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Date and Their Processing Byproducts as Substrates for Bioactive Compounds Production

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ABSTRACT

Date is the most popular fruit in middle-east countries. However, the date production process is accompanied by a substantial increase of loss during picking, storage, commercialization and conditioning process. Date and their byproducts have many essential elements for the growth of microorganisms. Thus, they can be converted into value-added compounds through biotechnology. In this paper, date and their processing byproducts used as substrates for producing value-added products such as organic acids, exopolysaccharide, antibiotics, date flavored probiotic fermented dairy, bakery yeast, etc, were reviewed.

Key words: Date, byproduct, biotechnology, fermentation, review

INTRODUCTION

As a high-value fruit, date is mainly produced in the South Asian and North African countries. The world production of date fruit is more than 7 million tons/year (FAO 2007). Carbohydrates are the major chemical constituents of date, mainly including glucose, fructose and small amounts of polysaccharides such as cellulose and starch (Al-Shahib and Marshall 2003; Al-Farsi et al. 2005). Date has high concentrations of protein (1-7%), which contains essential amino acids needed by human body (Al-Showiman 1998). Date has large amounts of dietary fiber. It also is thought to be a good source of some minerals such as iron, potassium and calcium et al. However, the date production process involves substantial loss of dates that occur during the picking, storage, marketing and conditioning process. Lost dates, termed as "by-products", which account for more than 2,000,000 tones every year, are discarded due to their inadequate texture (Besbes et al. 2005, 2006). Hence, it would be useful to develop the efficient and cost-effective method to utilize these dates for the production of value-added products through fermentation. This paper reviews the prospects of using date and their by-products as substrates for producing value-added products such as organic acids, exopolysaccharide, antibiotics, date flavored probiotic dairy, bakery yeast, etc.

DATE AND THEIR BY-PRODUCTS AS SUBSTRATES FOR THE PRODUCTION OF BIO-ACTIVE SUBSTANCES

Because date and their by-products have nutritive components for microbial growth, fermentation products using date and their by-products as substrates have been reported. Table 1 shows some important fermentation products prepared using date and their by-products as substrates.

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Fermentation products	Strains	References
Citric acid	A. niger	Mehyar et al. (2005), Mostafa and
		Alamri (2012)
Lactic acid	Lactobacillus sp. KCP01	Chauhan et al. (2007)
	Lactobacillus casei subsp. Rhamnosus	Nancib et al. (2005)
BC	Gluconacetobacter xylinus	Moosavi-Nasab and Yousefi (2011)
Curdlan	Rhizobium radiobacter ATCC 6466	Salah et al. (2011)
Xanthan gum	Xanthomonas campestris	Moosavi-Nasab et al. (2009), Salah
		et al. (2010), Salah et al. (2011)
BLM	Streptomyces mobaraensis	Radwan et al. (2010)
Fermented dairy product	Bifidobacterium lactis Bb-12, B. longum Bb-	Al-Otaibi and Saleh (2010)
	46 and Lactobacillus acidophilus La-5	Khan et al. (1995), Nancib et al.
V	S. cerevisiae	(1997), Al-Jasass et al. (2010)
Yeast	L. plantarum QS3	Elsanhoty et al. (2012)
Carotenoids	Corynebacterium glutamicum CECT690	
GA	Bacillus spp	Tavakkoli et al. (2012), Davati et al.
PHAs	Clostridium acetobutylicum ATCC 824 and	(2007)
ABE	Bacillus subtilis DSM 4451	
ADL .	Saccharomyces cerevisiae var. ellipsoideus	Khiyami et al. (2011)
	Candida utilis and Saccharomyces cerevisiae	Abd-Alla and Ei-Enany (2012)
Wine	LAB	Shi et al. (2005)
		5m et al. (2003)
SCP		Aleid et al. (2010)
Nisin		
Nisin		Khiyami et al. (2008)

Table 1 - Fermentation products from date and their by-products.

