



Review Paper

## Biofuel production from Jerusalem artichoke tuber inulins: a review

Samarthya Bhagia<sup>1</sup>, Hannah Akinosho<sup>2,3,4</sup>, Jorge F.S. Ferreira<sup>5</sup>, Arthur J. Ragauskas<sup>1,3,4,6,\*</sup>

<sup>1</sup> Department of Chemical & Biomolecular Engineering, University of Tennessee, Knoxville, TN, USA 37996.

<sup>2</sup> Georgia Institute of Technology, Renewable Bioproducts Institute and the School of Chemistry and Biochemistry, Atlanta, 30332, USA.

<sup>3</sup> Joint Institute of Biological Sciences, Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA 37831.

<sup>4</sup> Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA 37830.

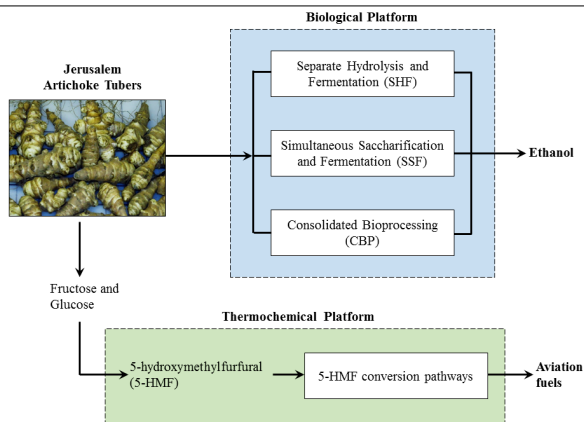
<sup>5</sup> US Salinity Laboratory, 450 W. Big Springs Rd., Riverside, CA, USA 92507

<sup>6</sup> Center for Renewable Carbon, Department of Forestry, Wildlife, and Fisheries, University of Tennessee Institute of Agriculture, Knoxville, TN, USA 37996.

### HIGHLIGHTS

- Jerusalem artichoke has high productivity of tubers that are rich in inulin.
- Inulins can be fermented into ethanol by SHF, SSF, and CBP approaches.
- Ethanol yields from Jerusalem artichoke can rival those from corn and sugarcane.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Jerusalem artichoke (JA) has a high productivity of tubers that are rich in inulins, a fructan polymer. These inulins can be easily broken down into fructose and glucose for conversion into ethanol by fermentation. This review discusses tuber and inulin yields, effect of cultivar and environment on tuber productivity, and approaches to fermentation for ethanol production. Consolidated bioprocessing with *Kluyveromyces marxianus* has been the most popular approach for fermentation into ethanol. Apart from ethanol, fructose can be dehydrated to 5-hydroxymethylfurfural followed by catalytic conversion into hydrocarbons. Findings from several studies indicate that this plant from tubers alone can produce ethanol at yields that rival corn and sugarcane ethanol. JA has tremendous potential for use as a bioenergy feedstock.

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\* Corresponding author at: Tel.: +1 (865) 974-2042  
 E-mail address: [aragausk@utk.edu](mailto:aragausk@utk.edu)

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### Abbreviations

ABE	Acetone-butanol-ethanol
CBP	Consolidated bioprocessing
DMSO	Dimethylsulfoxide
DP	Degree of polymerization
FOS	Fructooligosaccharides
GVL	$\gamma$ -valerolactone
JA	Jerusalem artichoke
5-HMF	5-hydroxymethylfurfural
LA	Levulinic acid
L/ha	Liters per Hectare
Mg/ha	Megagrams per Hectare
OF	Oligofructose
PLA	Poly(lactic acid)
RSM	Response surface methodology
SCFAs	Short chain fatty acids
SHF	Separate hydrolysis and fermentation
SSF	Simultaneous saccharification and fermentation



Fig.1. Tubers of Jerusalem artichoke cv. Stampede cultivated in large sand tanks at the US Salinity Lab (USDA-ARS), Riverside, CA.

## 1. Introduction

World's energy consumption will continue to increase significantly in the foreseeable future due to growing population, and energy consumption per capita in fast growing economies of India and China that house some 2.5 billion people (IEA, 2015). Renewable sources of energy need to be harnessed for meeting future energy demands (Bhagia, 2016). Roughly 60% of petroleum worldwide is consumed by the transportation sector and renewable fuels like cellulosic ethanol can be utilized to reduce dependency on petroleum. Moreover, low-cost, price-stable biofuels offer new opportunities for rural development and offer improved environmental benefits (EIA, 2016). However, today's relatively low oil prices reduce cost-competitiveness of cellulosic ethanol which have hindered the development of new energy technologies that need large financial commitments before they mature. Sugars locked as cellulose and hemicellulose in low-energy intensive plants like poplar and switchgrass can be converted by fermentation into ethanol at high yields, and advances in transgenic plants, pretreatment technologies, and fermentation have greatly reduced the process intensity of cellulosic ethanol (Ragauskas et al., 2006). Despite these benefits, the cost of ethanol made from lignocellulosic sources is currently higher than starch-based ethanol derived from food crops, corn, and sugarcane, due to the facile breakdown of starch into glucose. However, these food crops do not address the issue of climate change as corn ethanol reduces carbon dioxide emissions only by 13% compared to 83% by cellulosic ethanol (Farrell et al., 2006). This begs the question if it is possible to find a source of low cost fermentable sugars like glucose from corn and sugarcane, but unlike these feedstocks is a low-energy intensive crop which at the same time does not compete for food, can utilize low-grade agricultural soils, and is relatively productive and versatile to grow. One of these sources is Jerusalem artichoke (JA) (*Helianthus tuberosus*), a plant of the sunflower family that has high productivity of tubers in soil (Fuchs, 1987). Figure 1 shows an image of tubers from Stampede cultivar grown by the US Salinity Laboratory in Riverside, CA. JA was grown in North America along with strawberries, blueberries, cranberries, pecans, and sunflower seeds before Native Americans brought the "three sisters"; i.e., corn, beans, and squash from Mexico (Hurt, 2002; Nester, 2016). Its tubers carry high amounts of non-structural sugars mainly in the form of inulin (Fig. 2), a fructan polymer, that is easy to breakdown into fructose and glucose (Kosaric et al., 1984). These hexoses can be easily converted by microorganisms into ethanol at high yields which is the deciding factor for its successful application as high volume-low cost renewable transportation fuel. JA has been envisioned as an energy crop since the oil crisis in 1970s but never explored on a large-scale (Margari

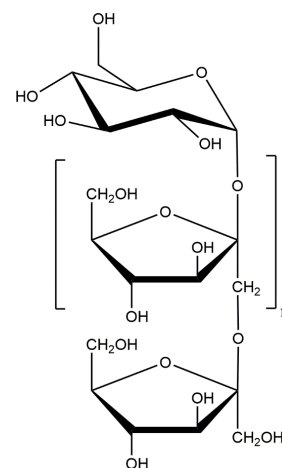


Fig.2. Structure of inulin (G-Fn) (Stevens et al., 2001).

and Bajpai, 1982c). Its advantage over other plants is its ability to thrive on less fertile land (Duvnjak et al., 1981), in saline and alkaline soils, survival in drought or cold conditions (Margaritis et al., 1981), and ability to resist pathogens (Denoroy, 1996).

