

Review

***Vitellaria paradoxa* fruit pulp bioethanol production potential: A review**

Abdul-Mumeen Iddrisu^{1*}, Zakpaa Hilary D.², Felix Charles Mills-Robertson² and Lowor Samuel T.³

¹Department of Biochemistry and Biotechnology, College of science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

²Department of Biochemistry and Biotechnology, Faculty of Biological Sciences, KNUST, Kumasi, Ghana.

³Department of Biochemistry and Plant Physiology, Cocoa Research Institute of Ghana (CRIG), TafoAkim, Ghana.

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In the last decade, bioethanol has become a powerful biofuel for the improvement of environmental pollution such as reduction in greenhouse gas levels. Yet, the source and type of substrate material plays a crucial role in the bioethanol production process due to the different compositional characteristics and availability of monomeric sugars. Different substrates of first, second and third generation fuel sources exist and may be used as reliable and sustainable substrates for bioethanol generation. The current review provides an overview of *Vitellaria* fruit pulp; its composition and characteristics for ethanol production. This study has examined literature on the background of the *Vitellaria paradoxa*, the characteristics and the potential of the shea nut pulp for fermentation to bioethanol. This review will be useful in harnessing the potentials of the shea pulp as industrially relevant substrate for use independently or in combination with other substrates in microbial fermentation processes for ethanol production.

Key words: *Vitellaria*, shea nut pulp, fermentation, composition, characterization, bioethanol.

INTRODUCTION

Bioethanol is a form of biofuel that has become a major source of bioenergy. Bioethanol is reported as a fuel devoid of pollutants usually mixed with gasoline to run vehicles without modifications to the engine or its design (Doble and Kruthiventi, 2007). It is one of the most commonly used biofuels in the transportation sector and contributes immensely to the reduction of greenhouse gases in the atmosphere (Tesfaw and Assefa, 2014) due to its distinct physico-chemical properties. The United

States for instance produced 16.1 billion gallons of clean-burning bioethanol with total consumption rising to 16.2 billion gallons in 2018, 300 million gallons more than 2017 (RFA, 2019). The production and use of bioethanol in automobiles has both economic significance and environmental safety and this remains an important prospect of biofuel generation.

Bioethanol can be produced directly or indirectly from biomass (FAO, 2004; Giampietro et al., 1997; IEA, 2011).

*Corresponding author. E-mail: jugu2004@yahoo.com.

It is of biological origin excluding material embedded in geological formations and transformed to fossil. Bioethanol is noted to be the most produced biofuel in the world (RFA, 2018) and the production from first generation source (Klanarong et al., 2012; Thalisa, 2010), second generation source (Zakpaa et al., 2010; Suhas et al., 2013) and third generation material sources (Abdul-Mumeen et al., 2016) have received the greatest attention worldwide. Ethanol generated from first generation crops or food crops or energy crops such as maize, cassava and sugar cane and beet has shown numerous benefits but has always done so with myriad of concerns. The large acreage of arable land required for first generation crop production to meet the requisite quantities of ethanol demand is a concern. The main reason is that it poses a huge toll of competition with food and animal feed in addition to other criticisms which highlight the raw material processing cost having the ability to take up to 40% of the total production cost. The use of industrial, agricultural, household and municipal waste or second generation source materials for ethanol production has become the immediate solution to the concerns of using food crops. Residual biomass can contain high carbohydrates content that can be converted to bioethanol. Fruit rinds remain one of the most abundant and affordable raw material source for second generation bioethanol production.

Bioethanol considered as liquid fuel is produced by fermentation - a process by which ethanol is made from sugars (Thomsen et al., 2003). All ethanol fermentation is still based, practically, on the use of the Baker's yeast or *Saccharomyces cerevisiae*, which requires monomeric sugars as the raw material. Fermentation using *S. cerevisiae* produces 0.51 kg of ethanol from 1 kg of any of the C6 sugars: glucose, mannose and sucrose (Thomsen et al., 2003). But *S. cerevisiae* and other microorganisms can also be used to produce ethanol from C5 sugars such as xylose. Ethanol produced by microbial fermentation is used blended or alone, primarily as a substitute for gasoline. Global ethanol usage is expected to increase by 17 billion liters by 2026 and 90% of this increase will take place in developing countries (OECD/FAO, 2017) although bioethanol usage is driven primarily by policies mandating usage levels (FAPRI-MU, 2018).

The *Vitellaria paradoxa* fruit pulp reported to be sweet is a rich source of sugars, minerals and proteins (Maranz et al., 2004) even though the exact monomeric sugars are not known. The shea fruit weighs from 10 to 57 g and its annual production is from 15 to 30 kg/tree (Agbahungba and Depommier, 1989). The pulp constitutes about 60 to 80% (w/w) of the total mass of the shea nut fruit. The *V. paradoxa* fruit pulp with its characteristic soft, smooth and easy to digest macrostructure has not been thoroughly examined for its fermentability to bioethanol. This current assessment describes the biochemical, minerals, soluble sugars,

amino acids and the general uses of the *Vitellaria* fruit pulp, the agronomy, production and the potential as feedstock for bioethanol production with a focus on enzyme-assisted and microbial aided fermentation processes. Enzymatic hydrolysis technology prior to fermentation has, in recent times, gained increased attention pertaining to the soluble sugar yield and bioethanol output of targeted substrates. Enzymatic use also allows for reduced cost of hydrolysis in the fermentation of fruit rinds and as a result holds the key to sustainable production of optimal bioethanol in Africa.