ORGANIC ACID

Citric acid

Citric acid has been widely used in food, pharmaceutical and chemical industries due to its low toxicity (Drysdale and Mckay 1995; Radwan et al. 2010). In 2008, the citric acid production was over 1.6 million tons and this trend was expected to increase (Husseiny et al. 2010). Because of its wide use, high quality of volumes and low production cost of citric acid are of great interest to the industries. In order to decrease the cost of the production, interest in using agro-industrial residues and by-products has increased. Mehyar et al. (2005) studied the ability of Aspergillus niger to produce citric acid from dates. Two strains of A. niger were grown in the media containing different concentrations of date extract fortified with whev. methanol and tricalcium phosphate. The results showed that the addition of whey, methanol and tricalcium phosphate to the date medium improved the production of citric acid. The concentrations of citric acid reached to 38.4 and 42.4 g/L, respectively in date medium fortified with methanol and tricalcium phosphate. Acourene et al. (2011) investigated the potential of date wastes as substrates for the production of citric acid using Saccharomyces cerevisiae ATCC 1102 and A. niger ATCC 16404.

The results showed that the optimal fermentation conditions were fermentation time 144 h, pH and temperature 3.5 and 30°C, respectively using a medium containing (g/L) sugar 150, ammonium nitrate 2.5 and potassium phosphate 2.5. Under optimal conditions, 126.4 g/L citric acid was produced. Mostafa and Alamri (2012) used immobilized A. niger cells to produce citric acid. One of the most important advantages of immobilized cells is their ability and stability to produce citric acid under a repeated batch culture. Over four repeated batches, the production of citric acid production was maintained for 24 days. The results also showed that the appropriate presence of nitrogen, phosphate and magnesium appeared to be important for the production of citric acid. All above obtained results confirmed that date and their by-products could be used as substrates for the production of citric acid.

Lactic acid

Lactic acid is a valuable industrial chemical that is used as a preservative in food and beverage industries. Lactic acid can be obtained through fermentation. While most of the studies on lactic acid fermentation are focused on using refined carbohydrates such as glucose (Ishida et al. 2006; Yadav et al. 2009), or starch hydrolysate (Tanaka et al. 2006), it has been found that the cost of the medium alone accounts for 60-80% of the total production cost, thus making it imperative to use economical agricultural residues and by-products. Chauhan et al. (2007) screened media components for the production of lactic acid by Lactobacillus sp. KCP01 and obtained 2.6 g/L of lactic acid using date juice alone at 5% sugar concentration. The increase of the concentration of lactic acid (15.1 g/L) was observed when salts and organic nitrogen sources were supplemented into the medium. Nancib et al. (2005) investigated the production of lactic acid by L. casei subsp. The addition of five B vitamins at less than 25 mg/L to date juice could enhance the production of lactic acid. Yadav et al. (2011) produced lactic acid using L. delbruckii, under optimal production medium comprising (%) 10 date syrup, 1 yeast extract, 0.6 sodium acetate, 0.5 KH₂PO₄, and 0.5 MgSO₄.7H₂O. High lactic acid productivity was obtained.

Acetic acid

Acetic acid is an important chemical product and has been widely used in food and pharmaceutical industries. Due to depleting natural resources, the microbial production of acetic acid by fermentation has attracted much interest. The production of acetic acid from dates mainly involves two stages. In the first stage, sugar in date juice is converted into alcohol by Saccharomyces sp. In the second stage, alcohol is converted into acetic acid by Acetobacter sp. The commonly used organisms for the production of acetic acid are S. cerevisiae, S. ellipsoideus, Acetobacter xylinum, and A. cycendens (Sokollek et al. 1998; Manickavasagan 2012).

EXO-POLYSACCHARIDES Bacterial Cellulose (BC)

BC is an exo-polysaccharide produced by various species of bacteria (Chawla et al. 2009), having good properties such as free of undesirable components (lignin and hemicelluloses), high polymer crystallinity and high degree of polymerization. Due to its unique properties and structure, BC has many applications in a variety of food formulations (Khan et al. 2007). Moosavi-Nasab and Yousefi (2011) utilized low quality date

the production of BC syrup for using Gluconacetobacter xylinus and obtained 4.35 g BC /100 mL of date syrup after 336 h fermentation. SEM results revealed more delicacy in the structure bacterial cellulose. X-ray of diffractometry demonstrated that the crystallinity level of standard cellulose (83.61%) was higher than that of bacterial cellulose (60.73%). This showed that date syrup could be used as a substrate for the production of BC.