Apart from ethanol production, there are several uses of JA tubers and its inulins. Inulins fulfill the role of prebiotics as its  $\beta$ -2,1 linkages cannot be cleaved in the gastrointestinal tract of humans (Bach Knudsen and Hessov, 1995) but can be broken down in the large intestine by bifidobacteria that have inulinase producing capabilities (Biedrzycka and Bielecka, 2004). These bacteria ferment inulins into short chain fatty acids (SCFAs) and organic compounds, and their proliferation helps metabolism and immunity of humans (Pokusaeva et al., 2011). They are applicable as sugar substitutes for people suffering from diabetes as inulins can have 30-50% of the sweetness of sucrose but very low calorific value (Kelly, 2008). Moreover, they are used as thickening or bulking agent in foods and find applications in drug delivery (Barclay et al., 2010). Tubers are fed to

animals like pigs for their high nutritional value (Buclaw, 2016; Dias et al., 2016). Juice of JA has been used in fermentation to produce succinic acid (Gunnarsson et al., 2014a), 2,3-butanediol (Gao et al., 2010; Li et al., 2010), butanol (Thaysen and Green, 1927; Chen et al., 2010; Sarchami and Rehmann, 2014), and single cell oil (Zhao et al., 2011). JA has been shown to produce L-lactate at a yield of 0.96 g/g reducing sugars with *Bacillus coagulans* XZL4 for renewable production of polylactic acid (PLA), a biodegradable polyester (Wang et al., 2013).

This review discusses findings from several studies on tuber and inulin yields, effect of cultivar and environment on tuber productivity, fermentation of sugars in tubers of JA, and conversion of fructose into renewable fuels and chemicals. Fermentation for ethanol production can be carried out as separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), and consolidated bioprocessing (CBP). Since the 1980s, several studies have investigated *Kluyveromyces marxianus*, a natural inulinase producer for breaking down inulins for conversion into ethanol. Recent works have introduced inulinase genes in *Saccharomyces cerevisiae* strains for efficient fermentation of juice from tubers of JA. The last section of this review discusses degradation of fructose into 5-hydroxymethylfurfural (5-HMF) that can serve as a platform for hydrocarbons and other intermediates.

## 2. Yields of tubers from JA

JA can be planted in spring and shoot emergence can take 2-5 weeks. Tubers formation initiates 5-13 weeks after planting around the same time the plant starts flowering. This is followed by continuous increase in size of tubers and storage of inulins while the foliage dies off. The maximum dry weight of tubers is achieved when the aerial biomass is completely dry (Denoroy, 1996). Harvesting aimed for maximum weight of tubers can vary between 4 and 9 months depending on early, mid, or late cultivar (Chabbert et al., 1985b; Baldini et al., 2004; Dias et al., 2016). Among the cultivars grown in Montpellier, France, that were planted in mid-March, *Fuseau 60* and *Nahodka* had maximum tuber yield in early October while *Violet de Rennes*, *Kharkowskii*, and *Medius* had maximum tuber yield at the end of November. *K8* cultivar had maximum yield in mid-January (Chabbert et al., 1985b). Gunnarsson et al. (2014b) grew 11 clones of JA in Alnarp, Sweden. They planted tubers in mid-May and found that highest yield of 44 Mg/ha occurred in December.

JA originated in temperate climate of North America but can be successfully grown in several environmental conditions (Losavio et al., 1996; Pimsaen et al., 2010). Fresh tuber yields ranging between 61.2 and 88.2 Mg/ha from cultivation in south Italy showed that JA can adapt well in hot and arid climate (Baldini et al., 2006). Like South Italy's Mediterranean climate, Bragança, Portugal, also has the same warm and dry summer climate (average summer temperature 18-22°C), where the local JA clone *Bragança* gave a fresh tuber yield of 65.6 Mg/ha when irrigated with water amount of 460 mm. Highest fresh tuber yield 30.3 Mg/ha was achieved from growing JA in a semi-arid tropical environment in Chaiyaphum, Thailand, that had minimum and maximum average temperatures of 19.7 and 29.3°C, respectively. (Pimsaen et al., 2010).

The average tuber yield from 20 genotypes varied between roughly 1.64 and 3.15 kg per plant tested over a period of 8 years (Zorić et al., 2016). Other reports found 1.7 kg/plant (Dias et al., 2016), 3.7-4.6 kg/plant (Paungbut et al., 2012), 1.5 kg/plant (Liu et al., 2011), 0.8-1.72 kg/plant (Slimestad et al., 2010). JA can be planted at density of about 40,000 to 55,000 plants/ha (Pimsaen et al., 2010; Dias et al., 2016). Table 1 summarizes tuber productivities (Mg/ha) from several studies. Based on these studies, tuber dry matter yields can range from 9 to 15 Mg/ha or 30 to 90 Mg/ha on a wet basis. Productivity of JA tubers can be affected by length of growing season, temperature, as well as water and salt stress (Paungbut, 2015; Dias et al., 2016).

Zorić et al. (2016)'s statistically inclined study on tuber variables with 20 cultivars over an eight year period had important findings (Zorić et al., 2016). Tuber yield per plant and number of tubers were dependent both on genotype and environmental conditions, but mass of individual tubers was dependent largely on genotype. Ruttanaprasert et al. (2016) studied the effect of water stress over a two year period on five cultivars of JA. Like the findings of Zorić et al. (2016), they too found significant variation among tuber and above-ground biomass yields among cultivars under water stress. In another study, researchers found that tuber dry matter weight had a positive correlation with drought tolerance index of root parameters like root weight, root diameter, etc. under mild and severe water stress (Ruttanaprasert et al., 2015).

**Table 1.**  
Jerusalem artichoke tuber yields.

Tuber Yield (Mg/ha)*	Reference
9 (dry) 45 (fresh)	Duvnjak et al. (1981)
42 (wet) 11 (dry)	Chabbert et al. (1983)
34.9-39.5 (wet) 6.8-9.1 (dry)	Chabbert et al. (1985b)
80 (wet) 16 (dry)	Conde et al. (1991)
13 (dry)	Newton et al. (1991)
47-61.8 (fresh)	Klug-Andersen (1992)
90 (fresh)	Swanton et al. (1992)
30-70 (fresh) 4-15 (dry)	Denoroy (1996)
37.6-41 (fresh) 9.8-10.7 (dry)	Losavio et al. (1996)
46.4-54.4 (fresh)	Schorr-Galindo and Guiraud (1997)
13 (dry)	Baldini et al. (2004)
55.5-80 (fresh)	Baldini et al. (2006)
65.6 (fresh) 18.4 (dry)	Rodrigues et al. (2007)
30.3 (fresh)	Pimsaen et al. (2010)
7.1 (dry)	Liu et al. (2011)
44 (fresh)	Gunnarsson et al. (2014b)
9.1-10.6 (dry)	Li et al. (2015)
92 (fresh)	Dias et al. (2016)

\* 1 Megagram equals 1 metric ton.

