



REVIEW

# Valorization of date palm (*Phoenix dactylifera*) fruit processing by-products and wastes using bioprocess technology – Review

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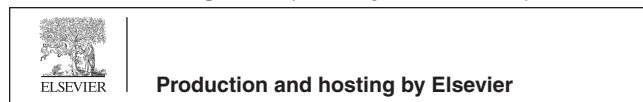
**Abstract** The date palm *Phoenix dactylifera* has played an important role in the day-to-day life of the people for the last 7000 years. Today worldwide production, utilization and industrialization of dates are continuously increasing since date fruits have earned great importance in human nutrition owing to their rich content of essential nutrients. Tons of date palm fruit wastes are discarded daily by the date processing industries leading to environmental problems. Wastes such as date pits represent an average of 10% of the date fruits. Thus, there is an urgent need to find suitable applications for this waste. In spite of several studies on date palm cultivation, their utilization and scope for utilizing date fruit in therapeutic applications, very few reviews are available and they are limited to the chemistry and pharmacology of the date fruits and phytochemical composition, nutritional significance and potential health benefits of date fruit consumption. In this context, in the present review the prospects of valorization of these date fruit processing by-products and wastes' employing fermentation and enzyme processing technologies towards total utilization of this valuable commodity for the production of biofuels, biopolymers, biosurfactants, organic acids, antibiotics, industrial enzymes and other possible industrial chemicals are discussed.

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## 1. Introduction

The date palm *Phoenix dactylifera*, a tropical and subtropical tree, belonging to the family Palmae (Arecaceae) is one of mankind's oldest cultivated plants, and in the Arabian Peninsula it has played an important role in the day-to-day life of the people for the last 7000 years (Ahmed et al., 1995). Today, worldwide production, utilization and industrialization of dates are continuously increasing and as per FAO (FAOSTAT, 2010) the production of date fruits is on the increase as recorded for some of the major date producing countries like Egypt (1,352,950 metric tons), Saudi Arabia (1,078, 300 metric tons), Iran (1,023,130 metric tons), UAE (775,000 metric tons) and Algeria (710,000 metric tons). Date fruit is marketed all over the world as a high value confectionery, and as a fresh fruit it remains an important subsistence crop in most of the desert areas. It is produced largely in the hot arid regions of the world particularly in Gulf Cooperation Council (GCC) countries, and Saudi Arabia is considered as one of the world's major producer of dates. Dates production in Saudi Arabia greatly increased over

the past two decades and is also paralleled by high consumption. *P. dactylifera* is the primary crop in Oman, which shares 82% of all fruit crops production in the country. Algeria produces more than 400 different varieties of dates with an annual production of over 400,000 tons (<http://faostat3.fao.org/home/index.html#VISUALIZE>).

The development of date fruits is divided into three stages, Khalal, Rutab and Tamr, and dates are generally harvested at the fully ripened Tamr stage, that is after the development of Total Soluble Solids (TSS) of 60–70 brix that are edible at this stage. Most dates are consumed at the Rutab (semi-ripe) and Tamr (fully-ripe) stages, with little or no processing. The quantities of processed dates have been rapidly and steadily growing in recent years because of encouragement and support provided to the date processing industry by the respective governments. Huge amount of wastes are generated from the Kabkab date and the wastes have potential for use in date syrup production with economical advantages (Al-Hooti et al., 1997).

Date trees produce large quantities of agricultural waste and according to one estimate, each date tree produces about

20 kg of dry leaves yearly. Other wastes such as date pits represent an average of 10% of the date fruits (Ministry of Agriculture, 1998; Barrevel, 1993). Although these agricultural wastes consist of cellulose, hemicelluloses, lignin and other compounds which could be used in many biological processes, they were burned in farms causing a serious threat to the environment. Although several investigators have studied date palm cultivation, their utilization and scope for utilizing date fruit in therapeutic applications, reviews available in the literature are rather limited to the chemistry and pharmacology of the date fruits (*P. dactylifera* L.) by Baliga et al. (2011), and phytochemical composition, nutritional significance and potential health benefits of date fruit consumption by Vayalil (2012). Hence, in this review the prospects of valorization of these date fruit processing by-products and wastes' employing bioprocessing technologies, are discussed towards total utilization of the date fruit processing wastes disposed into the environment. This review includes a comprehensive discussion on the nutritive value of date fruits and their biochemical characteristics, medicinal and pharmacological properties; date fruit processing and production of products and by-products; current practices and problems of waste management, scope for value addition using bioprocessing, fermented products derived from date palm fruits, enzyme processing of date fruits and deriving value added products besides a note on future trends in the valorization of date palm fruit processing by-products and wastes.

## 2. Nutritional value and biochemical composition

Date fruits assume great importance in human nutrition owing to their rich content of essential nutrients which include carbohydrates, salts and minerals, dietary fibre, vitamins, fatty acids, amino acids and protein. They have enormous scope and potential for use as food for generations to come due to their remarkable nutritional, health and economic value. The nutritional value of dates is due to their high sugar content (around 50–60%), potassium (2.5 times more than bananas), calcium, magnesium and iron as well as vitamins (B1, B2) and Niacin. Further, dates are rich in sugar ranging from 65% to 80% on dry weight basis mostly of inverted form (glucose and fructose). Fresh varieties have a higher content of inverted sugars, the semi dried varieties contain equal amount of inverted sugars and sucrose, while dried varieties contain higher sucrose. Water content is between 7% (dried) and 79% (fresh) depending on variety. Dried dates can easily be stored and preserved because of their naturally high sugar content. Thus date is considered as a nutritious fruit that contributes to human health when consumed with other food constituents (Lambiotte et al., 1982) and people eat fresh and/or dried dates. Recently Vayalil (2012) extensively reviewed the phytochemical composition, nutritional significance, and potential health benefits of date fruit consumption and discussed its great potential as a medicinal food for a number of diseases inflicting human beings. Some specific reports on the detailed biochemical composition of date fruits and date seeds are presented below in support of their desirable nutritive quality.

Fruits of the date palm (*P. dactylifera* L.) were reported to contain a high percentage of carbohydrate (total sugars, 44–88%), fat (0.2–0.5%), 15 salts and minerals, protein (2.3–5.6%), vitamins, and a high percentage of dietary fibre (6.4–

11.5%) (Al-Shahib and Marshall, 2003). Later reports indicated that date fruits (Tamr) contain moisture ranging from 10% to 22%, total sugars 62% to 75%, protein 2.2% to 2.7%, fibre 5% to 8%, fat 0.4% to 0.7%, ash 3.5% to 4.2%, total acidity 0.06% to 0.20%, and ascorbic acid 30.0% to 50.0 mg%, on dry weight basis, (Baraem et al., 2006; El-Sharounby et al., 2007). The flesh of dates contains 0.2–0.5% oil compared to 7.7–9.7% in the seed. Unsaturated fatty acids of the fruit include palmitoleic, oleic, linoleic and linolenic acids and the oleic acid content of the seeds varies from 41.1% to 58.8%, indicating that the seeds of date could be used as a source of oleic acid. The protein in dates contains 23 types of amino acids, some of which are not present in the most popular fruits such as oranges, apples and bananas. They contain at least six vitamins including a small amount of vitamin C, and vitamins B(1) thiamine, B(2) riboflavin, nicotinic acid (niacin) and vitamin A. Dates contain 0.5–3.9% pectin, which may have important health benefits. Thus dates are considered as an ideal food, providing a wide range of essential nutrients and potential health benefits (Al-Shahib and Marshall, 2003).

Proximate composition examined for the eight different sun dried date (*P. dactylifera* L.) varieties (1) Daki, (2) Aseel, (3) Coconut, (4) Khuzravi, (5) Halavi, (6) Zahidi, (7) Deglet Nour and (8) Barkavi indicated that all are rich in proteins, fibres, carbohydrates and net gross energy (352.329 kcal/100 g in Aseel to 425.147 kcal/100 g in Khuzravi) having suitable levels of lipids and low values of ash, moisture and oxalates. Further Na, K and Li were recorded as macro minerals while Cr, Cu, Ca, Mg, Ni, Zn and Mn were noted as micro minerals (Jamil et al., 2010).

The flesh of Bekrarray dates contained a higher percentage of carbohydrates (76.97%), vitamin C (8.50 mg/100 g) and potassium (6043.2 mg kg<sup>-1</sup>) compared to Deglet-nour variety which had a higher percentage of total sugar (73.25%), vitamin A (10.50 µg/100 g) and vitamin E (12.98 mg kg<sup>-1</sup>). Whereas, the Khathori variety showed a high percentage of vitamin B2, magnesium, calcium, sodium and ferum with concentrations of 824.98, 660.74, 614.21, 485.86 and 20.29 mg kg<sup>-1</sup>, respectively. The Bekrarray dates exhibited a significantly high concentration of Total Phenolic Content (TPC) ( $p < 0.05$ ) from those of the two other varieties (Hasan et al., 2010).