Curdlan

Curdlan is a neutral, water-insoluble and linear polysaccharide, which is primarily composed of β - (1-3)-linked glucose (Delattre et al. 2009; West 2009). Curdlan has been reported as a useful additive for a variety of food products, such as noodles, sauces, frozen foods and packaged meats (Arocas; Sanz and Fiszman 2009; Zhang and Nishinari 2009). The currently available commercialized curdlan is relatively expensive. This is mainly because of using expensive carbon sources, namely glucose and sucrose. So, in order to increase its economic attractiveness, its production cost should be decreased (Funami and Nishinari 2007; Zhang and Nishinari 2009). Salah et al. (2011) investigated the possibility of using date by-products for curdlan production by Rhizobium radiobacter ATCC 6466. The strain exhibited a good ability to use the substrate vielding 22.83 g curdlan/L under optimal conditions in a medium containing (g/L) ammonium sulphate 2, date glucose juice concentration 120, pH 7.0 temperature 30°C, agitation speed 180 r/min and fermentation time 51 h. The purified curdlan had a molecular weight of 180 kDa. Melting temperature (T_m) and glass transition temperature (T_g) were 1.24 and -3.55°C, respectively.

Xanthan Gum

Xanthan gum is the most commercially produced industrial gum (Kalogiannis et al. 2003). It has been widely used as a thickening, or stabilizing agent in food, pharmaceutical and oil-recovery industries. The main chain of xanthan consists of glucose molecules connected by β -1, 4 glycosidic links. Every second glucose unit carries a side chain that is composed of β -D-mannose, β -1, 4-Dglucoronic acid and α -1, 2-D-mannose together with a pyruvic acid unit (Rosalam and England 2006). Moosavi-Nasab et al. (2009) used *Xanthomonas campestris* for producing xanthan gum employing date syrup as a substrate. The results showed that gum concentration increased with an increase in fermentation time with a maximum yield of 0.89 g/100 mL after 96 h. The xanthan obtained showed a very similar chemical composition compared to commercial xanthan. Salah et al. (2010, 2012) investigated and optimized xanthan gum production by *X. campestris* NRRL B-1459. The results showed that this strain had a high ability to metabolize date byproducts. The optimal conditions were 84.68 g/L carbon source, 2.7 g/L nitrogen source, and 30.1°C temperature, which produced 43.35 g/L curdlan. This was close to the 42.96 g/L value predicted by the Response Surface Methodology (RSM) model.

Antibiotics

Oxytetracycline is a broad-spectrum antibiotic produced by different species of *Streptomyces*. The antibiotic is active on Gram-positive and Gram-negative bacteria. Abou-Zeid et al. (1993) utilized date constituents as a nutrient medium for the production of oxytetracycline. Their results showed that the addition of 1.0 g/L urea and dateseed lipid into the medium increased the efficacy for antibiotic formation. When MgSO₄, MnSO₄, FeSO₄ and ZnSO₄ components of the medium were replaced by 0.5 g/L date-seed ash, antibiotic output was also increased.

Bleomycin (BLM) is a family of glycopeptidederived antibiotics (Troost et al. 2009). BLM has a strong antitumor activity and therefore, it has been widely employed for the treatment of several including non-Hodgkin's malignancies, lymphoma, squamous cell carcinoma and testicular tumors (Aras and Dilsizian 2008; Evens et al. 2008). Radwan et al. (2010) optimized the composition and concentration of a liquid fermentation medium for the production of BLM by S. mobaraensis. When date syrup was used as an additional carbon source, higher BLM amount was obtained compared to glucose. Under the optimal conditions, BLM yield was 138 mg/L. The complex medium containing 40 g/L date syrup as additional carbon source enhanced the production of BLM by 73%.