THE SHEA NUT TREE

The shea tree (*Vitellaria*) is a member of the Sapotaceae family. It is divided into two subspecies: *paradoxa* and *nilotica* (Moore, 2008). Under the African culture of unwritten facts, known and told by griots, the shea nut tree has been known and used in several different ways for nearly two centuries now. That was the case until in the 18th century when Mungo Park, the British explorer, first came upon it in West Africa in 1796 and described the tree as a useful specie (Wilson, 2019). In 1807, Karl Friedrich Von Gaertner (1772 - 1850), a German Botanist, was the first to classify the shea nut tree as *V. paradoxa* (West African subspecies) and *Vitellaria nilotica* (East African subspecies). Karl Georg Theodore Kotschy (1813 - 1866) Ustron, Poland, an Australian botanist and explorer, reclassified the shea nut tree as *Butyrospermum parkii* for the West African subspecies and *Butyrospermum nilotica* for the East African subspecies.

In Northern Ghana, the shea nut tree, commonly called 'taanga' (Abdul-Mumeen, 2013), was discovered about two centuries ago. The Dogomba women of Northern Ghana were among the first to recognize the significance of the shea tree when they extracted fat from its nuts. During the latter part of the 20th century, shea butter was declared a potential substitute for cocoa butter (Moore, 2008). There was a marked increase in demand for shea butter from the cosmetics and pharmaceutical industries. Thus the shea nut tree was included on the list of tree species constituting African forest genetic resource priorities (FAO, 2014). Therefore, the Cocoa Research Institute of Ghana (CRIG), from 1981 to 1989 was tasked to increase botanical and genetic exploration with research, focusing on diversity, management and propagation of the shea tree (Amissah et al., 2013). Almost five decades down the line, the CRIG is repositioning itself and opening up stations in the three Northern regions to give more definition to the shea nut industry.

The shea tree, an indigenous fruit tree (Figure 1), is perennial and deciduous, and occurs mainly on dry open slopes (Yidana, 2004) and mostly on sandy-loamy soils (Abdulai et al., 2015). The shea tree begins to bear fruit

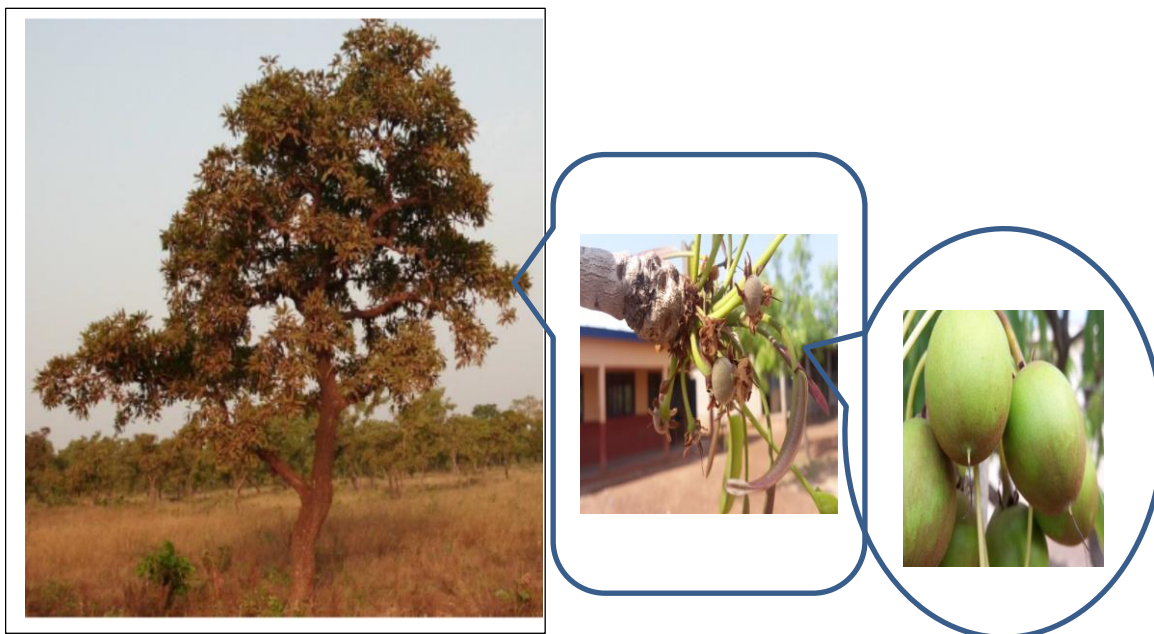


Figure 1. Shea (*Vitellaria*) tree species, shoots and fruits.