In a two-year field trial by Baldini et al. (2006) in three sites in Italy with JA and chicory (*Cichorium intybus*), one of the sites was in Bari, Italy which has a Mediterranean climate. This kind of climate in South Italy is characterized by hot and dry summer (average temperature of warmest month over 22°C) and mild and wet winter (average temperature of coldest month between 18 and 0°C). However, JA had the highest tuber productivity of 80 Mg/ha from this site where the total rainfall in the years 1999 and 2000 were 240 mm and 400 mm, respectively. The authors emphasized that low water availability can be compensated by good root depth and reachable water table in soil (Baldini et al., 2006). Apart from discovering higher yields from JA than chicory in all three sites, they found that high water availability totaling 826 mm from rainfall and irrigation in Bologna, Italy, was not favorable for JA tubers. Instead, excess water led to increase in above-ground biomass. With *Nahodka* cultivar field trial in Spain, tuber yield decreased only from 15.7 Mg/ha to 12.7 Mg/ha when irrigation was reduced from 1051 mm to continuous partial stress of 513 mm in the whole crop cycle. (Conde et al., 1991). A study by Zoric et al. (2016) in Bac'ki Petrovac, Serbia, reported lowest and highest precipitation of 139 mm and 663 mm in year 7 and year 8, respectively. In eight cultivars, tuber yields per plant positively correlated with precipitation. However, in six cultivars, there was no difference in tuber yield per plant in these two extreme cases of precipitation. Surprisingly, in the six remaining cultivars, tuber yields per plant were significantly higher in year 7 of low precipitation than year 8 of high precipitation. Overall, these studies indicate that drought as well as excess water can affect tuber yield while selection of the appropriate cultivar may mitigate water stress, excess water may only favor shoot over tuber accumulation. Moreover, one article mentioned that water availability may be the most important factor affecting yields, as this plant utilizes soil resources efficiently, and is quite resistant to pathogens and diseases (Denoroy, 1996).

JA is classified as a moderately salt-tolerant plant (Newton et al., 1991). The US Salinity Laboratory in Riverside, CA (Dias et al., 2016) recently studied effect of salinity between electrical conductivities of 1.2 and 9.3 dS/m (deci-Siemens/meter) in blended and sequential irrigation strategies. In the blended strategy, high and low (fresh) salinity water were mixed prior to irrigation. In the sequential strategy, low-salinity irrigation was followed by higher salinity irrigation thirty days after plantation, adjusted to match the final conductivities of blended strategy. In the blended strategy, when irrigation water electrical conductivity was increased from 1.2 dS/m to 12 dS/m, tuber yield per plant dropped from 1.66 kg to 0.88 kg. In their

experimental setup, an irrigation water conductivity of 9.3 dS/m corresponded to 3.97 dS/m in soil. Soils with conductivity of 4 dS/m are considered saline. For a perspective, sea water has a salinity around 55 dS/m (Gorham, 1992). One critical finding from this work was that in sequential irrigation strategy, tuber yields did not drop significantly as in 1.2 (control) and 9.3 dS/m (saline) tuber yields were 1.72 and 1.67 kg per plant, respectively. An irrigation water salinity of 6.6 dS/m in blended irrigation strategy resulted in a fresh weight tuber yield of 83 Mg/ha, only 11% lower than the tuber yield achieved with low-salinity water of 1.2 dS/m (Dias et al., 2016). Thus, with proper salinity management, this plant has the potential for cropping in regions of higher salinity where other crops fail to survive.

### 3. Characteristics of inulin and its yields from JA

Inulins are  $\beta(2\rightarrow1)$  fructans that often terminate in a glucose molecule linked by an  $\alpha(1\rightarrow2)$  bond. The fructose units in their furanose ring structure are linked together like a polyethylene oxide linear chain as shown in Figure 2. Inulins are non-reducing if they have glucose attached at the end of the fructan chain, however, lack thereof, makes them reducing (Mensink et al., 2015). Their solubility in water decreases with increase in degree of polymerization (DP). When their DP is 2-9, they can be classified as fructooligosaccharides (FOS) or oligofructose (OF) (Biedrzycka and Bielecka, 2004). Inulins become insoluble in water near 23 DP. They form a gel in water when concentration is higher than 10-15% at room temperature (Glibowski and Wasko, 2008). They are sparingly soluble in ethanol and isopropanol but highly soluble in dimethylsulfoxide (DMSO). The linkages of inulin are more susceptible to cleavage at low pH than neutral or high pH. L'homme et al. (2003) found first-order rate constants to be ten times higher at pH 4.0 than pH 7.0. At 80°C, half-lives of DP 5 FOS (glucose-fructoses) were 408 and 6178 min at pH 4.0 and 7.0 respectively. Matusek et al. (2009) tested stability of a commercially-available FOS between pH 2.7 and 3.3 at different temperatures and times. They found degradation to be very low at 60°C but increased significantly at 70°C and above. DP dropped to 1-2 at 90-100°C in 30-40 min.

Inulin production is initiated close to the flowering period. Li et al. (2015) found that inulin content was 3.5% in tuber 10 d before flowering and reached a maximum of 12.21% 40 d after flowering and decreased to 7.3%, 80 d after flowering, all percentages based on weight of wet tubers, and can have a wide-range of DP. Although DP of inulins has been reported to vary between 2 and 60, DP in JA tubers is at the lower end of this range. Dias et al. (2016) reported inulin DP between 6 and 8 for *Stampede* cultivar. Similar DP range was found for three cultivars in another study (Chabbert et al., 1985b). Li et al. (2015) found a maximum DP of 19. One study reported low average DP of 4 to 5 (Slimstad et al., 2010). Baldini et al. (2004) reported DP ranging between 4.8 and 11.2 (Baldini et al., 2004). Chabbert et al. (1983) found a DP of 12 (Chabbert et al., 1983). DP of inulins is not constant and changes depending on the stage of growth of the plant. Gunnarsson et al. (2014a) found a strong negative correlation between tuber yield and DP of inulin. Another study reported that content of inulin in tubers and their DP were correlated (Li et al., 2015). DP can be high in the initial period of tuber growth, but it can be relatively low around the time when tuber yield is at its maximum (Gunnarsson et al., 2014b).