Date probiotic fermented products

Fermented dairy products are beneficial for the health (Parodi 1999). Hashim (2001) studied the characteristics and acceptability of yogurt containing dates. The results indicated that addition of 10–20% of date paste with, or without

5% date syrup did not affect yogurt acidity, protein, or fat contents, but increased total solids significantly. Also, the addition of 15% date paste with 5% date syrup provided yogurt with desired sensory quality. Gad et al. (2010) used dates in reconstituting skimmed milk powder in processing yogurt with 14% total solids. The results showed that yogurt enriched with 10% dates had a significant sweetness, higher antioxidant values, higher in HCl soluble minerals and folate concentration compared to plain yogurt. The addition of date syrup to the probiotic yogurt enhanced the bifidobacterial count of the products and improved their survival during the cool storage period up to 10 days. This could be explained on the basis that date syrup contained some micronutrients, such as vitamins and minerals, which would enhance the growth of bifidobacteria. Al-Otaibi and Saleh (2010) probiotic incorporated microorganisms Bifidobacterium lactis Bb-12, B. longum Bb-46 and L. acidophilus La-5 into date pastes. All probiotic bacteria were capable of surviving in dates. The viable count of L. acidophilus remained above 6 \log_{10} CFU/g until the third week of storage. The viable count of B. lactis and B. *longum* remained above 6 log_{10} CFU/g until the eighth and tenth weeks of storage.

In order to get the information on the sugar utilization in fermenting milk, Hartati et al. (2012) added date into yogurt drink with different percentage. The result showed that adding date from 3.19 to 3.32%, reducing sugar increased persistently from 2.15 to 2.99%, and pH value decreased from 3.8 to 3.76. However, adding date into yogurt drink had no effect on sourness. This study indicated that lactose and reduced sugar concentration and pH value of fermented milk could be affected due to utilization of date by the lactic acid bacteria (LAB).

Yeast

Dates are a good potential substrate for the production of bakery yeast. Khan et al. (1995) used Saudi Arabian dates for the production of baking yeast. They propagated six different strains of *S. cerevisiae* in fermentation medium containing date extract (with 60% sugars), in addition to 2 g/L ammonium sulfate and 50 mg/L biotin. Yields about 42.8% of the theoretical were obtained. Nancib et al. (1997) used date byproducts for the production of baking yeast from *S. cerevisiae*. Although this medium was satisfactory

for the production of the yeast, yields obtained were very low with a maximum of 0.60 g/L biomass concentration in the fermentation medium compared to the optimum of about 40 g/L expected for an economical production. Al-

Jasass et al. (2010) used date syrup and molasses for the propagation of baking yeast strain S. cerevisiae. The results showed that the overall biomass yield from date syrup substrate was significantly lower than the yields from molasses. Reduced yields could be attributed to the effect of toxic organic acids contained in date syrup at high concentrations. Ei-Sharnouby et al. (2010) investigated the performance of the produced baker's yeasts on Arabic bread quality. Their results indicated that the yeast produced from date syrup was comparable to the yeast produced from molasses. There were insignificant differences in gassing power between the yeasts produced from date syrup and molasses. Thus, it was concluded that excellent quality baker's yeast could be produced using date syrup as a substrate.

OTHER VALUE-ADDED PRODUCTS Carotenoid

Carotenoids are natural pigments responsible for the pleasing colors of many foods. Some carotenoids are the precursors of vitamin A, which have beneficial effects on human health, including enhancement of the immune system and reduction of the risk for degenerative diseases such as cancer, cardiovascular diseases and cataract (Krinsky 2001; Garrido-Fernández et al. 2010). The commercial utilization of microorganisms to produce carotenoids is presently limited by the high cost of production. However, the cost of carotenoids production by fermentation could be minimized by using cheap industrial by-products (Aksu and Tugba 2005). Elsanhoty et al. (2012) used date wastes for the production of carotenoids. L. plantarum QS3 gave the highest production. Date syrup at 5% sugar concentration produced 16.21 mg/kg dry cell of carotenoids when used alone. Plackett-Burman design showed peptone, K₂HPO₄, sodium acetate and date juice affected the production of carotenoids. Carotenoids yield (54.89 mg/kg dry cell) was observed under optimized conditions.

Amino acid

Glutamate (GA) is mainly used as a flavoring agent (Nandakumar et al. 2003, Jyothi et al. 2005).