after about 15 to 30 years and can produce good-quality sweet fruits for up to 30 - 250 years (Hall et al., 1996; Dalziel, 1937). The fruits are produced from May to August; being subglobose to ovoid in shape and resemble small avocado fruits. Each fruit is covered with a pulp which is delicious when the fruit is ripe. Olaniyan and Oje (2007) describe the shea fruit to consist of a green epicarp, a fleshy mesocarp and a relatively hard shell (endocarp) which encloses the shea kernel (embryo) sometimes two or more (Ruyssen, 1957). The fruit weighs from 10 to 57 g and its annual production is from 15 to 30 kg/tree (Agbahungba and Depommier, 1989). The pulp of the fruit, which is sweet is widely consumed in areas where the species occurs. It is a rich source of sugars, protein, calcium, ascorbic acid, and iron (Maranz et al., 2004).

SHEA MORPHOLOGY, AGRONOMY AND PLANTATION

V. paradoxa has a wide range of appearance across sub-Saharan Africa. This species is ellipsoidal or a pyramidal crown in shape, a deciduous fruit tree of medium size with a white scar at one side (Alonge and Olaniyan, 2007; Moore, 2008). The morphological characteristics of the shea nut tree have proved to be significantly different from one tree to another by their characteristics. The height, girth, density, seed length and seed width of the shea tree are all different from one tree to the other (Moore, 2008). Generally the tree can attain a height of about 6.1 m under harsh conditions (Maranz, 2004). A fully matured tree under protected conditions will grow

from 10 to 20 m in height and rarely to 25 m (Maydell, 1990; Maranz, 2004). A shea nut tree has a cylindrical trunk of 0.5 to 2.5 m circumference, measuring 3 - 4 m before splitting into numerous branches with thick, fissured bark (Moore, 2008) but can also be of 61 cm girth when ravaged by bush fires (Yidana, 2004). The characteristics of the shea nut fruits and nuts, together with the nut length, leaf length, leaf width and petiole length are among the factors that contribute to the variation among shea nut trees (Enaberue et al., 2014). The elliptically shaped fruit measures 2.0- 8.0 cm long × 1.0 to 4.0 cm wide × 2.3 cm thick (Maranz and Wiseman, 2003; Alonge and Olaniyan, 2007). There is a kernel inside the nut which fits properly into the shell. The kernel is about 3.2 cm large, 2.3 cm wide × 0.1 - 2.1 cm thick in size (Alonge and Olaniyan, 2007; Olaniyan and Oje, 1999).

The shea tree can survive on a range of soil types (Hall et al., 1996) as it grows well in sandy soils, light sandy-loams and loamy soils but not in clay soils (Abdulai et al., 2015). The tree is not adaptable to lands susceptible to flooding (Agyente, 2010). *V. paradoxa* has excellent tolerance for drought and this has been well recognized by its ability to grow in impoverished soils and dry areas such as northern Ghana. *Vitellaria* has an extensively, moderately shallow rooting system. This aids the tree's adaptation to extended dry seasons or areas of unpredictable rainfall (Vermilye, 2004). *V. paradoxa* occurs naturally in the wild and grows slowly by seeds randomly dispersed by humans, birds, bats, wind or by gravitational force. The natural regeneration of *V. paradoxa* may be aided by appropriate land management

practices such as protection from bush fire or grazing of livestock (Kristensen and Lyke, 2003) or good farming practices. As a result *V. paradoxa* is considered a semi-domesticated crop (Boffa, 2015). Research has shown that in most areas of Northern Ghana, centuries of traditional land management has led to semi-domesticated *Vitellaria* plots unconsciously being selected (Lovett and Haq, 2000).

Artificial regeneration of the shea nut tree has not been very successful. Biotechnological improvement of the shea through the manipulation of its genetic code to enhance its long juvenile phase is affected by several factors such as its highly recalcitrant seeds, slow growth and the absence of both efficient conventional vegetative propagation and biotechnological methods. Several vegetative propagation studies have been carried out (Amisshah et al., 2013; Opoku-Ameyaw et al., 2002; Sanou et al., 2004; Yeboah et al., 2009) but the shea tree has proven to be recalcitrant, responding unfavorably to all known vegetative propagation techniques. However, *Vitellaria*, once matured, has an average life span of 250 years.

SHEA PRODUCTION AND FEEDSTOCK

The production and harvest of shea (*Vitellaria*) is in the African continent only. The estimated number of productive trees is some several hundred million (Lovett, 2004) and the potential number of shea trees in Africa's shea zone ranges from a couple of a billion (Naughton et al., 2014). *V. paradoxa* or *nilotica* grows across approximately 4 million square kilometers of sub-Saharan Africa (Julia et al., 2015) and stretches along almost 19 countries in west and central Africa (Scholz, 2009). The shea nut tree becomes therefore the largest tree population size of the economic tree species in the region. Africa produces about 1.76 million metric tons of raw shea nuts annually (Mohammed et al., 2013). There was an estimated 94 million shea nut trees in Ghana which were projected to produce at least 60,000 metric tons of shea nuts per annum for the production of all shea butter processed locally (Ofosu, 2009). The thickest of shea nut trees is in the Northern Savannah areas, covering over 80% of the woody vegetation (Lovett and Haq, 2000) and offering Ghana the potential to produce 90% of the world's shea nuts (Techno Serve Ghana, 2004).