*P. dactylifera* L. of two cultivars Aziza Bouzid and Assiane from Morocco were reported as a good source of sugar (71–80 g/100 g dry weight) and dietary fibre (10.22–12.79 g/100 g dry weight) on a dry matter basis. Further it was also observed that Aziza Bouzid contains significant quantities of some essential amino acids (lysine: 184, isoleucine 122, threonine 98 mg/100 g dry matter), while the Assiane variety was rich in macro-elements (potassium: 863, calcium 87 mg/100 g dry matter) (Elguerrouj et al., 2011). Another study reported that *P. dactylifera* L. fruit contains 4.94 ± 0.04% crude protein; 80.87 ± 0.05% carbohydrate; maltose (33.7 mg%), fructose (22.8 mg%) and glucose (22.3 mg%) as the dominant sugars; 5.24 ± 0.05% moisture, 1.54 ± 0.01% ether extract value; 4.34 ± 0.03% crude fibre content; 710.0 mg% potassium as the most abundant mineral; and oleic acid (44.51 g/100 g) > palmitic acid (23.05 g/100 g) > linoleic acid (11.66 g/100 g) as the most concentrated fatty acids (Ogungbenle, 2011).

Egyptian date fruits (*P. dactylifera* L.), were reported to contain a variety of B-complex vitamins B1, B2, nicotinic acid and vitamin A and the date palm extracts contained 13.80%

moisture, 86.50% total solid, 2.13% ash and 5.20% crude fibres, 3.00% protein, 73.00% carbohydrates and 2.90% lipids contents. High performance liquid chromatography (HPLC) analysis showed that, the carbohydrate content was constituted by a large amount of glucose, fructose and sucrose. High concentrations of aspartic acid, proline, glycine, histidine, valine, leucine and arginine; low concentrations of threonine, serine, methionine, isoleucine, tyrosine, phenylalanine; and lysine and very low concentrations of alanine were also recorded (El-Sohaimy and Hafez, 2010).

A comparative analysis of water soluble vitamins viz., B1 (thiamine HCl), B2 (riboflavin), B3 (nicotinamide), B5 (pantothenic acid), B6 (pyridoxine HCl), B9 (folic acid), and B12 (cyanocobalamin) carried out in fruits (immature, semi mature and mature) of six date palm (*P. dactylifera L.*) cultivars (Barhee, Khalasah, Muzati, Shishi, Zart, and Zardai) growing in the United Arab Emirates (UAE) by HPLC indicated a significant variation within the different cultivars and the developing stages of date palm fruit. Vitamin B1 (Khalash, Shishi), B3 (Zardai, Zart and Barhee), and B5 (Shishi) were maximum (1 g/100 g fresh weight) at their matured stage while maximum of vitamin B2 (Khalash, Barhee and Shishi), B6 (Muzati, shishi, Khalash), B9 (Khalash and Zardai), and B12 (Khalash, and Shishi) were detected in immature fruits (Aslam et al., 2011).

Seeds of the date palm were found to contain boron, calcium, cobalt, copper, fluorine, iron, magnesium, manganese, potassium, phosphorous, sodium and zinc in addition to aluminium, cadmium, chloride, lead and sulphur in various proportions. The seeds of two date palm (*P. dactylifera L.*) cultivars, Deglet Nour and Allig, from the Degach region – Tunisia, were observed to contain (on a dry-weight basis) respectively: protein 5.56% and 5.17%, oil 10.19% and 12.67%, ash 1.15% and 1.12%, and total carbohydrate 83.1% and 81.0%; oleic acid (41.3–47.7%) and lauric acid (17.8%) in the Deglet Nour cultivar, and palmitic acid in the Allig cultivar (15.0%); besides capric, myristic, myristoleic, palmitoleic, stearic, linoleic and linolenic acids (Besbes et al., 2004). Results of their study indicated that date seed oil could be used in cosmetics, pharmaceuticals and food products.

### 3. Pharmacological properties and applications

In spite of the fact that date fruits serve humanity as the staple food for millions of people around the world for several centuries, systematic studies on the health benefits are inadequate and dates are hardly recognized as a healthy food by the health professionals and the public (Vayalil, 2012). Nevertheless, in the folk-lore, date fruits have been ascribed to have many medicinal properties when consumed either alone or in combination with other herbs. In recent years dates have drawn greater attention owing to their numerous health benefits and consequently many *in vitro* and animal studies besides the identification and quantification of various classes of phytochemicals are being pursued worldwide (Vayalil, 2012). A comprehensive analysis of the phytochemistry and validated pharmacological properties of date fruits (*P. dactylifera L.*) and the seeds were extensively reviewed recently (Baliga et al., 2011). Phytochemical investigations have revealed that the fruits contain anthocyanins, phenolics, sterols, carotenoids, procyanidins and flavonoids, known to possess multiple beneficial effects. Preclinical studies have shown that the date fruits possess free radical scavenging, antioxidant, anti-

mutagenic, antimicrobial, anti-inflammatory, gastro protective, hepatoprotective, nephroprotective, anticancer and immunostimulant activities. These reviews indicated that in addition to its dietary use the dates have medicinal value and are extensively used to treat a variety of ailments in the various traditional systems of medicine.

### 4. Date palm fruits processing products and by-products

The whole date fruits are traditionally used to prepare a wide range of products such as date juice concentrates (spread, syrup and liquid sugar), fermented date products (wine, alcohol, vinegar, and organic acids) and date pastes for different uses (e.g. bakery and confectionary) besides their direct consumption. Date processing industries manufacture a variety of date products such as date-paste, date-syrup, date dip, date-honey, date-jam and date-vinegar. Date pectin, dietary fibre and syrup are some of the date substances which find a plethora of applications as a thickener or gelling agent in processed foods, i.e. confectionery products, jams, table jellies, soft cheeses, yoghurts, etc. Date juice extraction and date syrup were extensively studied by several investigators (El-Shaarawy, 1989; Ramadan et al., 1995; Al-Hooti et al., 2002).

The dates are generally steamed, destoned, macerated, and converted to a semi-solid form known as paste with approximately 20–23% moisture content and a water activity below 0.6 (Ahmed et al., 2005). Date paste has been used as a filler and as a substitute sugar in many food formulations and confectioneries have been utilizing date paste as one of their major ingredients (Alhamdan and Hassan, 1999).

Date syrup (dibs), the main and general by-product of date, is being used in the preparation of foodstuffs such as jams, marmalades, concentrated beverages, chocolates, ice cream, confectioneries, sweets, snacks, bakery products and health foods (Riedel, 1986). It is produced as an incidental by-product when bagged humid dates are heaped for several months, during which some syrup oozes out by the force of its own weight. In the date syrup industry, the fruits are mixed with water and heated for around 1 h at 50°C and the main component, sugars, are then extracted. It is also produced in homes and in villages by extraction and boiling down of juice and on a semi and full industrial scale (FAO, 1992). Mature date fruits are also processed into products such as date bars, date syrup, etc. (Abd El-Mohsen and Nezam El-Din, 1995).

Second -grade dates (with a hard texture) from three potential Tunisian cultivars (Deglet Nour, Allig and Kentichi) were reported (Besbes et al., 2009) to contain the same levels of sugar (~73.30–89.55 g/100 g dry matter), fibre (~7.95–18.83 g/100 g dry matter) and total phenolics (~280.6–681.8 mg of GAE/100 g), and dates of high quality add value to these raw materials by using them in jam production. They also observed that the date variety had a significant effect on the composition and physical characteristics of date jams produced. Further Allig jam was richer in reducing sugars and was characterized by its higher firmness and water retention capacity. Comparative evaluation of these new products for acceptability, with quince jam (the most consumed in Tunisia) showed that Allig and Kentichi jams presented a higher overall acceptability indicating scope for the commercialization of date jam (Besbes et al., 2009).



Few by-products are also produced through chemical modifications of date seeds such as polyol and mayonnaise. Polyols, known as sugar-free sweeteners, are compounds with multiple hydroxyl functional groups available for organic reactions and are commonly added to foods because of their lower calorific content than sugars. Polyols were produced from date seeds through oxypropylation and liquefaction techniques using organic solvents in the presence of a catalyst (Briones et al., 2011).

Mayonnaise is a thick creamy sauce often used as a condiment and it is made by slowly adding oil to an egg yolk, while whisking vigorously to disperse the oil. The oil extracted from residual pits from the date (*P. dactylifera* L.) variety khalas from Saudi Arabia when tried as a replacement for conventionally used corn oil showed that mayonnaise containing date pit oil was superior in sensory characteristics as compared with control manufactured from corn oil. The study advocated the use of the date pit oil as non-traditional oil in mayonnaise products (Basuny and Al-Marzooq, 2011).