Carbohydrate potential from tubers can be anywhere between 5 to 14 Mg/ha (Chabbert et al., 1985b; Swanton et al., 1992; De Mastro et al., 2004; Dias et al., 2016). A field trial by De Mastro et al. (2004) in hot arid climate in South Italy (Mediterranean region) with *Violetto di Rennes* cultivar concluded that for sugar yield from tubers, the best time for harvest is late November to early December. They argued that if the goal is to harvest the whole plant, a mid-October harvest would give the highest yields. However, if only above-ground biomass needs to be recovered for a multi-year crop, then harvesting should be done around the time JA starts flowering, i.e., between August and September (Baldini et al., 2004). This was also reported for the cultivar *Stampede* when grown in sand tanks in southern California (Dias et al., 2016).

### 4. Fermentation of sugars from tubers of JA

Yeasts like *S. cerevisiae* and *K. marxianus* and bacterium *Zymomonas mobilis* have native metabolic pathways for efficient conversion of hexoses into pyruvate. *S. cerevisiae* carries out this function through the Embden-Meyerhof-Parnas (EMP) glycolytic pathway whereas *Zymomonas* does it through Entner-Doudoroff (ED) glycolytic pathway. The latter pathway generates only 1 ATP (adenosine triphosphate) compared with 2 ATP in the EMP pathway, which results in less cell mass and high ethanol productivity in

fermentation with *Zymomonas* (Clomburg and Gonzalez, 2010). Under anaerobic conditions and/or high glucose concentrations, for fast energy production, metabolic flux is driven to produce acetaldehyde through the action of pyruvate decarboxylase which is then converted by alcohol dehydrogenase to produce ethanol and carbon dioxide (Otterstedt et al., 2004). However, these organisms do not naturally metabolize pentoses like xylose and arabinose, and even though much progress has been made to introduce pentose conversion pathways, achieving high yields from these sugars are still challenging. This is a problem for lignocellulosic feedstocks as they can have hemicellulose content of 15-30% that is largely made of five carbon sugars like xylose (Aristidou and Penttilä, 2000). This is not a problem in fermentation of JA tuber inulins as they are made only of fructose and glucose. Moreover, JA flour or juice may contain 6-7% protein that reduces demand for adding nitrogen externally for optimum growth of cells (Cieslik et al., 2011). However, one issue with fermentation of JA sugars is that they are rich in fructose compared with glucose. A portion of sugars can remain unhydrolyzed in the fermenter hexose transporters of *K. marxianus* that prefer glucose over fructose. Activation of these transporters need to be investigated for more efficient ethanol fermentation (Yuan et al., 2012). Table 2 summarizes values reported for ethanol productivity (L/ha or kg/acre) from several studies. Table 3 provides a comprehensive list of organisms, substrate, ethanol yield, and volumetric ethanol productivity from studies that carried out fermentation of JA tubers.

**Table 2.**  
Ethanol yields from Jerusalem artichoke tubers.

Ethanol production (L/ha)	Reference
3131-7513	Margaritis et al. (1981)
2500-6500	Guiraud et al. (1981)
5509	Sachs et al. (1981)
3900-4500	Duvnjak et al. (1981)
4383-8452	Margaritis and Bajpai (1982c)
5635-9392	Margaritis and Bajpai (1983a)
5000	Chabbert et al. (1985b)
4678	Kim and Handy (1986)
6498	Newton et al. (1991)
3060-11000	Gunnarsson et al. (2014b)

*Z. mobilis* may have a higher sugar uptake and ethanol yield and productivity, lower cell biomass, tolerance at higher ethanol concentrations, and easier genetic manipulation than *Saccharomyces* (Rogers et al., 1982; Hobley and Pamment, 1994). However, *S. cerevisiae* can produce high ethanol yields but can tolerate higher concentration of inhibitors like 5-hydroxymethylfurfural (5-HMF) (Lujan-Rhenals et al., 2014). With acid or high temperature hydrolysis, a small portion of glucose and fructose are degraded to 5-HMF but it is less inhibitory to yeast than furfural generated by degradation of xylose which lowers yield of ethanol in lignocellulosic biomass based fermentation processes (Sanchez and Bautista, 1988). *K. marxianus* has lower ethanol yield and tolerance compared with *S. cerevisiae* (Wang et al., 2016).

Inulins from JA tubers need to be broken down into monomeric fructose and glucose as the starting point for their conversion into ethanol. One way is to hydrolyze the inulins uses acidic conditions at higher temperatures and the second way employs inulinase to break them down enzymatically prior to fermentation. These are the two approaches of SHF. The third way of CBP uses microorganisms that synthesize inulinases for the dual role of inulin depolymerization followed by metabolism. The fourth way of SSF involves adding inulinase externally in the fermentation reactor for a one-pot process. This may also include addition of culture of inulinase producing organism, like *Aspergillus niger*, along with ethanologenic organism. One problem of the SSF approach is that microorganisms prefer temperatures of 25-35°C while inulinases have optimum activity at