The shea nut pulp (SNP) constitutes about 60- 80% of fruit weight of the shea nut fruit. During the processing of the shea nut fruit for shea butter extraction, the SNP is first removed by a process known as depulping through unguided fermentation (Abdulai et al., 2015) in mass quantities. For instance, for every 1000 Kg of wet mass of shea fruits picked, about 600 to 800 Kg of wet mass of SNP is generated. The quantum of waste generated is thus huge and therefore the shea nut pulp is best described as an industrial *residue* or forestry waste in

Abundance (Figure 3).

OVERVIEW OF ATTRIBUTES AND COMPOSITION OF SHEA NUT FRUIT

The shea nut fruit is a berry, it is hard when raw and soft when ripe but generally green from outside when raw or ripe. The shea nut fruit (SNF) is a naturally profiled layer of four. It consists of a thin epicarp and a soft mesocarp enclosing a single seed, sometimes two or more (Ruysen, 1957). The thin epicarp and the soft mesocarp constitute the pulp which is very sweet and highly nutritious when ripe (Maranz et al., 2004). The pulp is widely consumed in areas where the shea tree species occurs. It is a rich source of sugars, proteins, calcium, ascorbic acid, and iron (Maranz et al., 2004). The pulp surrounds a relatively large oily-rich oval, brown seed, referred to as shea nut (Figure 2) from which shea butter is extracted (Mohammed et al., 2013; Moore, 2008).

The enclosed nut has a shiny, smooth surface and comprises about 50% of the fresh weight of the fruit (Maranz and Wiseman, 2003). The shea nut has 2 layers: a brown testa or shell and an endosperm or (an oil-bearing) kernel from which shea butter is extracted. SNP is a polysaccharide and has been examined to consist of many biochemical constituents or nutritional elements such as carbohydrates, protein, lipids and fibre (Enaberue et al., 2014; Aguzue et al., 2013; Okullo et al., 2010; Ugese et al., 2008b; Mbaiguinam et al., 2007; Ojo and Adebayo, 2013; Omujal, 2009). The presence of carbohydrates in the SNP which is mostly deduced by difference varies across the shea regional zones from a minimum of 8.10 g/100g to 62.68 g/100 g (Table 1). The difference in carbohydrate levels of the SNP is explained by the fact that soil variation impacts shea nut fruit composition (Abdulai et al., 2015) as well as the stage of harvest and the source or location (Ugese et al., 2008a; Mbaiguinam et al., 2007). The presence and levels of carbohydrates suggest that the SNP contains monosaccharide units such as glucose or its isomers, once it is treated to produce as such. In addition, SNP contains some amount of protein ranging between 4.2 g/100g to 5.6 g/100 g, Table 1, suggesting that the SNP will contain traces of protein biosynthetic precursors, amino acids. Likewise, the amino acid profile (Table 1) will differ from place to place (Mbaiguinam et al., 2007; Dakora and Naab, 2014) mainly due to differences in the location, harvest stage and soil variation (Abdulai et al., 2015). SNP is hydrolysable and has been reported to produce soluble sugars, glucose and fructose especially, also contains sucrose and Mannitol (Dakora and Naab, 2014). The sucrose and glucose levels have been reported very high, 151 g/100g and 157 g/100g respectively, as well as the levels of fructose (145g/100g) and Mannitol (139g/100g). The literature reports several mineral compositions (Table 1) of the SNP; Dakora and Naab (2014) found 6 macronutrients (P, K, Ca, Mg,

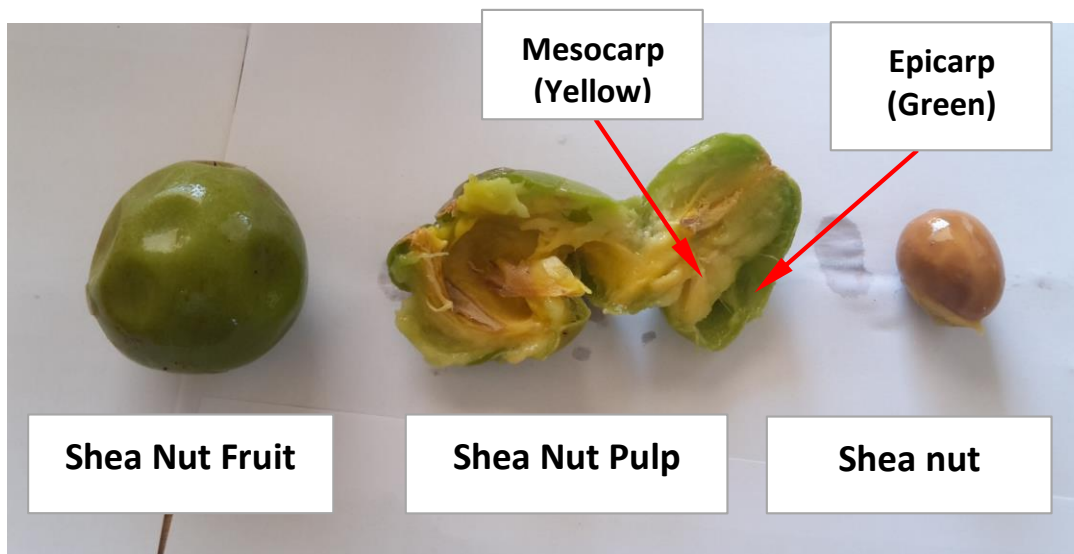


Figure 2. Shea nut fruit, pulp and the nut (seed).
Source: Abdul-Mumeen et al. (2019).