## 5. Waste management—current practice and problems

Enormous wastes mainly in the form of date fruits that fell from the tree before maturity, date fruit seeds (pits), and date press cakes are being generated by the date palm agro industry and date processing industries. These wastes pose serious environmental problems besides contributing to a great loss of raw materials. Date palm fruit harvesting is often accompanied by substantial fruit losses that occur during the picking, storage and conditioning processes. Due to their inadequate texture (too soft), the lost dates, commonly named “date by-products”, are not edible and are often discarded. Currently these by-products are used for limited purposes such as animal feed (Besbes et al., 2006). Within agricultural systems dates falling down from palms before maturity, date press cake (by-product of date juice production) and date pits are used as animal feed-stuff (Abou-Zied et al., 1991; Al-Hooti et al., 1997; Youssif and Alghamdi, 1999). In some date-processing countries, such as Tunisia, date seeds are discarded or used as fodder for domestic farm animals. In Tunisia, the mean annual yield of date fruits is about 100,000 tons and from this, around 1000 tons of date seeds oil would be extracted.

In fact all organic waste materials arising from date processing have potential for use as a component for compost preparation. A composting mixture consisting of 70% date palm wastes and date palm pits and 30% shrimp and crab shell wastes was reported to take about 13 weeks to mature and at the end of maturation the compost had all the quality of a good fertilizer marked by its various constituents. The final compost product was found to have 57.1% moisture content with a pH of 7.9 and organic matter of 891.0 g/kg dry matter with significant amounts of 2.2, 0.82, 14.3, and 1.3 g/kg calcium, phosphorus, potassium, and sodium, respectively (Khyami et al., 2008a,b).

## 6. Scope for value addition using bioprocessing

Bioprocessing technologies generally include the application of microorganisms as whole cells in submerged fermentation (SmF), solid state fermentation (SSF) or slurry state fermentation (SLF); use of whole cells of the microorganism under

immobilized condition; and use of enzymes under Free State or immobilized state for bioconversions or bio-transformations of organic materials into desired end products or biomass production. These technologies have been developed and employed in food industries over time and holds immense scope and potential for exploiting organic residues that include agro-industrial wastes, fruit wastes and unutilized and discarded fruit by-products. The fruit processing industries employ enzymes widely for varied applications including the clarification of fruit juices and deriving sugars. Since the literature on basic principles, characteristic features, methodologies and applications of fermentation technologies and enzyme technologies are vast, a detailed discussion on these technologies is out of scope here and the reader is recommended to refer such specific literature on these techniques for more detailed information and use.

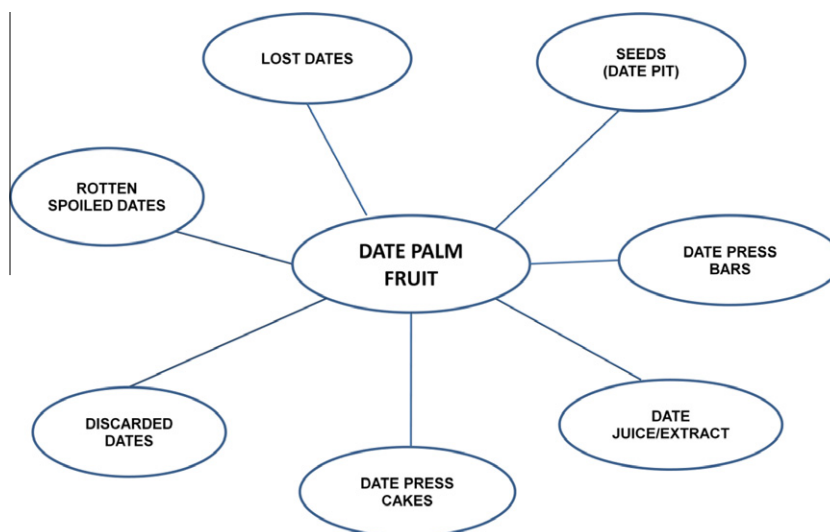
It must be noted that thousands of tons of date wastes are discarded daily by the date processing industries leading to environmental problems. Thus, there is an urgent need to find suitable applications for this waste. Date can be a good source of sugar, and its use as raw material can result in a relatively cheaper fermentation process as it does not require any special treatment like acid hydrolysis, steam explosion, or enzymatic treatment to release sugars in fermentable form. The date wastes (Figure 1) especially date pits and date press cakes have immense scope for value addition using bioprocess technologies considering their biochemical components and quality as substrate for solid state fermentation by microorganisms or for enzyme processing of materials for the augmentation of value added products.

## 7. Fermented products derived from date palm fruits

Fermentation technology is one of the oldest technologies ever employed for the preservation of food and deriving value added food products from food materials across the globe. Microbial fermentations yield several value added products for varied applications besides the augmentation of foods such as bread, yoghurt, vinegar, alcoholic beverages etc. that are directly consumed. Almost all fruits, plant and animal products that qualify to be used for consumption as food have been subjected to fermentation by microorganisms in order to derive further by-products and products such as organic acids, amino acids, vitamins, etc. In this context date fruit products and wastes have been considered as potential raw material for deriving value added products employing fermentation. The various products derived from date fruit by-products and wastes employing different microorganisms are presented in Table 1 for instant reference. The details of the reports available in the literature on the various products derived from date fruits by-products and wastes are discussed below.

### 7.1. Microbes associated with date palm fruits

Microorganisms capable of fermenting wild date palm (*Phoenix sylvestris*) sap into wine (toddy) were isolated and identified (Shamala and Sreekantiah, 1988). *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Acetobacter aceti*, *Acetobacter rancens*, *Acetobacter suboxydans*, *Leuconostoc dextranicum*, *Micrococcus* sp., *Pediococcus* sp., *Bacillus* sp. and *Sarcina* sp. were encountered in the freshly tapped sap



**Figure 1** Date palm fruit by-products and wastes.

while a majority of these microorganisms were also isolated from the traditionally fermented fresh toddy samples. Further certain variations in the growth pattern of these microorganisms and in the amount of ethanol, volatile acid, non-volatile acid and esters produced during these fermentations were also observed during a comparative study on the fermentation of fresh sap and fresh toddy (Shamala and Sreekantiah, 1988). The information available on qualitative micro flora associated with date fruit is limited and it is indicated that the flora described here could play a significant role in harnessing the same as potential microorganisms for use as biocatalysts in the bio-processing of the date fruit by-products and wastes.

## 7.2. Biopolymers

### 7.2.1. Xanthan gum

Date fruit by-products are known to be useful for the production of high value-added components such as xanthan gum, which has been authorized by the Food and Drug Authority (FDA) in 1969, for use in food products (Besbes et al., 2006). In fact, the cost of the fermentation medium has always been a major concern in the commercial production of xanthan gum and consequently search for cheaper natural alternatives for the currently used substrates, namely glucose or sucrose has drawn the attention of researchers towards the economic production of the final product. Accordingly, the Allig date palm by-products, which are abundantly available in nature as a waste of palm date harvesting, storing and conditioning processes, were tried as a cheap substrate for xanthan gum production by *Xanthomonas campestris* in batch experiments and the process variables were optimized employing response surface methodology (Besbes et al., 2006). Three main independent variables, namely carbon sources, nitrogen sources and temperature were evaluated in terms of their individual and combined effects on optimum xanthan gum and biomass production. The study concluded that the date palm juice by-products have potential and promising properties towards the production of efficient and cost-effective xanthan gum at industrial scale (Besbes et al., 2006). Later xanthan gum production by *X. campestris* NRRL B-1459 using date palm juice

by-products was optimized by Response Surface Methodology complemented with a Central Composite Orthogonal Design (Salah et al., 2010). The results obtained indicated that 84.68 g l<sup>-1</sup> carbon source, 2.7 g l<sup>-1</sup> nitrogen source, and 30.1°C were optimal conditions for production of 43.35 g l<sup>-1</sup> of xanthan gum under the conditions, which was close to the value (42.96 g l<sup>-1</sup>) predicted by the model and higher yields of biomass production could be obtained at 46.68 g l<sup>-1</sup> carbon source, 4.58 g l<sup>-1</sup> nitrogen source and 30°C.

### 7.2.2. Poly (3-hydroxybutyrate)

Polyhydroxyalkanoates (PHAs) are biocompatible, non toxic and biodegradable materials, which are accumulated to store carbon and energy in various microorganisms. PHAs have the potential to replace petroleum-based plastics; as biomedical materials for use in surgical pins, sutures, staples, blood vessel replacements, bone replacements and plates, medical implants and drug delivery devices owing to their superior biodegradability and biocompatibility. Among the candidates for biodegradable plastics, PHAs have drawn much attention due to their complete biodegradability and the similarity of their material properties to conventional plastics (Luengo et al., 2003). *Bacillus megaterium*, isolated from the sludge of a sewage treatment plant in Makkah, Saudi-Arabia, was optimized for growth and PHB accumulation in medium enriched with 5% (w/v) date syrup or beet molasses supplemented with NH<sub>4</sub>Cl. It was observed that when date syrup and beet molasses were used alone without an additional nitrogen source, the bacterium registered a cell density of about 3 g l<sup>-1</sup> with a PHB content of the cells of 50% (w/w). Further, the addition of NH<sub>4</sub>NO<sub>3</sub> followed by ammonium acetate and then NH<sub>4</sub>Cl was found to support cell growth up to 4.8 g l<sup>-1</sup>, while PHB accumulation increased with NH<sub>4</sub>Cl followed by ammonium acetate, NH<sub>4</sub>NO<sub>3</sub> and then (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> to a PHB content of nearly 42% (w/w) (Omar et al., 2001).