**Table 3.**  
Ethanol fermentation of Jerusalem artichoke tuber sugars.

Type	Organism	Substrate	% of theoretical ethanol yield	Time (h)	Ethanol productivity (g/L/h)	Other remarks	Reference
CBP	<i>Kluveromyces fragilis</i>		98	-	-	-	
CBP	<i>Kluveromyces marxianus</i>	Juice extract	97.5	-	-	-	Guiraud et al. (1981)
CBP	<i>Torulopsis colliculosa</i>		92	-	-	-	
CBP	<i>Kluveromyces marxianus</i>		87.5	30	1.68	-	
CBP	<i>Kluveromyces cicerisporus</i>	Unhydrolyzed juice	85.7	30	1.55	-	Duvnjak et al. (1981)
CBP	<i>Kluveromyces fragilis</i>		79	30	1.25	-	
CBP	<i>Kluveromyces fragilis</i> ATCC 28244	Juice extract	96	-	13.5	-	Margaritis and Bajpai (1981)
SHF	<i>Saccharomyces cerevisiae</i> 125	Acid hydrolyzed juice	78	20	-	-	
SHF	<i>Saccharomyces diastaticus</i>	Acid hydrolyzed juice	84	20	-	-	Duvnjak et al. (1982)
CBP	<i>Kluveromyces fragilis</i>	Unhydrolyzed juice	92	50	-	-	
CBP	<i>Kluveromyces marxianus</i>	Juice extract	-	-	104	Immobilized cells	Margaritis and Bajpai (1982b)
CBP	<i>Kluveromyces marxianus</i> UCD (FST) 55-82	Juice extract	90	-	7	CSTR	Margaritis and Bajpai (1982a)
CBP	<i>Kluveromyces marxianus</i> UCD (FST) 55-82	Unhydrolyzed juice	88	-	-	-	Margaritis and Bajpai (1982c)
CBP	<i>Saccharomyces rosei</i> UWO (PS) 80-38		88	-	-	-	
CBP	<i>Kluveromyces marxianus</i> LG	Juice extract	98% sugar conversion	-	-	-	Chabbert et al. (1983)
CBP	<i>Kluveromyces marxianus</i>	Juice extract	-	-	118	Immobilized	Margaritis and Bajpai (1983b)
CBP	<i>Kluveromyces marxianus</i> UCD (FST) 55-82	Juice extract	0.45 g/g sugars utilized	-	-	-	Margaritis and Bajpai (1983a)
CBP	Flocculent cells of <i>Kluveromyces marxianus</i> SM 16-10	20% sugars from acid hydrolysis of juice	94	-	17.21-21	Continuous fermentation	Bajpai and Margaritis (1986)
CBP	<i>Kluveromyces marxianus</i> IGC2671	215 g/L total sugars	78	30	0.35	-	Rosa et al. (1987)
CBP	<i>Kluveromyces marxianus</i>	100-300 g/L sugars from acid hydrolysis of juice	86	24	11	-	Bajpai and Margaritis (1987)
SHF	<i>Zymomonas mobilis</i> ZM4F	100 g/L	0.41 g/g sugars	-	67.2	-	Allias et al. (1987)
SHF	<i>Zymomonas mobilis</i> ZM4	Acid hydrolyzed juice	78	-	-	-	
SHF	<i>Zymomonas mobilis</i> ZM4	Enzymatically hydrolyzed juice	88	-	-	-	Kim and Rhee (1989)
SSF	<i>Aspergillus ficuum</i> inulinase + <i>Zymomonas mobilis</i> ZM4	-	96	-	3.7	-	
SSF	<i>Aspergillus niger</i> 817 inulinase + <i>Saccharomyces cerevisiae</i> 1200	Ground tubers	92	15	5.5	-	
SSF	<i>Aspergillus niger</i> 817 inulinase + <i>Saccharomyces cerevisiae</i> 1200	Juice concentrate	52	72	1.7	-	Nakamura et al. (1996)
SSF	<i>Aspergillus niger</i> 817 culture + <i>Saccharomyces cerevisiae</i> 1200	Ball-milled tuber flour	80	120	1.3	-	
SHF	<i>Kluveromyces fragilis</i> + <i>Saccharomyces cerevisiae</i> Bc16a	Enzymatically hydrolyzed tubers (Rubik cultivar)	86	72	-	-	Szambelan et al. (2004)

**Table 3.**  
(Continued)

Type	Organism	Substrate	% of theoretical ethanol yield	Time (h)	Ethanol productivity (g/L/h)	Other remarks	Reference
SHF	<i>Kluveromyces fragilis</i> + <i>Zymomonas mobilis</i> 3883	Enzymatically hydrolyzed tubers (Rubik cultivar)	94	72	-	-	
SHF	<i>Kluveromyces fragilis</i> + <i>Saccharomyces cerevisiae</i> Bc16a	Enzymatically hydrolyzed tubers (Albik cultivar)	82	72	-	-	Szambelan et al. (2004)
SHF	<i>Kluveromyces fragilis</i> + <i>Zymomonas mobilis</i> 3883	Enzymatically hydrolyzed tubers (Albik cultivar)	88	72	-	-	
CBP	<i>Kluveromyces marxianus</i> ATCC8554	200 g/L tuber flour	91.5	60	1.05	-	Yuan et al. (2008)
SHF	<i>Saccharomyces</i> sp. W0	Sugars after enzymatic hydrolysis	0.384 g of ethanol/g of inulin	96	-	-	Zhang et al. (2010)
CBP	<i>Saccharomyces</i> sp. W0/YCPlac33 PGK/CYC1-INU1	0.5 g/ml tuber meal	0.319 g ethanol/g sugar	144	-	-	
CBP	<i>Kluveromyces cicerisporus</i> Y179	22% w/v total sugars tuber meal	86.9	144	-	-	Yu et al. (2010)
SHF	<i>Zymomonas mobilis</i> TISTR 548	Juice after acid hydrolysis, 250 g/L total sugars+0.5 g/L ammonium nitrate	98	-	1.98	-	Thanonkeo et al. (2011)
SHF	<i>Saccharomyces cerevisiae</i>	Juice after acid hydrolysis, 16% reducing sugar	94	72	1.01	-	Razmovski et al. (2011)
CBP	<i>Saccharomyces cerevisiae</i> KCCM50549	135 g/L JA flour	70	-	1.06	-	Lim et al. (2011)
CBP	<i>Saccharomyces cerevisiae</i> JZ1C	200 g/L tuber flour	79.7	48	0.91	-	Hu et al. (2012)
CBP	<i>Kluveromyces marxianus</i> PT-1	200 g/L tuber flour	90	48	1.53	-	
CBP	<i>Kluveromyces marxianus</i>	230 g/L inulin	93.7 g/L	84	1.12	-	
CBP	<i>Kluveromyces marxianus</i> K/INU2	230 g/L inulin	96.2 g/L	72	1.34	-	
CBP	<i>Kluveromyces marxianus</i>	176 g/L JA flour	62 g/L	48	1.29	-	Yuan et al. (2013c)
CBP	<i>Kluveromyces marxianus</i> K/INU2	176 g/L JA flour	69 g/L	48	1.44	-	
CBP	<i>Saccharomyces cerevisiae</i> JZ1C	200 g/L inulin	0.34 g/g JA sugars	48	1.19	-	
CBP	<i>Saccharomyces cerevisiae</i> JZ1C-inuKM	200 g/L inulin	0.34 g/g JA sugars	48	1.22	-	
CBP	<i>Saccharomyces cerevisiae</i> JZ1C-inuCK	200 g/L inulin	0.38 g/g JA sugars	48	1.35	-	Yuan et al. (2013a)
CBP	<i>Saccharomyces cerevisiae</i> JZ1C	200 g/L JA flour	0.43 g/g JA sugars	36	1.02	-	
CBP	<i>Saccharomyces cerevisiae</i> JZ1C-inuKM	200 g/L JA flour	0.46 g/g JA sugars	36	1.54	-	
CBP	<i>Saccharomyces cerevisiae</i> JZ1C-inuCK	200 g/L JA flour	0.47 g/g JA sugars	36	1.62	-	
SSF	<i>Saccharomyces</i> sp. W0	25% w/v inulin+0.75% w/v malt extract	11.1 % w/v	120	-	-	
CBP	<i>Saccharomyces</i> sp. W0 - <i>Arthrobacter</i> sp. Endoinulinase mutant	25% w/v inulin+0.75% w/v malt extract	12.8 % w/v	120	-	-	Li et al. (2013)
SSF	<i>Saccharomyces</i> sp. W0	30% w/v inulin+0.75% w/v malt extract	12.4 % w/v	120	-	-	