S and Na) and 6 trace elements (Fe, Cu, Zn, Mn, B and Al) in the SNP.

SNP FOR BIOETHANOL PRODUCTION

It has been demonstrated that the SNP is a potential source of reducing sugars (Table 1). The major source of bioethanol is the conversion of bioethanol from reducing sugars, by fermentation. Unfortunately, there has not been any report on converting the SNP to bioethanol by any means possible yet bioethanol has become one of the safest fuels for combating climate change factors. Bioethanol is a form of biofuel that generates bioenergy and it is one of the most commonly used biofuels in the transport sector to reduce greenhouse gases (Tesfaw and Assefa, 2014). The FAO (2004) defines biofuel as “fuel produced directly or indirectly from biomass”. Biomass is a material of biological origin, excluding material embedded in geological formations, transformed to fossil (FAO, 2004). Biofuel is also considered as any solid, liquid or gaseous fuel that is produced from biomass (Giampietro et al., 1997; IEA, 2010). Therefore, bioenergy is all the energies derived from biofuels. Bioethanol is mainly obtained by the processes of fermentation.

Bioethanol is produced by the fermentation of materials of sugar or starch source. The most common substrates are sugar cane, corn, wheat, sugar beet, seaweeds and fruit pulps. Cellulosic biomass such as grasses, woody crops, and organic wastes can also be used to produce bioethanol through advanced processing. Several studies (Grohman, 1995; Hammond, 1996; Grohmann, 1998; Sharma et al., 2007; Tesfaw and Assefa, 2014)

investigating cellulosic biomass have been carried out for bioethanol production. Bioethanol production from green seaweeds (Abdul-Mumeen et al., 2016), banana and citrus waste (Sharma et al., 2007) has also been investigated but limited literature is available on ethanol production from the SNP. The SNP is in abundance and with its characteristic soft, smooth and easy to digest macrostructure, requires no any special treatment prior to fermentation. Therefore, the energy needs of the bioethanol production process using the SNP as substrate may be curtailed and as a result, the pulp remains a huge potential for bioethanol production.

MICROBIAL/ENZYMATIC SACCHARIFICATION OF SHEA NUT PULP

Fruit pulps like the shea nut pulp are composed of carbohydrates or sugars, best described as monosaccharides, disaccharides and polysaccharides. Monosaccharides are simple sugars that cannot be broken down further while disaccharides and polysaccharides have glycosidic bonds between every 2 simple sugar molecules. They require further breakdown by the requisite mechanisms to obtain reasonable amounts of monomeric units, prior to fermentation. According to Thomas et al. (1993), the sugar content in fuel ethanol fermentation can be categorized to normal when the sugar composition is between 20-22% of the substrate or very high when the sugar level is greater than 27% of the total substrate weight.

Several saccharification methods have been used to breakdown the glycosidic bonds holding together, monomeric units in polysaccharides. The methods could be physical, chemical or biological breakdown of

Table 1. Summary of the biochemical characteristics, mineral content, soluble sugars and the amino acid profile mapping of shea nut pulp (SNP).

Characteristics	Range of values			References
	Min	Mean	Max	
Biochemical composition (g/100 g)				
Carbohydrates	8.10	22.60	62.68	Enaberue et al. (2014)
Crude protein	4.20	5.20	5.60	Aguzue et al. (2013)
Crude lipid	1.30	1.30	34.53	Okullo et al. (2010)
Crude fiber	42.20	42.20	42.20	Ugese et al. (2008b)
Ash	4.92	5.06	5.20	Mbaiguinam et al. (2015)
Energy	248.16	250.25	252.29	Ojo and Adebayo (2013)
Mineral (mg/100 g)				
Na	10.79	19.30	52.30	
Ca	0.19	70.30	117.30	
Mg	0.50	21.78	57.20	
K	1.40	51.38	830.30	
Cu	0.14	0.62	1.10	Enaberue et al. (2014)
Fe	0.01	14.15	28.29	Aguzue et al. (2013)
Mn	0.20	0.64	1.07	Okullo et al. (2010)
P	0.07	35.74	71.40	Ugese et al. (2008b)
Zn	0.50	2.25	4.00	Mbaiguinam et al. (2007)
Ni	-	0.86	-	Dakora and Naab (2014)
Cd	-	0.04	-	Omuja (2009)
Co	-	0.80	-	
S	-	0.05	-	
B	-	0.90	-	
Al	-	14.26	-	
Soluble sugar (g/100 g)				
Fructose	40	87	145	
Mannitol	47	91	139	
Glucose	51	103	157	Dakora and Naab (2014)
Sucrose	38	96	151	
Amino acids (g/100 g)				
Alanine	2.21	63.32	120.00	
Arginine	2.93	91.40	174.00	
Asparagine	6.03	95.05	172.00	
Cysteine	0.97	1.12	1.28	
Glycine	1.93	2.18	2.44	
Glutamine	4.98	5.59	6.28	
Histidine	1.03	1.23	1.37	
Isoleucine	1.87	17.81	30.00	
Leucine	2.88	27.82	47.00	Mbaiguinam et al. (2007)
Lysine	1.67	1.79	1.91	Dakora and Naab (2014)
Methionine	0.07	0.09	0.12	
Phenylalanine	1.29	1.44	1.65	
Proline	3.56	599.84	1189.00	
Serine	1.71	42.57	80.00	
Threonine	1.53	13.80	23.00	
Tyrosine	1.41	14.62	25.00	
Valine	2.25	29.88	53.00	