### 7.2.3. Curdlan

Curdlan is a high molecular weight polysaccharide consisting of β-1,3-linked glucose units, produced by pure-culture fermentation from a non-pathogenic and non-toxicogenic strain of *Agrobacterium biovar 1* or *Agrobacterium radiobacter*.

**Table 1** Products derived from date fruit byproducts and wastes through Microbial fermentations.

Product	Substrate	Microorganisms	Reference
<i>Biopolymers</i>			
Xanthan gum	Date palm juice	<i>Xanthomonas campestris</i>	Besbes et al. (2006)
	Date palm juice	<i>X. campestris</i> NRRL B-1459	Salah et al. (2010)
PHB	Date syrup	<i>Bacillus megaterium</i>	Omar et al. (2001)
Curdlan	Date palm juice	<i>Rhizobium radiobacter</i> ATCC 6466	Salah et al. (2011)
Carotenoid	Date syrup	<i>Lactobacillus plantarum</i> QS3	Elsanhoty et al. (2012)
<i>Biofuel</i>			
Hydrogen	Rotten date	<i>E. coli</i> EGY	Abd-Alla et al. (2011)
		<i>Clostridium acetobutylicum</i> ATCC824	Abd-Alla et al. (2011)
		<i>Rhodobacter capsulatus</i> DSM 1710	Abd-Alla et al., 2011
Ethanol	Date extract	<i>Saccharomyces cerevisiae</i> ATCC 36858	Gupta and Kushwaha (2011)
		<i>S. cerevisiae</i> STAR brand	Gupta and Kushwaha (2011)
Butanol	Date wastes	<i>Saccharomyces cerevisiae</i> SDB	Acourene and Ammouche (2012)
	Spoiled date fruit	<i>Clostridium acetobutylicum</i> ATCC824	Abd-Alla and El-Enany, 2012
Biosurfactant	Date molasses	<i>Bacillus subtilis</i> DSM 4451	Abd-Alla and El-Enany (2012)
		<i>Bacillus subtilis</i> 20B	Al-Bahry et al., 2012
<i>Organic acids</i>			
Citric acid	Date extract/molasses	<i>Aspergillus niger</i> ATCC 6275 & 9642	Mehyar et al. (2005)
	Date wastes	<i>Aspergillus niger</i> ANSS-B5	Acourene and Ammouche (2012)
Lactic acid	Date juice	<i>Lactobacillus casei</i> subsp. rhamnosus	Nancib et al. (2001)
	Date juice	<i>Lactobacillus delbrucki</i>	Yadav et al. (2011)
<i>Amino acid</i>			
Glutamic acid	Date fruit waste	<i>Corynebacterium glutamicum</i> CECT 690 & CECT77	Davati et al. (2007)
	Date fruit waste	<i>Corynebacterium glutamicum</i> CECT 690	Tavakkoli et al. (2012)
<i>Biomass</i>			
Baker's yeast	Dates sugars	<i>Saccharomyces cerevisiae</i> (I)	Khan et al. (1995)
		<i>S. dastorianus</i> NRRL Y12693	Khan et al. (1995)
		<i>S. cerevisiae</i> (III)	Khan et al., 1995
		<i>S. dayanus</i> NRRL Y-12624	Khan et al. (1995)
		<i>S. cerevisiae</i> NRRL Y-12632	Khan et al. (1995)
		<i>S. lodgwii</i>	Nancib et al., 1997
<i>Probiotics</i>			
lactobacilli	Date powder	<i>Lactobacillus casei</i> ATCC 334	Shahravy et al. (2012)
<i>Antibiotics</i>			
Bleomycin	Date syrup	<i>Streptomyces mobaraensis</i>	Radwan et al. (2010)
Oxytetracycline	Date-coat sugar extract & date-seed hydrolysate	<i>Streptomyces rimosus</i>	Abou-Zeid et al. (1993)
<i>Enzymes</i>			
Pectinase	Date syrup	<i>Bacillus subtilis</i> EFRL 01	Qureshi et al. (2012)
Endopectinase	Date pomace	<i>Aspergillus niger</i> PC5	Bari et al. (2010)
Alpha amylase	Date wastes	<i>Candida guilliermondii</i> CGL-A10	Acourene and Ammouche (2012)

Curdlan consists of  $\beta$ -(1,3)-linked glucose residues and has the unusual property of forming an elastic gel upon heating its aqueous suspension. It is a food additive that is used as firming agent, gelling agent, stabilizer, and thickener in the food industry.

*Rhizobium radiobacter* ATCC 6466 was reported to produce curdlan using date palm juice by-products with an yield of  $22.83 \text{ g l}^{-1}$  when cultivated in a medium with optimal conditions of pH 7;  $2 \text{ g l}^{-1}$  of ammonium sulphate;  $120 \text{ g l}^{-1}$  of date glucose juice concentration,  $30^\circ\text{C}$ ; inoculum ratio of 5 ml/100 ml; 180 rpm agitation, and a fermentation period of 51 h (Salah et al., 2011). The purified date by-products-curdlan (DBP-curdlan) had a molecular weight of 180 kDa, a linear structure composed exclusively of  $\beta$ -(1,3)-glucosidic linkages,

a melting temperature ( $T_m$ ) and glass transition temperature ( $T_g$ ) of 1.24 and  $-3.55^\circ\text{C}$ , respectively (Salah et al., 2011).

#### 7.2.4. Carotenoid

Carotenoids are a group of red, yellow, or orange highly unsaturated pigments that are found in foods such as carrots, sweet potatoes, and leafy green vegetables. Carotenoids are tetra terpenoid organic pigments that are naturally occurring in the chloroplasts and chromoplasts of plants and some other photosynthetic organisms like algae. They are also produced by few microorganisms during the course of fermentation and are being industrially exploited. With respect to date fruit by-products as substrates for the fermentation production of carotenoids there are no other reports other than the one on optimization of the

medium components by Plackett–Burman design for carotenoid production using date (*P. dactylifera*) wastes by *Lactobacillus plantarum* QS3 (Elsanhoty et al., 2012). Results of the study indicated that Date syrup at 5% sugar concentration resulted in 16.21 mg kg<sup>-1</sup> dry cell of carotenoids when used alone, and there was increase in carotenoids production (54.89 mg kg<sup>-1</sup> dry cell) with supplementation of the MRS medium with salts and organic nitrogen after optimization of pH and temperature using date syrup as a carbon source.

### 7.3. Biofuels

#### 7.3.1. Hydrogen production

Hydrogen is preferred to biogas or methane as an alternative biofuel since H<sub>2</sub> is not chemically bound to carbon, and therefore burning does not contribute to greenhouse gases or acid rain (Nath and Das, 2004). Hydrogen is produced mainly from natural gas, a finite resource, through steam reforming, a process that generates large quantities of carbon dioxide (CO<sub>2</sub>) which is a principal cause of global warming. Therefore, it is imperative that an alternative but cleaner process that relies on renewable feedstock must be developed. Hydrogen can be produced by anaerobic bacteria through dark fermentation by species of *Enterobacter* (Nath et al., 2006), *Bacillus* (Kotay and Das, 2007), *Clostridium* (Ferchichi et al., 2005; Zhang et al., 2006) and *Thermotoga* (Schröder et al., 1994; Van Ooteghem et al., 2002). In fact hydrogen production through dark or photo-fermentative conversion of organic substrates is of great interest due to its dual function of waste reduction and clean energy production, thereby acting as a promising option for bio hydrogen production (Lata et al., 2007; Gadhamshetty et al., 2008; Tao et al., 2008). In terms of economical benefits, the H<sub>2</sub> production from rotten dates by mixed culture without anaerobic pre-treatment (reducing agent and flashing with argon) has a great potential for H<sub>2</sub> production at industrial scale.

Hydrogen production from rotten dates by sequential three stage fermentation was studied using three different bacteria (Abd-Alla et al., 2011). A facultative anaerobe, *Escherichia coli* EGY was used in the first stage to consume O<sub>2</sub> and maintain strict anaerobic conditions for a second stage dark fermentative H<sub>2</sub> production by the strictly anaerobic *Clostridium acetobutylicum* ATCC 824. Subsequently, a third stage photofermentation using *Rhodobacter capsulatus* DSM 1710 was conducted for the H<sub>2</sub> production. The total H<sub>2</sub> yield of the three stages was observed to be 7.8 mol H<sub>2</sub> per mol of sucrose when 5 g l<sup>-1</sup> of sucrose was supplemented as rotten date fruits. Results of their study indicated that rotten dates can be efficiently used for commercial H<sub>2</sub> production without the need for addition of a reducing agent or flashing with argon, both of which are expensive.