**Table 3.**  
(Continued)

Type	Organism	Substrate	% of theoretical ethanol yield	Time (h)	Ethanol productivity (g/L/h)	Reference
CBP	<i>Saccharomyces</i> sp. W0- <i>Arthro bacter</i> sp. Endoinulinase mutant	30% w/v inulin+0.75% w/v malt extract	13.5% w/v	120	-	Li et al. (2013)
CBP	<i>Kluyveromyces marxianus</i> CBS1555	10% w/v pretreated stalk + 1% w/v tubers batch SSF	0.497 g ethanol/g glucose.	27	1.08	Kim et al. (2013)
CBP	<i>Kluyveromyces marxianus</i> CBS1555	5% w/v pretreated stalk + 0.5% w/v tubers batch SSF	0.361 g ethanol/g glucose.	76	0.924	-
CBP	<i>Saccharomyces cerevisiae</i> DQ1	35% w/w tubers	73.5	72	-	High solids loading Guo et al. (2013)
CBP	<i>Kluyveromyces marxianus</i> DBKKU Y-102	-	90	-	2.63	Charoensopharat et al. (2015)
CBP	Inulinase engineered <i>Saccharomyces cerevisiae</i>	250 g/L tuber flour	95	-	3.2	Wang et al. (2016)

40-60°C which affects rates and yields of ethanol. However, *K. marxianus* is a thermostable yeast compared with *S. cerevisiae* and maybe the preferred choice in SSF. In one study, optimum growth temperatures of *K. marxianus* PT-1 and *S. cerevisiae* JZ1C were 42°C and 37°C, respectively (Hu et al., 2012). Coincidentally, SSF of lignocellulosic biomass with *S. cerevisiae* presents the same issue as cellulases employed for cellulose conversion into glucose have an optimum activity at 50°C (Olofsson et al., 2008).

In the 1980s, fermentations were carried out on juice recovered after pressing the tubers in a hydraulic or screw press (Guiraud et al., 1981; Duvnjak et al., 1982; Rosa et al., 1987). Several publications from Margaritis and co-workers incubated 1:1 ratio of ½” sliced tubers:water at 75°C for 1 h that led to 94% inulin recovery followed by filtration and sterilization at 120°C for 20 min (Margaritis et al., 1981; Bajpai and Margaritis, 1982; Margaritis and Bajpai, 1982c; Bajpai and Bajpai, 1991). Chabbert and co-workers extracted inulins by a continuous diffusion process using boiling water (Chabbert et al., 1983; Chabbert et al., 1985a; Chabbert et al., 1985b). Relatively new studies employed the CBP approach for ethanol production using JA tuber flour. In these studies, tubers were washed, cut or chopped in a grinder, dried, and then milled to obtain tuber flour (Yuan et al., 2008; Liu et al., 2011; Guo et al., 2013; Sarchami and Rehmman, 2014; Khatun et al., 2016). In two studies, dried slices (Yuan et al., 2013b) or tuber mash after grinding (Charoensopharat et al., 2015) were the source of inulins.

Acid hydrolysis for the SHF approach offers benefits such as shorter reaction times. Kim and Hamdy (1986) suggested that acid hydrolysis on inulin from JA tubers be carried out in 0.1 N hydrochloric acid for 15 min at 97°C for maximum recovery of reducing sugar. Sarchami and Rehmman (2015) maximized fructose recovery (98.5%) from inulin derived from JA at pH 2 and 97°C in 35 min using mineral acids. However, the authors did observe byproduct formation that worsened with the reaction duration. Nasab et al. (2009) used response surface methodology (RSM) and found that maximum inulin hydrolysis using hydrochloric acid needed pH <2 for 60 min at temperatures greater than 90°C. Additionally, catalysts are often used in chemical hydrolysis to improve product selectivity. Abasaheed and Lee (1995) reported moderate fructose recovery (75%) from JA, when hydrolysis was carried out with an acidic Zeolite LZ-M-8 catalyst over 150 min. Interestingly, the byproducts that were identified as problematic in the Sarchami and Rehmman (2015)'s study were not detected even though the reaction was four times as long (Abasaheed and Lee, 1995). Razmovski et al. (2011) found that a temperature of 126°C for 60 min with 1:1 ratio of JA:water at pH 2.0 with HCl was the best for optimum inulin hydrolysis and it also kept 5-HMF concentrations less than 0.2 g/L.

For enzymatic SHF, Parekh and Margaritis (1986a and b) carried out enzymatic hydrolysis of JA inulin through use of immobilized dead cells of *K. marxianus* in alginate beads. In a packed bed reactor, a volumetric productivity of 136 g/L/h of total reducing sugars was found with 98% conversion of inulins.

Enzyme activity half-life was 28 d. The same biocatalyst gave a half-life of 14 d when recycled in a batch process with 20% JA fructans and 98% sugar conversion. Kim and Rhee (1990) carried out fermentation with free or immobilized *Z. mobilis* ZM4. In two of the methods, inulin was pre-hydrolyzed either with sulfuric acid at pH 1.5 or enzymatically with inulinase from *Aspergillus ficuum* at 60°C for 48 h, followed by autoclaving at 121°C for 15 min in both methods. Enzymatic hydrolysis was superior to acid hydrolysis due to formation of the byproducts in the latter that reduced ethanol yields from 88% to 78%. In the batch SSF method, enzyme-free cells yielded 92.8% yield. Since inulinase had an optimum temperature of 60-65°C but fermentation was carried out at 30-35°C, they further increased inulinase dose to achieve 97% ethanol yield. Furthermore, immobilization of inulinase on chitin and bacterium on sodium alginate, and co-immobilization through trapping of inulinase on chitin in sodium alginate had 94 and 91% ethanol yield, respectively, possibly due to mass-transfer limitations.

Szambelan et al. (2004) carried out SHF with co-cultures of *Kluyveromyces fragilis* with either *S. cerevisiae* or *Z. mobilis* and found that mixed cultures had 2-12% higher ethanol yield than single cultures, tested on tubers from *Albik* and *Rubik* cultivars that were hydrolyzed by inulinase from *A. niger* (20 mg enzyme/kg tubers) prior to fermentation. Moreover, *Z. mobilis* combination yielded 4-8% more ethanol than *S. cerevisiae* combination. Kim et al. (2013) used both above-ground biomass and tuber for ethanol production. They pretreated above ground biomass with 0.5% H<sub>2</sub>SO<sub>4</sub> at 121°C for 60 min followed by 1M NaOH for another 121°C for 60 min, then mixed the pretreated solids with tubers in a 10:1 ratio and performed batch SSF and fed-batch SSF with Cellic® CTec2 cellulase (80 FPU per g total biomass) and *K. marxianus* CBS1555 at 37°C. They found 0.497 g and 0.361 g ethanol/g glucose that corresponded to 83.6% and 70.8% sugar conversion in batch and fed-batch SSF, respectively.

