic analysis and environmental impact assessment of three different fermentation processes for the production of FOS by *A. japonicus* were also evaluated (Mussatto, Aguiar, Marinha, Jorge, and Ferreira (2015)). Sucrose solution with free or immobilized cells in corn cobs was tested under SmF and coffee silverskin was tested as support and nutrient source under SSF. The scale-up of the process was projected using data obtained at laboratory scale and considering an annual productivity goal of 200 t. Among the evaluated processes, SSF resulted as the most feasible process due to its higher productivity (232.6 t), purity (98.6%), and the fastest payback time of 2.27 years. It also turned out to be more favorable for the environment generating a lower carbon footprint (0.728 kg/kg, expressed in mass of CO_2 equivalent per mass of FOS) and less waste of water during the process. Therefore, it can be concluded that SSF process as great potential and suitability to be employed at industrial scale, providing not only an eco-friendly but also a profitable process giving use to coffee waste generated in the industry.

4.4. Cassava wastes

Cassava (*Manihot esculanta*) is recognized as one of the most essential agricultural commodities in several countries, is known as the biggest source of carbohydrate in the tropics after rice and maize and the most consumed tuber crop worldwide (Panda, Swain, Singh, & Ray, 2013). Cassava is commonly used as raw material to produce cassava starches and traditional foods and cakes. A large amount of solid wastes are generated during cassava starch processing, these include the outer skins, inner rinds and fibrous residues (Ray and Ward, 2006), which direct disposal may cause severe problems to the environment (Bhatnagar et al., 2015).

Lateef and Gueguim Kana (2012) studied the capacity of a newly isolated strain - *Rhizopus stolonifer* LAU 07, for the FTase production using cassava waste as substrate in SSF (cassava peel) and SmF (cassava steep liquor). The enzyme was produced in more than 20 U/g when 5–15% inoculum sizes were evaluated with minimal supplementation of cassava peels with yeast extract. Cassava steep liquor presented the highest FTase yield of 32.87 U/mL at 96 h under SmF. A fermentation yield of 34% FOS (1-kestose and nystose) was obtained using 60% (w/v) sucrose as substrate. This bioprocess offers an economical alternative obtained through cassava wastes, for the production of FOS, that does not require major supplementation for enzyme production, with acceptable titer (Ganaie et al., 2017; Guimar & Terenzi, 2007; Paiva Alegre, de Moraes Polizeli, Terenzi, Jorge, & Souza Guimaraes, 2009).

4.5. Apple pomace

Large amounts of wastes are produced in the apple juice industry, only 75% of apple is utilized for juice and the remainder 25% are apple pomace by-products (Shalini & Gupta, 2010). Apple pomace is the residual mixture of peels, seeds and pulp from apple. Pomace and its extracts are largely available during the harvesting season and have great potential for biotechnology industry; millions of pounds of apple waste are generated each year (Wolfe & Liu, 2003), representing a high cost of dumping and adverse impact to the environment (Mirabella, Castellani, & Sala, 2014).

Apple pomace contains a high proportion of water and insoluble carbohydrates, such as hemicellulose, cellulose and lignin. It is constituted by simple sugars (glucose, fructose, and sucrose) and other desirable components such as pectin, crude fiber, proteins, vitamins and minerals, which are worth to be recovered since they represent a good source of nutrients to be bio-transformed by microorganisms strains able to used them as nutrient source for growth (Kosseva, 2011).

Arfelli et al. (2016) explored the production and characterization of an extracellular FFase by the isolated strain *Aspergillus versicolor* using a media supplemented with apple pomace. The process was optimized with a central composite design and response surface methodology. Best fermentation conditions found were: 3% (w/v) apple pomace, 7.5 initial pH and incubation time of 12 days. FFase showed thermal stability under 55–60 °C, activity under acidic conditions (pH 3.0–6.0), and resistance to metal ions, solvents and detergents. Ganaie et al. (2017) also evaluated apple pomace for FTase production by *A. flavus* NFCCI 2364. A FTase activity of 73.62 ± 1.38 (U/gds) and a FOS formation of 4.72 ± 0.80% (w/w) were obtained. Thus, apple pomace suitability as an alternative low-cost carbon source was demonstrated.

4.6. Wheat bran

Wheat bran is a low-cost by-product of the milling industry produced in abundance. It is estimated that this by-product could generate nearly about 150 million tons of bran per year. The valorization of wheat bran is of interest, as there are different areas of opportunity to produce value-added products. Wheat bran is mainly comprised by dietary fiber, sugars and their derivatives, starch and proteins. These compounds may be exploited by microbial fermentation for generation of enzymes such as FOS producing enzymes at a low cost.

Gonçalves, Jorge, and Guimarães (2016) used wheat bran as a carbon source to produce a potential FOS producing enzyme, β-D-FFase, with *Fusarium graminearum* HB0810, under SSF conditions. *F. graminearum* colonized the substrate completely producing high levels of extracellular invertase (109 U/gds). Results demonstrated the capability of the fungus to utilize wheat bran as carbon source.