#### 7.3.2. Bioethanol

The most commonly used metabolically derived liquid bio-energy compounds are ethanol and butanol. Ethanol derived from biomass has the potential as a substitute for fossil fuel which is renewable, non-toxic, biodegradable and more ecofriendly. Ethanol is an important renewable and sustainable alternative clean fuel source (Balat et al., 2008; Sassner et al., 2008) and the world fuel bioethanol production exceeds 20,000 millions of gallons per year (Renewable Fuels Association, 2010; Astudillo and Alzate, 2011). In fermentation processes, several micro-organ-

isms (*S. cerevisiae* and *Zymomonas mobilis*) can be used to produce fructose and bioethanol. Generally, the use of mutants of these microorganisms decreases the consumption of fructose (Atiyeh and Duvnjak, 2001, 2002, 2003). The three major classes of feedstocks used for ethanol production are sugars, starches and lignocelluloses.

Date palm (*P. dactylifera*) sap which is highly nutritive and has high sugar content (60–70%) is a very good source for microbial fermentation (Gupta and Kushwaha, 2011). Hence, fermentation of date extracts to ethanol and vinegar in batch and continuous membrane reactors (Mehaia and Cheryan, 1991) and date syrup and wastes were tried for the production of ethanol (Besbes et al., 2009). Palm-wine fermentation is always alcoholic–lactic–acetic acid fermentation involving mainly yeasts and lactic acid bacteria. Studies on the production of fructose and/or bioethanol from date's extract by fermentation at a fixed temperature using a mutant strain *S. cerevisiae* ATCC 36858 and a commercial *S. cerevisiae* (STAR brand) activated at different periods demonstrated that *S. cerevisiae* ATCC 36858 could selectively convert glucose to ethanol and biomass with minimal fructose conversion (Gupta and Kushwaha, 2011). A high fructose yield above 91% of the original fructose was obtained with ATCC 36858 in addition to the ethanol yield which was found to be 63% of the theoretical (Gaily et al., 2012). Ethanol production, by *S. cerevisiae* SDB showed that an optimum yield of 136.00 ± 0.66 g l<sup>-1</sup> could be obtained under optimum conditions of an incubation period of 72 h, inoculum content of 4% (w/v), sugars concentration of 180.0 g l<sup>-1</sup>, and ammonium phosphate concentration of 1.0 g l<sup>-1</sup> (Acourene and Ammouche, 2012).

#### 7.3.3. Butanol

Acetone, butanol and ethanol (ABE) are commonly used solvents in many important industries and have a high potential for replacing petrochemical derived energy. Butanol, along with small amounts of acetone and ethanol, is produced biologically from renewable biomass by *Clostridium* spp. under strictly anaerobic conditions (Jones and Woods, 1986; Qureshi and Ezeji, 2008). There are several possible ways to reduce the costs of producing ABE from fermentation, which include use of a low cost fermentation substrate or optimizing the fermentation conditions to improve the efficiency of converting substrate to ABE.

Among the cheap and readily available substrates for ABE production, spoiled date fruits are possibly one of the better choices. The spoilage of dates is due to increased infestation of pests and diseases, improper transporting, handling, lack of cold stores, and lack of marketing and export (Al Jasser, 2010). Spoiled date fruits (*P. dactylifera* L.) were evaluated as a potential substrate for ABE fermentation employing mixed cultures of *C. acetobutylicum* ATCC 824 and *Bacillus subtilis* DSM 4451 (Abd-Alla and El-Enany, 2012). Mixed cultures of *C. acetobutylicum* ATCC 824 and *B. subtilis* DSM 4451 were observed to consume sugar, amino acid and NPK as well as any available oxygen during growth and reduced the need to develop anaerobic conditions to commence fermentation. The study also indicated that the aerobic *B. subtilis* DSM 5541 can be used to install and maintain anaerobic conditions for strictly anaerobic *C. acetobutylicum* ATCC 824 and the supplementation of yeast extract or ammonium nitrate to spoiled date fruits homogenate markedly increased the total



ABE production. The results suggested that rotten date palm fruits homogenate can be efficiently used as substrate without the need for any pre-treatment or hydrolysis and without requirement to add any costly reducing agent to the medium or flushing with  $N_2$  to ensure anaerobic condition for commercial ABE production by mixed cultures (Abd-Alla and El-Enany, 2012). Date fruit as carbon source in RCM-modified medium to produce biobutanol by *C. acetobutylicum* NCIMB 13357 was also reported (Khamaiseh et al., 2012).

#### 7.4. Biosurfactant

Biosurfactants are used widely in agricultural, cosmetics, food, pharmaceuticals, and petrochemical industries besides in environmental applications (Desai and Banat, 1997; Makkar and Cameotra, 2002). Biosurfactants are less toxic, highly biodegradable, have better foaming properties, and greater stability under harsh environmental conditions, as compared to chemical surfactants (Desai and Banat, 1997). They are more suited for bioremediation of polluted sites, oil spill management, and enhanced oil recovery (Banat, 1995; Al-Sulaimani et al., 2011). However, high production cost and low yield of biosurfactants affect their wide spread applications (Deleu and Paquot, 2004). The success of biosurfactant production at an industrial scale depends on several strategies, which includes among others the development of cheaper scale-up processes, and the use of inexpensive raw materials, which generally account for 10–30% of the overall cost (Joshi et al., 2008a,b).

Date molasses was used as a novel substrate and sole source of carbon for biosurfactant production ( $2.29 \pm 0.38 \text{ g l}^{-1}$ ) by *B. subtilis* 20B (Al-Bahry et al., 2012). The biosurfactant reduced surface tension and interfacial tension from 60–25 mN/m to 27 and 5.02 mN/m respectively, besides showing significant stability under a wide range of temperatures, pH and salt concentrations. Further, it was observed that the produced biosurfactant could yield additional 9.7% oil through core-flood studies, under reservoir conditions indicating potential for use in enhancing oil recovery.

#### 7.5. Organic acids

##### 7.5.1. Citric acid

Among the organic acids industrially produced, citric acid is the most important in quantitative terms, with an estimated annual production of about 1.4 million tons (Al-Shehri and Mostafa, 2006). Citric acid is the major organic acid produced by fermentation with *Aspergillus niger* and is widely used in the food, beverage, chemical, pharmaceutical, and other industries. The production of citric acid by *A. niger* is one of the most commercially utilized examples of fungal overflow metabolism although *Candida lipolytica* was experimented for the utilization of dates in the fermentative formation of citric acid (Abou Zied and Khoja, 1993).

*A. niger* (ATCC 6275 and 9642) produced citric acid in media containing different concentrations of date extract or molasses fortified with whey, methanol and tricalcium phosphate, at 25°C for 12 days (Mehyar et al., 2005). A high level of citric acid ( $32.4 \text{ g l}^{-1}$ ) was produced by *A. niger* ATCC 6275 in 20% molasses in whey after growth and the addition of methanol and tricalcium phosphate led to a significant increase in citric acid production ( $P < 0.05$ ). Citric acid concen-

trations were 38.4 and  $42.4 \text{ g l}^{-1}$  in media fortified with methanol and tricalcium phosphate, respectively (Mehyar et al., 2005).

*A. niger* obtained from soil samples of different locations in Saudi Arabia was screened for its ability to produce citric acid in date syrup medium under free and immobilized cells systems. Results of the study indicated that the maximum production of citric acid, about 1.44 times higher, could be obtained with the immobilized system compared to free cells. *A. niger* j4 isolated from the Jazan region was selected as a good producer for optimization of citric acid fermentation in immobilized cells technique. The investigators reported that citric acid production increased gradually and reached a maximum ( $30.6 \text{ g l}^{-1}$ ) after 6 days of cultivation and the optimum production conditions included 15% sugar concentration with consumed sugar of 49.5%. They also observed the highest value ( $42.5 \text{ g l}^{-1}$ ) of citric acid at pH 5.5 by increasing the consumed sugar to 61.7%. Further a positive relationship between citric acid production and incubation temperature was also observed up to 30°C (Al-Shehri and Mostafa, 2006).

Date syrup and wastes were experimented for the production of biomass and citric acid (Besbes et al., 2009). Citric acid production by *A. niger* ANSS-B5 was found to be influenced by the cumulative effect of temperature (30°C), sugar concentration of  $150.0 \text{ g l}^{-1}$ , methanol concentration of 3.0%, initial pH of 3.5, ammonium nitrate concentration of  $2.5 \text{ g l}^{-1}$ , and potassium phosphate concentration of  $2.5 \text{ g l}^{-1}$ . Thus under the said conditions during the fermentation process of date wastes syrup the citric acid production was increased to  $98.42 \pm 1.41 \text{ g l}^{-1}$  (Acourene and Ammouche, 2012).