polysaccharides into their base monomer units. Any such treatment; acidic or alkaline, enzymes or microorganisms, size reduction or softening by beating, or thermal application aimed at breaking down the cell wall, hemicellulose, cellulose or lignin for the release of soluble sugars; pentose or hexose, is also referred to as pretreatment. That is, in fermentation processes, the terms saccharification, hydrolysis and pretreatment are sometimes used interchangeably. Microbial or enzymatic hydrolysis is by far the mildest and the most environmentally safe process for the release of monomeric sugars from fruit pulps (Figure 2).

Enzymes are vegetable or animal extracts or just microorganisms. They have been used as such throughout civilization. Microbes or their enzymes have been widely used for breaking glycosidic bonds in complex sugars to produce monomeric sugars. Some plant materials such as lignin may be very recalcitrant to microbial or enzymatic attack. The production of bioethanol from maize agro-wastes (lignocellulose) with cellulase as the saccharifying agent is crucial and relatively expensive cost-wise since enzyme cost alone contributes about 40% (zakpaa et al., 2010; Howard et al., 2003; Miyamoto, 1997) of the production cost.

Zakpaa et al. (2010), in search of low cost saccharifying organisms for corncob, assayed cellulolytic isolates on corncob based broth media. *Aspergillus niger* had the highest significant filter paper activity (0.37 FPU/ml), carboxymethyl cellulose activity (0.7025 U/ml) and protein concentration (5.62 mg/ml) although *Trichoderma*, *Penicillium*, *Mucor*, *Fusarium Rhodotorula*, *Acremonium* and *Coccidioides* were all isolated and assayed for their saccharification potentials (Table 2). Thus, the use of cellulase-producing organisms (Bon and Ferrara, 2007) is one way of reducing the higher production cost which also remains one of the ways to increasing available sugar in the fermentation media. Suhas et al. (2013) utilizing fruit rinds from four fruits (Pineapple, Jackfruit, Watermelon and Muskmelon) as possible source of cellulosic ethanol under anaerobic conditions, employed *Trichoderma viride* for saccharification of the powdered substrate. Significant amounts of reducing sugars were obtained at the end of the saccharification process, with the microbe being most effective on jackfruit and pineapple rinds, resulting in a monomeric sugar recovery of 10.28 mg/ml and 10.18 mg/ml respectively.

Microbial saccharification of sugary substrates is common in the natural environment. SNP easily decays from microbial attack of its high sugar quantities (Caroline et al., 2009). The fungal attack of SNP does not only deteriorate the pulp but also affects the oil content of the oil bearing nut and must be removed during shea butter processing to prevent further fungal growth (Caroline et al., 2009).

Many fungal species have been identified to be associated with the saccharification of SNP. Eight fungi

species during the bio-deterioration of the shea nut pulp were isolated from the fruit natural environment (Ojo and Adebayo, 2013). *Aspergillus flavus*, *Aspergillus niger*, *Botrydiploia theombromae*, *Botryosphaeria* spp., *Colletotrichum gleosporioides*, *Lisidiplodia* spp., *Pseudofasicocum* spp. and *Trichoderma viridae* were mentioned (Ojo and Adebayo, 2013). *Aspergillus niger* developed the most extensive saccharifiable ability when the microbes were inoculated directly on the shea nut fruit. Nwufu and Mba (1987) also mentioned *Aspergillus niger* as part of the fungi found associated with the decomposing seeds of African shea butter fruit in Nigeria. Similarly (Aculey et al., 2012) noted during an investigation of the deteriorating parboiled shea nut kernels that the frequently encountered moulds were of *Aspergillus* and the *Rhizopus* species. Thus, *Aspergillus niger* has by far proven to be causing the most rot once inoculated alongside other fungi species, common at shea nut pulp environments, producing the highest significant filter paper activity, carboxymethyl cellulose activity and protein concentration.