(Paiva Alegre et al., 2009) evaluated the use of several agro-industrial residues, such as wheat bran, sugar cane bagasse, oat meal, among others, by the strain *Aspergillus caespitosus*. Its ability to produce intracellular and extracellular invertases via SmF and SSF was evaluated. The extracellular activity using wheat bran under SSF was about 5.5-fold higher as compared to SmF (Khanna medium) using the same carbon source. However, the enzyme production with wheat bran combined with oat meal was 2.2-fold higher than with wheat bran alone. Nitrogen and phosphate affected the enzyme production. Remarkably, when glucose was added to the media, the extracellular activity was decreased in both SmF and SSF but the intracellular enzymes was enhanced 3-5-fold. For the above-mentioned, enzyme production using the fungal strain *A. caespitosus* and agro-industrial residues allow to produce invertase by means of SSF, reducing production costs and enhancing enzyme productivity.

Sathish and Prakasham (2013) evaluated solid substrate mixtures on FTase and FOS production *by Aspergillus awamori* GHRTS. The production of FTases was optimized via simplex lattice design. Corn cobs, wheat bran and rice bran had major impact on FTase production. The mixture of them in a ratio of 45:26:29 (corn cobs: wheat bran: rice bran) resulted in the maximum production of FTases.

4.7. Miscellaneous

Partially dry foodstuff may be obtained by osmotic dehydration. However, the remaining spent osmotic solution at the end of the process may contain large amount of organic compounds contributing as waste with high environmental impact. Ruiz et al., 2014 evaluated the use of these spent osmotic solutions (SOS) from osmotic dehydration of Andes berry (*Rubus glaucus*) and tamarillo (*Cyphomandra betacea*) to produce FOS by *A. oryzae* N74. At bioreactor scale a FOS yield of 58.51 ± 1.73% was achieved, and 305.94 g/L of FOS were produced with SOS resultant from tamarillo osmotic dehydration. While SOS from Andes berry osmotic dehydration yielded 49.17 ± 2.82% of FOS in 267.24 g/L concentration. The results showed that producing FOS from SOS can be a viable strategy to valorize this food industry residue.

Smaali et al. (2012) synthesized FOS from date by-products with immobilized FFase enzymes from *Aspergillus awamori* NBRC 4033. The effect of water-extraction volume of dates on the sucrose conversion to FOS was studied. The best results found were with an extraction volume

of 150 mL/100 g of date by-products (231.94 g/L of sucrose extracted) giving the best FOS production and productivity (123 g/L and 18.5 g/h/100 g, respectively), resulting in a sucrose conversion of 53.26%. Results offer a possible alternative to re-valorize date by-products and suggest it as a promising material with potential to be used as a low-cost source for biotechnological production of FOS.

With the aim of reducing neo-FOS production costs, Ning et al. (2012) optimized culture conditions and medium composition for 6G-FFase and astaxanthin production with *Xanthophyllomyces dendrorhous*. It was found that sucrose and corn steep liquor (CSL) were the best carbon and nitrogen sources, respectively. CSL 52.5 mL/L and pH 7.89 were stablished by central composite design. Under the optimized medium conditions Neo-FOS were produced in an amount of 238.12 g/L. A substrate cost reduction of 66.3% in comparison with that before optimization was suggested by cost analysis. A very interesting process of Neo-FOS and astaxanthin production was provided with great economic potential.

5. Conclusions and future prospects

There is a tendency towards the incorporation of agro-industrial residues in bioprocesses to generate FOS and FOS producing enzymes. In the industrial world where the implementation of low-cost processes is mandatory, and the environmental impact must be addressed, take full advantage of the resources is of huge relevance. Thus, alternative low-cost sources such as agro-wastes offer a good solution to both. Since each region in the world have different agricultural sectors, specific residues are also generated and a wide variety of alternative novel sources of nutrients emerge, such as carbohydrates, protein and minerals. Different bio-processes have been exploited based on the waste substrate accessibility and in strains able to exploit these agro-sources. Multiple strategies have been discussed to substitute synthetic carbon and nitrogen sources. Agro-wastes such as molasses, syrups, peels, bagasse, etc. produced around the world have been employed as alternative substrates to produce FOS and FOS producing enzymes. Nevertheless, there are still technical challenges to overcome, such as the cost of the raw material pre-treatment, the downstream processing and yield impact on the bioproduct cost. Persistent efforts from researchers and government interest on these bioprocesses are still needed to make the transition and produce an impact in the industry. Interdisciplinary research combining biotechnology, microbiology and engineering, focused on the development of standardized processes for use these agro-wastes as raw materials, addressing specific needs on pre-treatments and downstream processing, will allow to produce value-add products as FOS and FOS enzymes at high yields. The cost-effective standardized processes will allow their transference from laboratory to industrial scale.

Acknowledgements

Authors thank to National Council of Science and Technology (CONACYT, México) for the financial support (CVU 860996) given to the Master Program in Food Science and Technology offered by the Autonomous University of Coahuila, Mexico. This study was also supported by the Portuguese Foundation for Science and Technology under the scope of the strategic funding of UID/BIO/04469/2019 unit and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte also, Project ColOsH 02/SAICT/2017 (POCI-01-0145-FEDER-030071).

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