##### 7.5.2. Lactic acid

Lactic acid has significant applications in the pharmaceutical, cosmetics and food industries (Göksungur and Güvenc, 1997). Indeed new applications, such as degradable plastics made from poly (lactic) acid, have a great potential, particularly if economic processes can be developed (Payot et al., 1999). Lactic acid bacteria are currently used in the dairy industry for cheese and fermented milk manufacture. In spite of the fact that several substrates were experimented for lactic acid production by lactic acid bacteria date fruits wastes or by-products are minimally experimented. In this context yeast extract has a prominent role in the medium as source of nitrogen for enhanced lactic acid production compared to other sources of nitrogen supplement. But yeast extract supplementation is not economically attractive and hence different proportions of other nitrogen supplements were used in combination with yeast extract. Studies on date juice as nitrogen supplement for the production of lactic acid by *Lactobacillus casei* subsp. *rhamnosus* showed that lactic acid production in date juice supplemented with  $20 \text{ g l}^{-1}$  yeast extract (0:5) was at same levels of lactic acid produced with the elemental nitrogen ratio of ammonium sulphate to yeast extract at 4:1 ratio (Nancib et al., 2001). Various nitrogen sources were compared with yeast extract for efficient lactic acid production by *L. casei* subsp. *rhamnosus*. Further, during the course of a study on the joint effect of nitrogen sources and B vitamin supplementation of date juice on lactic acid production by *L. casei* subsp. *rhamnosus* it was observed that among the different nitrogen sources added to date juice (yeast extract, ammonium sulphate, tryptic soy, urea, peptone, and casein hydrolysate),

yeast extract was found to be the most efficient (Nancib et al., 2005). Addition of five B vitamins at less than 25 mg l<sup>-1</sup> to date juice with any nitrogen source also led to the enhancement of lactic acid production to some extent except for date juice with yeast extract or urea or peptone. It was also observed that half of the yeast extract content (10 g l<sup>-1</sup>) in a supplemented date juice could be replaced by a mixture of B vitamins at less than 25 mg l<sup>-1</sup>, and ammonium sulphate at 2.6 g l<sup>-1</sup> with no significant decrease in lactic acid production (Nancib et al., 2005). Plackett–Burman design was employed for the statistical screening of medium components using date juice for lactic acid production by *Lactobacillus* sp. KCP01 (Chauhan et al., 2007). Lactic acid production by *Lactobacillus delbrueckii* was evaluated in an optimal production medium comprised of a 10% carbon source derived from sweet sorghum (SS) (*Sorghum bicolor*)/golden syrup (GS; molasses after glucose crystallization)/date palm (DP) juice (*P. dactylifera* L.), 1% yeast extract, 0.6% sodium acetate, 0.5% KH<sub>2</sub>PO<sub>4</sub>, and 0.5% MgSO<sub>4</sub> · 7H<sub>2</sub>O, in batch mode, at 45 ± 1°C, 150 rpm, anaerobic condition, and pH 5.5 ± 0.1. The lactic acid (LA) productivity was reported to be higher with GS and less with SS as well as DP juice (Yadav et al., 2011).

## 7.6. Amino acids

### 7.6.1. Glutamic acid

Glutamic acid has a wide range of applications in food industry, in pharmacology and cosmetics. Date waste generated from date processing industry was recognized as a substrate for the growth and GA production (maximum of 8 mg ml<sup>-1</sup>) by *Corynebacterium glutamicum* (Davati et al., 2007). Two mutants of *C. glutamicum* CECT690 and CECT77 were found to produce amino acids from date wastes and the amino acids measured by HPLC showed that many amino acids such as alanine, valine, lysine, proline, tyrosine, phenylalanine, leucine and isoleucine apart from glutamic acid and threonine were produced. Based on the statistical analysis, the study concluded that the most effective variables on glutamic acid production are the concentration of date fruit wastes, time of penicillin addition, phosphate concentration and type of microorganism respectively and the least effective variables on glutamic acid production are biotin amount, temperature and nitrogen source (Davati et al., 2007).

Glutamic acid production using palm date waste as substrate by *C. glutamicum* CECT690 under submerged fermentation conditions was optimized for the variables inoculum size, substrate concentration, penicillin concentration, phosphate concentration, and inoculum age for enhancing GA production employing response surface methodology (Tavakkoli et al., 2012). The RSM model predicted 39.32 mg ml<sup>-1</sup> as the maximum GA and the first stage of fermentation under optimized conditions of inoculum size 2% (v/v), substrate concentration 25% (w/v), penicillin concentration 1 U ml<sup>-1</sup>, phosphate concentration 4 g l<sup>-1</sup>, and inoculum age 10 h yielded 36.64 mg GA ml<sup>-1</sup> which was in agreement with the predicted model. In the second stage of the study the rate of GA was found to be increased in response to enhanced rate of air flow in a 5 l fermenter (batch mode) which was run in optimized conditions and 118.75, 142.25, and 95.83 mg of

GA ml<sup>-1</sup> were observed for the three levels of air flow rate of 0.6, 1.2, and 1.6 vvm, respectively. The study concluded that date waste juice has the potential of a substrate for GA production by *C. glutamicum* (Tavakkoli et al., 2012).

## 7.7. Biomass

Industrial production of microorganisms as biomass for varied applications is practiced over the years particularly with reference to yeasts that serve as biocatalysts or source of products in bakeries, breweries; lactic acid bacteria as starter cultures for use in dairy product manufacture; probiotics for application in dairy industries, animal feed and aquafeed production among other applications. Large scale production of biomass necessitates the use of cheap substrates for economic production of the commodities by fermentation technologies. While substrates like sugar cane molasses, soya bean meal, and other agro industrial wastes are experimented date fruit wastes remains to be exploited. In this context few investigations have been reported on the use of date fruit by-products and wastes for the production of biomass such as baker's yeast, probiotic lactobacilli and thermophilic dairy starters strain *Streptococcus thermophilus* (Nancib et al., 1999).

### 7.7.1. Baker's yeast

“Baker's yeast” is the common name for the strains of yeast, *S. cerevisiae*, commonly used as a leavening agent in baking bread and bakery products, where it converts the fermentable sugars present in the dough into carbon dioxide and ethanol. Bread is consumed as a major food item worldwide and bakeries are one among the major food industries distributed globally. These industries thrive on baker's yeast for their manufacture of bakery products. Consequently baker's yeast has become a commodity and the yeast biomass is produced in large scale by food industries. Although several substrates are used as a component in the cultivation of yeast biomass in large scale date fruit by-products and wastes were not exploited significantly. In this context dates were evaluated as an alternative substrate for black strap molasses in the production of yeast biomass. Different fermentation media were tried with six different yeasts namely *S. cerevisiae* (I); *S. dastorianus* NRRL Y12693; *S. cerevisiae* (III); *S. dayanus* NRRL Y-12624; *S. cerevisiae* NRRL Y-12632 and *S. lodgwii* and among the strains *S. cerevisiae* (I) recorded the highest biomass production and Sefry Beesha dates sugars which contained 60% total reducing sugars, were found to be suitable similar to that of the molasses in the production of yeast biomass. The study concluded that 50.0 mg of date sugar/ml, 2.0 mg ammonium sulphate/ml as nitrogen source, and 50 pg Biotin/l as growth stimulator were best for the production of yeast biomass (*S. cerevisiae* (I)). The ash of *S. cerevisiae* (I) containing Na, Mg, Ca, Fe, Zn and Cu. Cobalt and Ni was not detected (Khan et al., 1995).

### 7.8. Probiotic lactobacilli

Functional foods containing probiotic bacteria such as lactobacilli (*Lactobacillus casei*) have earned utmost significance in the food industry since lactobacilli enhance the microbial safety and offer nutritional and health benefits to the consumer (Leroy and De Vuyst, 2004). As a consequence of the large-

scale production of fermented foods incorporated with probiotics, the industrial production of these bacteria is becoming more important. Whereas, commercial production of *L. casei* at industrial scale necessitates cheap production medium towards economic production of the bacteria among several other decisive factors (Oh et al., 1995). In this context, date palm (*P. dactylifera*) fruit which contains high amounts of sucrose as well as reducing sugars (especially glucose and fructose) offers scope as a potentially convenient and inexpensive substitute for the glucose required by the lactobacilli during their growth and biomass production (Al-Shahib and Marshall, 2003). Dates syrup and date pits were reported to have positive influence as nutrients for the cultivation of *Lactococcus lactis* and hence were suggested a suitable substrate for the cultivation of microorganisms (Khiyami et al., 2008b).