The use of saccharifying microbes during simultaneous fermentation processes however occurs, however, with some demerits. In many situations, the most secreted proteins by the microorganisms are not that particularly thermostable or that the native β -glucosidase released in the fermentation media is sufficient for the hydrolysis of most substrates. Once produced in the fermentation medium, sometimes the native GH61 proteins are not highly expressed and are not particularly active in the medium to cause the needed breakdown of the substrate. Other organisms during the fermentation can produce enzymes that are individually superior.

CONDITIONS FOR SUBSTRATE FERMENTATION WITH *SACCHAROMYCES CEREVISIAE*

A substrate for bioethanol production refers to any plant material or algae that have the potential of releasing soluble sugars in solution for fermentation to proceed. Such biomasses as forestry wastes, corn stalk and cobs, wheat straw, grasses and rice straw have been mentioned. Fermentation is the core process in ethanol production from a given substrate. Fermentation occurs through the activity of a variety of microorganisms including fungi, bacteria, and yeasts. Ethanol production from kinnow waste and banana peels by simultaneous saccharification and fermentation using cellulase and co-culture of *S. cerevisiae* G and *Pachysolen tannophilus* MTCC 1077 has been carried out by Sharma et al. (2007) at optimized conditions. Certain fermentation parameters such as inoculum, enzyme and substrate concentration besides optimum pH, temperature, time, agitation among others play an important role in obtaining good ethanol yield (Sharma et al., 2007). The biomass after enzymatic saccharification containing 63 gL^{-1} reducing sugars was

Table 2. Saccharifying abilities of different fungi species associated with the deterioration of shea nut pulp, corn cobs and shea nut kernels.

Substrate	Microrganism	Saccharifying ability	References
Shea nut pulp	<i>Aspergillus niger</i>	Most extensive	Ojo and Adebayo (2013) Aculey et al. (2012) Nwufu and Mba (1987)
	<i>Rhizopus species</i>	Most extensive	
	<i>Aspergillus flavus</i>	Extensive	
	<i>Botryodiplodia theobromae</i>	More extensive	
	<i>Botryosphaeria</i> spp	Extensive	
	<i>Collectotrichum gloeosporioides</i>	Extensive	
	<i>Lisidiplodia theobromae</i>	Extensive	
	<i>Pestalotiopsis</i> spp.	Extensive	
	<i>Pseudofasicocum</i> spp.	Extensive	
	<i>Trichoderma viridae</i>	Extensive	
Corn cob	<i>Aspergillusniger</i>	Highest	Zakpaa et al. (2010)
	<i>Trichodermaviridae</i>	Higher	
	<i>Penicillium</i>	High	
	<i>Mucor</i>	High	
	<i>FusariumRhodotorula</i>	High	
	<i>Acremonium</i>	High	
	<i>Coccidioides</i>	High	
Shea nuts and kernel	<i>Aspergillusniger</i>	Most extensive	Esiegbuya et al. (2014)
	<i>Aspergillusflavus</i>	Most extensive	
	<i>Aspergilluspersii</i>	Most extensive	
	<i>Mucor</i> sp	Extensive	
	<i>Fusarium</i> sp	Extensive	
	<i>Phomas</i> p	Extensive	
	<i>Xylaria</i> sp	Extensive	

fermented with both hexose and pentose fermenting yeast strains, resulting in ethanol production, ethanol yield and ethanol fermentation efficiency of 26.84 and 0.426 gg^{-1} and 83.52% respectively. Suhas et al. (2013) carried out fermentation on fruit rinds using *S. cerevisiae*. The amount of ethanol produced after fermentation was analyzed by gas chromatography and found to be the highest for jackfruits and pineapple rind fruits with yields of 4.64 and 4.38 g/L respectively. Coculturing *S. cerevisiae* with other yeasts or microbes is targeted to optimize ethanol production, shorten fermentation time, and reduce process cost.

To increase the yield of ethanol by microbial fermentation, the use of ideal microbial strain, appropriate fermentation substrate and suitable process technology remains essential (Benjamin et al., 2014). Also, one of the efforts to increase the production of ethanol is the engineering of the microbial genetic composition or the modification of fermentation media, or combination of both (Chan-u-tit et al., 2013; Deesuth et al., 2012; Krause et al., 2007; Nikolić et al., 2009; Takagi et al., 2005; Xue et al., 2008). A considerable amount of literature has

been published on microbial fermentation. These studies (Benitez et al., 1983; Diwanya et al., 1992) have suggested that an ideal microorganism for bioethanol production must have rapid fermentative potential, is thermo-stable, has improved flocculating ability, appropriate osmo-tolerance and can withstand high ethanol concentrations.

Recent research findings suggest that *S. cerevisiae* is one of the widely studied and used yeasts at both industry and household levels with bioethanol generated as the main fermentation product (Tesfaw and Assefa, 2014). Over the past decade, most research on the use of the right microorganism for fermentation process had emphasized the use of *S. cerevisiae* (Zakpaa et al., 2009; Hossain et al., 2011; Benjamin et al., 2014; Abdul-Mumeen et al., 2016).