According to Shimizu and Hirasawa (2006), the production of GA was more than 1.5 million tons per year worldwide (Shimizu and Hirasawa 2006). Efforts have been made to improve the GA fermentation process, especially from the standpoint of saving the production cost. Tavakkoli et al. (2012) used RSM to evaluate the effect of fermentation parameters for the production of GA using date waste as a substrate. The optimized fermentation conditions were inoculum size 2 % (v/v), substrate concentration 25 % (w/v), penicillin concentration 1 U/mL, phosphate concentration 4 g/L, and inoculum age 10 h, the resulting maximum GA (39.32 mg/mL). Davati et al. (2007) used two mutants C. glutamicum CECT690 and CECT77 to study the possibility of producing amino acids from date wastes. According to the results, the most effective factors on the production of GA were the amount of date fruit wastes, time of penicillin addition, phosphate amount and type of microorganism. The results demonstrated date waste juice as a substrate has the potential for producing GA.

Polyhydroxyalkanoates (PHAs)

PHAs are a group of renewable and biodegradable biopolymers that have thermoplastic properties. The production cost of biopolymers is a crucial parameter compared with that of chemical synthetic non-biodegradable plastics (Bengtsson et al. 2010). Hitherto, the low cost raw materials, such as date syrup, industrial waste and municipal waste et al., could be significant parameter for reducing the production cost. Khiyami et al. (2011) tested the accumulation ability of PHAs in Bacillus spp using date syrup as substrate and obtained 8.3 g/L cellular dry matter with almost 70.5% PHA content. The average molecular weight range was between 3.7 to 4.1×10^{5} g/mol. This study demonstrated that date syrup could be evaluated as a complex media supplement to meet the nutritional requirements for the production of PHA.

Biofuel

The most commonly used metabolically derived liquid biofuels are acetone, butanol and ethanol (ABE). ABE are commonly used solvents in many important industries and have a high potential for replacing the petrochemical derived energy. Substrate costs can make up to about 63% of the total cost of ABE production (Kumar et al. 2011). Hence, substrates such as agricultural residues offer potential alternatives (Qureshi et al. 2010 a, b, c). Abd-Alla and EI Enany (2012) studied spoilage date palm (*Phoenix* dactylifera L.) fruit as substrate for ABE production by mixed culture of *C. acetobutylicum* ATCC 824 and *B. subtilis* DSM 4451 and achieved 21.56 g/L ABE from 75 g/L spoilage date fruit homogenate. The addition of yeast extract (5 g/L), or ammonium nitrate (1.6 g/L) significantly enhanced ABE production. These results suggested that spoilage date fruit could be efficiently used for commercial ABE production.

Wine

Wine is a drink derived from the fruits and sometimes vegetables. Being rich in fermentable sugars, date juice could be a good raw material for wine making. A suitable strain for preparing a good quality date wine (12% ethanol, 0.35-0.54% acidity, and pH of 4.0–4.2) is *S. cerevisiae* var. *ellipsoideus* (Shi et al. 2005). In the production of wine, alcohol is one of the constituents of the end-products and some by-products such as CO₂ are also produced.

Single Cell Protein (SCP)

SCP, a protein source, is used to replace protein concentrates in animal feeds. SCP can be produced from bacteria, yeasts, molds and algae using different substrates as sources of carbon. Aleid et al. (2010) described *Candida utilis* and *S. cerevisiae* for the production of SCP using date syrup as a substrate. Biomass yields could reach more than 90% of the theoretical, and the protein content of the biomass was about 50%. The amino acid composition of the essential amino acids, especially lysine and the sulfur containing amino acids.

Nisin

Nisin is the most common kind of bacteriocins, produced by certain types of LAB. Khiyami et al. (2008) studied the potential of using a variety of dates and their byproducts date pits as complex medium for the production of nisin. Low concentration of date syrup 5.0% enhanced the yield of nisin. The production of nisin began after 2 h of incubation and reached maximum after 12 h of incubation. The optimal concentration of dates pits and dates syrup for LAB growth and nisin production was 1.5 and 5.0%, respectively.

CONCLUSIONS

Many reports have indicated that date and its processing by-products are good substrates for the production of fermentation products. Utilizing dates and their by-products in industrial biotechnology could offer economic, social and environmental benefits. Efforts should be made to explore new and improved microorganisms that can convert date into end-products and new bioactive compounds.

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