Date powder (commercial product obtained from a production facility from Jam Co., Iran) was used for the first time as a low-cost main carbon source during the optimization of culture conditions for the economic production of a probiotic bacterium, *L. casei* ATCC 334. The effect of eleven factors on bacterial growth was investigated using the Taguchi experimental design, and three factors including palm date powder ( $38 \text{ g l}^{-1}$ ), tryptone ( $30 \text{ g l}^{-1}$ ) and agitation rate (320 rpm) were found to be the most significant parameters by Response surface methodology of Box-Behnken (Shahravy et al., 2012).

### 7.9. Antibiotics

Date fruit by-products and wastes were explored as possible substrates for the production of few antibiotics by investigators. From the literature available it was inferred that date fruits could be successfully used in the production medium as substrate for the production of Bleomycin and Oxytetracycline by species of *Streptomyces*.

#### 7.9.1. Bleomycin

Bleomycin (BLM) is a family of glycopeptide-derived antibiotics. BLM exhibits a strong antitumor activity and therefore, it has been widely employed for the treatment of several malignancies, including non-Hodgkin's lymphoma, squamous cell carcinoma and testicular tumors. Date syrup was used as an additional carbon source in the medium for the production of BLM by *Streptomyces mobaraensis* and a combined statistical approach of orthogonal design and polynomial regression was applied to optimize the composition and concentration of a liquid fermentation medium. Results of the investigation indicated that when date syrup was used as an additional carbon source, higher BLM amount could be obtained compared to glucose. Further it was noted that soyabean meal was the optimum nitrogen source. The study concluded that  $40 \text{ g ml}^{-1}$  of date syrup as additional carbon source in the complex medium enhanced the production of BLM by 73% (Radwan et al., 2010).

#### 7.9.2. Oxytetracycline

Oxytetracycline is a broad spectrum antibiotic that is active against a wide variety of bacteria. Oxytetracycline is still used to treat infections caused by chlamydia (e.g. the chest infection psittacosis, the eye infection trachoma, and the genital infection urethritis) and infections caused by mycoplasma organisms (e.g. pneumonia). Barni date-coat (fruit-flesh) sugar

extract, date-seed hydrolysate, date-seed lipid, and date-seed ash were experimented as substitutes for the components of the synthetic fermentation medium, especially carbon, nitrogen, and mineral sources for the production of oxytetracycline by *Streptomyces rimosus*. Date-coat sugar extract was rich in sugars; while date-seed hydrolysate contained more nitrogen sources. Both date sources were utilized by the organism to give high titres of the antibiotic. The deficiency in nitrogen was overcome by the addition of urea. It was found that date-seed ash in a concentration of  $0.5 \text{ mg ml}^{-1}$  could replace  $\text{MgSO}_4$ ,  $\text{MnSO}_4$ ,  $\text{FeSO}_4$ , and  $\text{ZnSO}_4$  when depleted from the medium. Further studies on a combination of date sources (date-coat sugar extract, date-seed hydrolysate, date-seed lipid, and date-seed ash) to formulate a natural medium for the formation of oxytetracycline indicated that the date sources in the natural medium were a convenient substrate for the biosynthesis of oxytetracycline by *S. rimosus* (Abou-Zeid et al., 1993). Later studies on date syrup and wastes as substrates for the production of biomass and oxytetracycline also yielded positive results (Besbes et al., 2009).

### 7.10. Enzymes

Enzyme industry is the second largest industry that depends on fermentation production using microbes as sources next only to pharmaceuticals. Industrial production of commercially important enzymes such as amylases, proteases, lipases, cellulases, pectinases, xylanases, invertases etc., largely depend on cheap enzyme production media formulated with simple and cheap substrates for the economic production of enzymes. While substrates like sugar cane molasses, soya bean meal, and other agro industrial wastes are experimented, date fruit wastes remains to be exploited although few enzymes were experimented. Date fruit by-products and wastes were also experimented as complementary substrates in the enzyme production medium for few industrial enzymes by investigators. From the literature available it was inferred that date fruits could be successfully used in the production medium as substrate for the production of pectinases, endopectinases and alpha amylase by few microorganisms.

#### 7.10.1. Pectinases

Pectinase enzymes hydrolyse pectins, the soluble complex polysaccharides that occur widely in plant cell walls. They are commercially used in many processes and nearly 25% of the global enzyme sales are attributed to pectinases. As a bulk enzyme, pectinase must be produced cheaply and is not recovered after use. Hence the economic production of pectinase demands the use of cheap substrates for their industrial production. Several pectin rich substrates have been previously used to produce microbial pectinases by fermentation. In this context it is anticipated that the use of date waste syrup as substrate for the production of pectinases can contribute to efficient management of date fruit processing wastes. Further this approach could reduce the cost of importing expensive pectinases for use in the production of cotton textiles. Date syrup has been utilized for producing pectinases, for possible use in cotton scouring, by *B. subtilis* EFRL 01. A mineral medium based on waste date syrup ( $15 \text{ g l}^{-1}$ ) as the carbon source and yeast extract ( $7.5 \text{ g l}^{-1}$ ) as the nitrogen source proved to be the most effective. Batch fermentation in this medium for 48 h with a start-



ing pH of 8.0 produced a pectinase titre of  $\sim 2700$  U ml<sup>-1</sup> at the optimal fermentation temperature of 45°C (Qureshi et al., 2012).

Production of endopectinase from date pomace by *A. niger* PC5 was studied using a two-level fractional factorial design for screening of the most important factors among concentrations of ammonium sulphate, potassium dihydrogen phosphate and date pomace, pH, total spore amount, aeration rate and fermentation time. The results of the first stage experiment indicated ammonium sulphate (0.25–0.45%), pH (4.82–6.12) and fermentation time (50–90 h) as critical factors and hence they were considered for further studies. In the second stage, response surface methodology was used to determine the optimum fermentation conditions for production of the enzyme. Second stage results showed that, fermentation time was the most significant factor on endopectinase activity. Modelling of the fermentation process indicated that maximum amounts of endopectinase (10.88 U ml<sup>-1</sup>) could be obtained with pH 5, 0.3% ammonium sulphate and 76.05 h of incubation (Bari et al., 2010).

#### 7.10.2. $\alpha$ -Amylase

Amylases are enzymes that act upon starch and find potential application in a number of industrial processes such as starch processing, food processing, fermentation, textile, paper industries, and detergent industries. It is one of the major industrial enzyme produced globally employing fungi and bacteria through fermentation processes. However amylase producing microorganisms are not yet reported to produce amylase using date fruit products as substrates in their enzyme production medium. In this context yeast *Candida guilliermondii* CGL-A10 was reported to produce  $\alpha$ -amylase using date wastes (Acourene and Ammouche, 2012). The results of the study showed that the starch components in the date wastes strongly induced the production of  $\alpha$ -amylase with a maximum at 5.0 g l<sup>-1</sup>. Among the various nitrogen sources tested, urea at 5.0 g l<sup>-1</sup> supported maximum biomass (5.76  $\pm$  0.56 g l<sup>-1</sup>) and  $\alpha$ -amylase (2.304.19  $\pm$  31.08  $\mu$ mol l<sup>-1</sup> min<sup>-1</sup>), after 72 h incubation at 30°C, with an initial pH of 6.0 and potassium phosphate concentration of 6.0 g l<sup>-1</sup> (Acourene and Ammouche, 2012).

## 8. Enzymic processing

Enzymes have been in use for processing of biomaterials over the years and enzyme processing of fruits for extraction and clarification is practised in industries. In spite of the fact that several polysaccharases, lipases and proteinases are used in industries for varied applications, the use of cellulases and pec-

tinases has been an integral part of modern fruit processing technology. These two enzymes are applied for the treatment of fruit mashes as they not only facilitate easy pressing and increase in juice recovery, but also ensure high quality of the derived end products. These enzymes not only help in softening the plant tissue but also lead to the release of cell contents that may be recovered with high yield (Sreenath et al., 1994). They have been used for separating serum from pulpy fruits like banana, grapes, mango etc. (Joshi et al., 1991). In fact literature available on the use of enzymes for processing of date fruit is rather scanty and those reports available are presented below. Table 2 present the details of different products derived from date fruit by-products and wastes employing enzymes.

### 8.1. Production of high-fructose syrup (HFS)

Fructose is 60% sweeter than sucrose and 150% more than glucose. The major use of fructose syrup is in food and beverage industries at relatively high concentrations. Most of the carbohydrates in dates are in the form of reduced sugars, mainly fructose and glucose (Al-Farsi et al., 2007; Kulkarni et al., 2008). High fructose syrups (HFS) are produced from different raw materials including corn starch, sugar cane, and sugar beet, in addition to other starchy raw materials like rice and dates (Hanover and White, 1993; Vuilleumier, 1993). Discarded dates witnessed a remarkable increase in recent times and the amounts reached an average of 50,000 tons per year. In this context, valorizing these discarded dates by using them as substrates for the production of low-cost fructose-rich syrups is an ideal option for the agro-industries.