Species of the yeast *K. marxianus*, *K. cicerisporus*, and *K. fragilis* naturally produce inulinase that allows CBP of JA inulins. *K. marxianus* ATCC 12708 produced the highest ethanol yield of 87.4% of theoretical limit and productivity of 1.68 g/g/h compared with the other two species (Duvnjak et al., 1981). In one study, *K. marxianus* UCD (FST) 55-82 and *Saccharomyces rosei* UWO (PS) 80-38 both had 88% ethanol yield but the former had higher growth rates, ethanol concentration, and sugar utilization (Margaritis and Bajpai, 1982c). Bajpai and Margaritis (1986) performed fermentation of JA juice with recycling of *K. marxianus* SM 16-10 cells that had the tendency to flocculate. They mentioned that flocculent cells can be used repeatedly without loss of activity, reduce fermentation time, and increase ethanol yields. Moreover, downstream operations can be easier and may lead to significant cost savings. The volumetric ethanol productivity in this type of fermentation was 17-21 g/L/h with a 94% of theoretical ethanol yield. Zhang et al. (2010) took both the SHF and CBP

approaches. In SHF, *Pichia pastoris* X-33 with the cloned INU1 gene for inulinase production and ethanol production by *Saccharomyces sp.* W0. In CBP, *Saccharomyces sp.* W0/YCPlac33 PGK/CYC1-INU1 carrying the inulinase gene from *Pichia guilliermondii* strain 1 was used. Yields of 0.384 g ethanol/g inulin and 0.319 g/g sugar were found in the SHF and CBP methods, respectively.

Recent undertakings have successfully engineered inulinase synthesis in *S. cerevisiae* for CBP. Wang et al. (2016) introduced inulinase gene from *K. marxianus* into *S. cerevisiae* diploid strain JCD, and repressed vacuolar proteinase gene PEP4 to increase heterologous protein production. This resulting strain JZD-InuMKCP showed highest ethanol productivity of 3.2 g/L/h in 24 h and 2.44 g/L/h in 36 h with 95% theoretical ethanol yield solely fermented on JA tuber flour. Guo et al. (2013) used engineered *S. cerevisiae* DQ1 for CBP at optimum conditions of 30°C and pH 5.5. The optimum activity of the inulinase from this strain was at 50–55°C and pH 5.0. This study is noteworthy as ethanol yield of 73.5% was achieved at the highest tested tuber loading of 35% w/w on a dry basis in helical ribbon bioreactor that allowed better mixing at high solids loading. Yuan et al. (2013c) performed chromosome integration of inulinase gene in *K. marxianus* ATCC 8554 to produce K/INU2 strain that increased inulinase secretion from 2.4 to 3.7 U/mL when fermented on JA inulin and 3.1 to 6.8 U/mL on JA tuber mash. Ethanol productivity improved from 1.12 to 1.34 g/L/h when fed on JA inulin and 1.29 to 1.44 g/L/h on JA tuber mash. They mentioned that inulinase production improved using a similar approach in *S. cerevisiae* but ethanol production was not affected.

CBP using *K. marxianus* immobilized in alginate beads has also been carried out (Margaritis and Bajpai, 1983b). The result of immobilization was high ethanol productivity of 118 g/L/h with JA tuber extract in a continuous packed bed reactor. Only 15% loss of ethanol productivity occurred after 30 d. In another study, they reported a half-life of 72 d for the same immobilized yeast cells with a volumetric ethanol productivity of 104 g/L/h (Margaritis and Bajpai, 1982b). In yet another one of the works of Margaritis and Bajpai, immobilized *K. fragilis* had 96% of theoretical ethanol yield and ethanol productivity of 13.5 g/L/h (Margaritis and Bajpai, 1981).

In one SSF approach, Nakamura et al. (1996) used inulinase producing *A. niger* 817 that had four-fold higher inulinase activity than the wild-type strain along with *S. cerevisiae* 1200 for ethanol production. In their study, inulinase powder from *A. niger* and mashed tubers yielded 92% ethanol yield but only 52% yield using inulinase powder and juice concentrate with *S. cerevisiae*, reasoned to be due to inhibition from reducing sugars in the latter case. With *A. niger* culture and *S. cerevisiae* they found 80% ethanol yield from ball-milled JA tuber flour.

For acetone-butanol-ethanol (ABE) generation, a recent study carried out SHF using inulinase from *A. niger* (Novozymes Inc.) and fermentation with *Clostridium saccharobutylicum* DSM 13864. This study used RSM and achieved 94.5% inulin hydrolysis in 24 h in optimum conditions of pH 4.8, 48°C, inulin substrate concentration of 60 g/L, and achieved 85% of theoretical ABE yield (Sarchami and Rehmann, 2014).

Fermentation with yeast can produce microbial biomass for its protein value. Guiraud et al. (1981) estimated that 150–400 kg/ha of yeasts and protein residues may be produced from fermentation using *K. marxianus*. Margaritis and co-workers estimated dry cell weights in a similar range of 120–250 kg/acre

## 5. Conversion of fructose to 5-HMF for renewable chemicals and fuels

A big advantage of JA as a feedstock for production of renewable chemicals and jet-grade fuels is that inulins are largely made from fructose. After the acidic or enzymatic hydrolysis of inulin, high concentrations of fructose can be recovered in solution. Fructose has significantly higher rates of dehydration into 5-HMF as glucose has a more stable ring structure. The rate determining step in the production of 5-HMF is the enolization of hexoses (Kabyemela et al., 1999). Table 4 compares yields of 5-HMF obtained from conversion of fructose and glucose. This platform chemical serves as an intermediate for several pharmaceuticals and other valuable chemicals. Fructose can be converted to 5-HMF by acid hydrolysis, and there are diverse catalysts and solvents available for this conversion (Table 5). For example, ammonium chloride in isopropanol yielded 68% 5-HMF from fructose (Liu et al., 2012), whereas lignosulfonic acid in 1-butyl-3-methylimidazolium chloride converted up to 93.4% of fructose to 5-HMF (Xie et al., 2012). Coupling catalysts with environmentally friendly solvents is a growing area of research (Benoit et al., 2010; Qi et al., 2014). Additionally, one-pot conversions of inulin to 5-HMF in ionic liquids (Hu et al., 2009) offers an attractive alternative to fructose isolation and conversion.

**Table 4.** Comparison of fructose and glucose conversions and 5-HMF yields during acid hydrolysis.

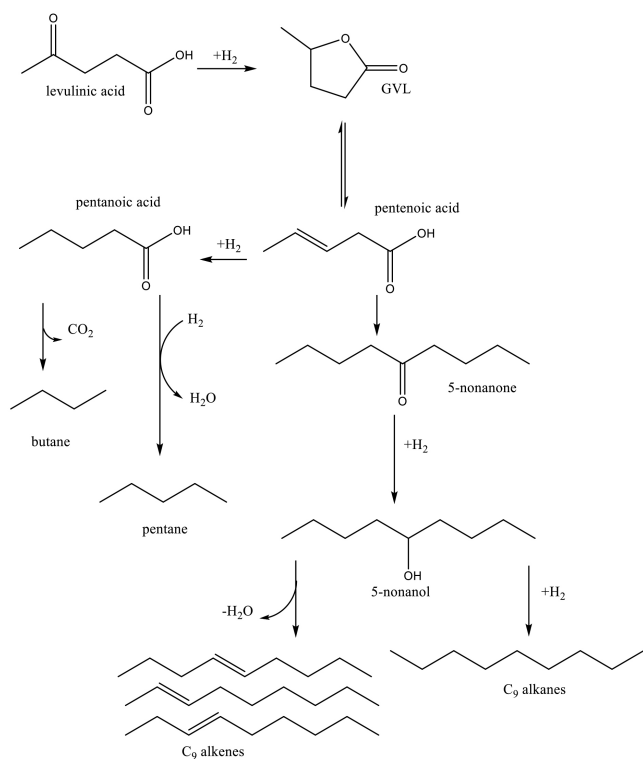
Sugar type	Catalyst and loading	Reaction conditions	Conversion (%)	5-HMF yield (%)	Reference
Fructose	H <sub>2</sub> SO <sub>4</sub> , 1% w/w	200°C, 5 min	97.3	47.0	Qi et al. (2008)
Glucose		200°C, 3 min	10.6	2.4	
Fructose	TiO <sub>2</sub> , 1% w/w	200°C, 5 min	83.6	38.2	
Glucose		200°C, 3 min	41.6	7.68	
Fructose	H <sub>2</sub> SO <sub>4</sub> , 5 M	130°C, 5 min	100	73	Qi et al. (2014)
Glucose			100	41	
Fructose	Lignin-derived carbonaceous catalyst, 5% w/w	110°C, 10 min	99	82	Guo et al. (2012)
Glucose		160°C, 50 min	99	68	
Fructose	Cellulose-derived carbonaceous catalyst, 40% w/w	160°C, 15 min	-	81.4	Hu et al. (2013)
Glucose			-	46.4	

**Table 5.** Examples of catalysts and solvents used during the conversion of fructose to 5-HMF.

Catalyst	Solvent	Reaction conditions	Fructose conversion (%)	5-HMF yield (%)	Reference
Sulfated zirconia	Acetone–dimethylsulfoxide (DMSO)	20 min at 180°C	93.6	72.8	Qi et al. (2009a)
Sulfonic ion-exchange resin	1-butyl-3-methyl imidazolium chloride	1 min at 120°C	100	82.2	Qi et al. (2009b)
		10 min at 80°C	98.6	83.3	
Germanium (IV) chloride	Dimethyl sulfoxide/1-butyl-3-methyl imidazolium chloride	25°C	-	70.0	Zhang et al. (2012)
None	Methyl isobutyl ketone/water	2 h at 160°C	96.8	73.6	Ma et al. (2015)
Iron (III) Phosphate	Tetrahydrofuran/water/sodium chloride	15 min at 140°C	99.9	71.5	Yang et al. (2015)
Phosphoric acid	Water	30 min at 140°C	97.4	44.5	



points to avoid the deposition of crystalline wax (Jiménez-Díaz et al., 2017). Branched alkanes are particularly relevant as they lower freezing points (Jiménez-Díaz et al., 2017). Aviation fuels (C<sub>9</sub>-C<sub>16</sub>) contain approximately 32% straight chain alkanes, 31% branched alkanes, 16% cycloalkanes, and 21% aromatics (Speight, 2005).



**Fig.3.** Example reaction pathways for the production of alkanes from levulinic acid adapted from Pileidis and Titirici (2016).

5-HMF can be converted to another intermediate, levulinic acid (LA) to generate industrially relevant hydrocarbons (Fig. 3). LA is produced from the acid hydrolysis of 5-HMF and derives its functionality from its ketone and carboxylic acid moieties. These functional groups render LA amenable to a variety of transformations (Hayes et al., 2008). Fructose is beneficial in that it can form difructo-disaccharides which can block the formation of additional condensation products (Van de Vyver et al., 2011). Reactions between aldoses and ketoses generate undesirable self-condensation products during the formation of HMF (Van de Vyver et al., 2011). LA can further undergo additional reactions to generate intermediates such as  $\gamma$ -valerolactone, often in the presence of a catalyst, that are relevant to chemical upgrading (Table 6). While intermediates such as  $\gamma$ -valerolactone (GVL) are of interest for the production of transportation fuels, the conversion of LA to hydrocarbons is highly desirable. LA can be converted to butene oligomers (Sen et al., 2012) and other alkenes (Bond et al., 2010) using the GVL intermediate. In other cases, the GVL intermediate is not necessary. For example, Case et al. (2012) employed a thermal deoxygenation with formic acid and LA to yield a hydrocarbon mixture that contained alkanes, alkenes, and aromatics. The dehydration of LA into angelica-lactone followed by its conversion with catalysts such as Ir-ReO<sub>3</sub>/SiO<sub>2</sub> and Pt-ReO<sub>3</sub>/C achieved 100% conversion to generate C<sub>7</sub>-C<sub>10</sub> hydrocarbons. Approximately 70% of the hydrocarbons generated were C<sub>10</sub> (Mascal et al., 2014). Thus, LA represents an especially valuable intermediate for conversion to transportation fuels. JA is an excellent source of fructose and a versatile starting material for aviation fuels.

**Table 6.**

Various catalysts used for the conversion of LA to GVL.

Catalyst	Loading	LA conversion (%)	GVL yield (%)	Reference
Ru/C	5% w/w	98	83	Piskun et al. (2016)
SnO <sub>2</sub> /SBA-15	4% w/w	85.1	95.2	Xu et al. (2017)
Cu-Mo/C	5% w/w	48	100	Pinto et al. (2017)
Ni-Mo/C	5% w/w	100	100	
40%Ni/Al <sub>2</sub> O <sub>3</sub>	10% w/w	100	99.2	Jie et al. (2016)
Ni-Sn (4.0)		>99	>99	
Ni-Sn (1.4)/AlOH	30% w/w	99	99	Rodiansono et al. (2015)
Pd/C		75	75	

## 6. Conclusions

Findings from several studies indicate that tuber yields of 9-15 Mg/ha on dry basis or 30-90 Mg/ha on wet basis, with a carbohydrate potential of 5-14 Mg/ha can be expected from tubers of JA. Tuber and carbohydrate yields can vary greatly with cultivar, climate, and soil parameters. Tubers can give ethanol yields of 2500-6500 L/ha. By comparison, yields of corn ethanol in the USA and sugar cane ethanol in Brazil can be around 4182 L/ha and 6471 L/ha, respectively (Goldemberg and Guardabassi, 2010). Research in fermentation is further needed for fast and efficient conversion of inulins into ethanol, and in high concentrations to keep costs low. Studies are needed for finding cultivars of JA for maximizing sugar yields. High yields of fructose from JA make it a valuable feedstock for production of 5-HMF.

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