Enzymatic isomerisation techniques are used to convert glucose into fructose, but the conversion is equilibrium-limited at around 42% fructose (Zhang et al., 2004). Invertase isolated from date palm fruits from the Tunisian oasis of Gabes and Jerid was mixed with an aqueous extract rich in sucrose prepared from the Deglet Nour variety and obtained a high-fructose syrup (HFS) after 30 min of incubation (Chaira et al., 2011).

High-fructose syrup was produced from date by-products (discarded dates) of the sucrose-rich variety of 'Deglet Nour' in a packed bed bioreactor using a novel thermostable invertase obtained from *Aspergillus awamori* under immobilized conditions (Smaali et al., 2011). The enzyme immobilized on acetic acid-solubilized chitosan by covalent binding using glutaraldehyde when deployed in a packed bed reactor (PBR) enabled the production of HFS efficiently and continuously from sucrose derived from aqueous date extracts. It was reported that feeding with an extract initially containing 139.2 g l<sup>-1</sup> of total sugar with 78.6 g l<sup>-1</sup> sucrose at a flow rate of 17 ml h<sup>-1</sup>, 50°C

**Table 2** Products derived from date fruit by-products and wastes through enzyme processing.

Product	Substrate	Enzymes	Reference
Date juice concentrate	Immature date fruit	Pectinase	Kulkarni et al. (2010)
	Date fruit	Pectinase/cellulase	Bahramian et al. (2011)
Date syrup	Date fruit	Pectinase/cellulase	El-Sharnouby et al. (2007)
High Fructose syrup	Discarded dates	Invertase	Chaira et al. (2011)
	Discarded dates	Invertase ( <i>Aspergillus awamori</i> ) immobilized enzymes	Smaali et al. (2011)
Fructooligosaccharides (FOS)	Date by-products	$\beta$ -Fructofuranosidase (Ffase)	Smaali et al. (2012)



and pH 6 resulted in a conversion factor of 0.95 and a final fructose content in the syrup of  $69 \text{ g l}^{-1}$  (Smaali et al., 2011).

### 8.2. Production of fructooligosaccharides (FOS)

Fructooligosaccharides (FOS) are commonly used as prebiotics. FOS was synthesized using aqueous extracts of date by-products of the sucrose-rich variety “Deglet Nour” as a starting substrate employing crude  $\beta$ -fructofuranosidase (Ffase) obtained from *A. awamori* NBRC4033 immobilized on chitosan by covalent binding through glutaraldehyde linkages. The studies on the effect of water-extraction volume on the FOS synthesis by transfructosylation indicated that 150 ml/100 g of date by-products could yield the best FOS concentration and productivity ( $123 \text{ g l}^{-1}$  and  $18.5 \text{ g/h/100 g}$  respectively), related to an optimal sucrose conversion of 53.26%. The main FOS product was purified via a biogel-P2 gel filtration column and its structure was determined as 1-kestose:  $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-fructofuranosyl-(2 $\rightarrow$ 1)- $\beta$ -D-fructofuranoside by a combination of  $^1\text{H}$ ,  $^{13}\text{C}$  and 2D-NMR techniques. The study concluded that date by-products could be an alternative source of sucrose for the enzymatic synthesis of FOS (Smaali et al., 2012).

### 8.3. Date juice concentrate

Pectinases can hydrolyse pectin and cause pectin–protein complexes to flocculate, so the resulting juice has a much lower amount of pectin and also a lower viscosity, which is advantageous for the filtration process (Rai et al., 2007). Pectinases were evaluated for the preparation of juice concentrates from date fruits (*P. dactylifera*). Fruits of date palm (*P. dactylifera*), widely cultivated in Kutch district of Gujarat, are harvested at an immature stage before the onset of monsoon to prevent spoilage. These immature date fruits with less commercial value were processed into date juice concentrate by treatment with 0.1% pectinase enzyme for 120 min to obtain maximum juice (Kulkarni et al., 2010). The study indicated that the prepared date juice was rich in reducing sugars (16.1%) and total sugars (18.3%). The prepared juice was pasteurized at  $85^\circ\text{C}$  to inactivate the enzyme, cooled and centrifuged at 3000 rpm to get clear juice. The juice was concentrated in a thin film evaporator to total soluble solids (TSS) of 76 Brix in 2 passes. Date juice concentrate thus prepared was found to be stable over 6 months of storage and could be reconstituted for preparing ready-to-serve beverages with acceptable sensory quality (Kulkarni et al., 2010).

Kabkab, a date cultivar from the Kerman province in Iran, which is industrially used for extraction of its sugar, was selected for enzymatic extraction using pectinases and cellulases (Bahramian et al., 2011). Comparison of samples, pre-treated by either Pectinex®Smash XXL or Cellubrix® L, with untreated date fruits, showed that the amount of both extracted sugar and clarity of juices thus produced, was affected by enzymatic pre-treatment of fruits. It was reported that the pre treatment of fruits by each of the two enzymes caused equally about 18% increase in the amount of extracted sugars, and a precisely determined mixture of two enzymes under suitable conditions, resulted in a further increase of sugar to about 46%, in relation to untreated samples. Regarding the clarity of the juice, the results indicated that increased sugar content of the

extracts positively affects the clarity of juices, with some exceptions (Bahramian et al., 2011).

### 8.4. Date syrup

Reziz date (Soft variety) fruits (*P. dactylifera*) cultivated in the Kingdom of Saudi Arabia were used for the production of high quality date syrup, since they contain high total sugar content (about 83.51% on dry basis). Pectinase and cellulase were employed to obtain the maximum date syrup extraction at different ratios of water/date (2:1, 2.5:1 and 3:1) (El-Sharnouby et al., 2009). The extraction rate of sugars increased as the water/flesh ratio increased and the use of pectinase/cellulase facilitated the highest recovery of total soluble solids (65.6–70.7%) compared with control (50.5–56.30%). Results of the organoleptic evaluation proved that date syrup was highly desirable than cane syrup and indicated the possibility of employing pectinase/cellulase to produce concentrated date syrup from tamr fruits for use in food product development. Further, it was also noted that this syrup was found suitable for the manufacture of different food products (El-Sharnouby et al., 2009).

## 9. Future trends

The review presented here points out the fact that there is an intense research activity around the world, both in countries that produce date palm as well as those in countries where it is not grown but consumed a lot, towards harnessing the various by-products of date palm fruit and its wastes efficiently. Whereas the diversity in terms of probable products achieved via valorization is rather narrow and there are lots of opportunities for experimenting with a new range of products considering their vast and diverse biomolecules that may hold potential for use in pharmaceutical industries as active pharmaceutical ingredient (API), or in the development of nutraceuticals besides using them as substrates for the production of a vast array of antibiotics, economically useful amino acids, organic acids, biopolymers, biofuels etc. It is anticipated that there will be intensive drive in research development activities in the coming future especially in exploring bioprocessing technologies for their possible application in large scale production, particularly by food and pharmaceutical industries. The authors are of the opinion that future prospects in research and development on the valorization of date palm fruit by-products may include the following specific targets:

- ✓ Identification of active pharmaceutical ingredients in date fruits for use in the development of new pharmaceutical drugs.
- ✓ Development of new functional foods based on date fruit biomolecules.
- ✓ Use of date fruit ingredients as cheap substrates and components in cultivation media in place of costly substances that form the constituent of cultivation media for growing several microorganisms, and cell lines.
- ✓ Identification of potential biocatalysts, both microbes and enzymes for the total utilization of date fruit components and production of novel products through value addition.

- ✓ Development of appropriate bioprocesses and bioreactors for the efficient utilization of date fruit by-products towards the economic production of desired industrial chemical or biomass such as probiotics.

## 10. Conclusion

Date palm fruit, one of the most nutritive and comprehensive fruits in terms of health benefits is an ideal substrate for deriving a range of value added products in food and nutraceutical industries in the coming future employing bioprocessing technologies which have immense scope for application in the valorization of date fruit by-products and wastes. The enormous amount of date seeds thrown into the environment besides the discarded dates and spoiled wastes hold immense potential as raw material for bioprocessing and augmentation of new range of products as indicated in this review. There is ample scope for the emergence of new bio industries and bioentrepreneurs in the date growing countries towards total utilization of the date palm in addition to efficient and effective date palm fruit waste management. Further there is also scope for socioeconomic and rural development in those countries where date palm is cultivated in large scale by the effective management of date palm fruit resources. Taking into consideration the quantum and quality of scientific literature available on the valorization of date palm fruits by-products and wastes, there is need for undertaking intensive research and development activities in identifying new products from date fruits considering their rich and diverse biomolecules that holds potential to return value added compounds for commercial utilization. Further new biocatalysts and development of appropriate bioprocesses, downstream processes and modelling studies need to be recognized and developed for such a noble mission.

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