S. cerevisiae is chosen for most fermentation experiments since it is a well understood fermentative organism (Lamb et al., 2018). *S.cerevisiae*, a natural evolution meant for efficient consumption of sugars especially sucrose, remains one of the most important cell factories due to its robustness, stress tolerance,

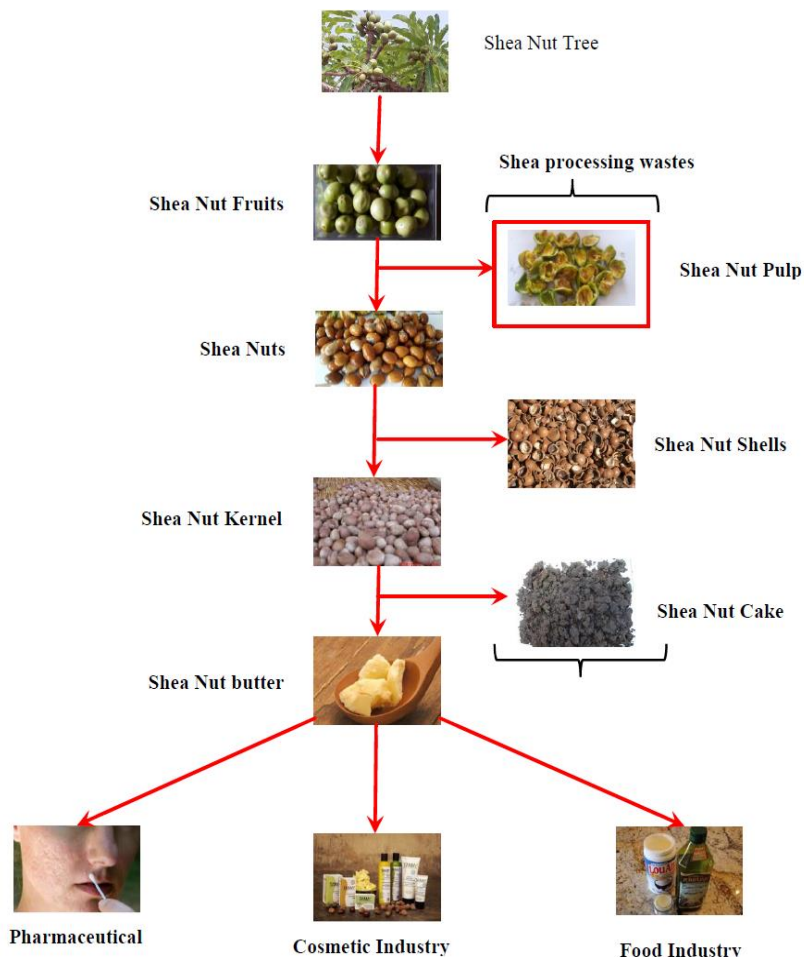


Figure 3. Various waste substances generated from shea butter processing.

genetic accessibility, simple nutrient requirements and long history as an industrial workhorse. Fermentation performance of the yeast *S. cerevisiae* is however influenced, among others, by growth media composition (Djajasoepena et al., 2015). Complex nitrogen source media tend to give better fermentation performance. Djajasoepena et al. (2015) confirm the effect of growth media composition, especially media with complex nitrogen source tends to increase fermentation performance of the yeast *S. cerevisiae*. Paul (2010) suggests the growth curve of *S. cerevisiae* with the right media composition at 30°C for 12 h with absorbance reading at 600 nm to be the result, as shown in Figure 4.

For the several good factors about *S. cerevisiae* many researchers (Lamb et al., 2018; Tropea et al., 2014; Almeida and Angelis, 2016; Suhas et al., 2013; Togarepi et al., 2012; Ofori-Appiah et al., 2016; Sharma et al., 2007) have relied on the microbe for the fermentation of different substrates for ethanol generation, Table 3. *S. cerevisiae* is superior to bacteria, other yeasts, and filamentous fungi in various physiological characteristics regarding ethanol production in industrial context. It

tolerates a wide range of pH (Lin et al., 2012) operates at optimum acidity (Ortiz-Muñiz et al., 2010) and its robust. It also tolerates ethanol better than other ethanol producing microorganisms (Prasertwasu et al., 2014). The use of *S. cerevisiae* in fermentation is safe and less susceptible to infection since it is extensively used for human consumption.

With the use of *S. cerevisiae* on dried *Ziziphus mauritiana* (Chinese date) fruit pulp for instance, at pH of 6, with optimum temperature at 30°C, the yeast concentration of 8 g/20g (0.4 g/g) fruit pulp yielded the optimum rate of fermentation after the stipulated seven days, Table 3. Using a free cell batch fermentation process, *Zymomonas mobilis* reached 59.95% of the theoretical yield. Immobilized cells reached 68.53% using a batch and 74.49% using a continuous fermentation process. Under the same conditions, *Saccharomyces cerevisiae* reached 70.03, 77.10 and 78.47% of the theoretical yield respectively. Higher yields were achieved for both microorganisms using mixed culture fermentation, compared to pure cultures. Under the same conditions for both pure cultures, mixed cultures reached

Table 3. Review of fermentation conditions, mechanisms and ethanol yield.

Microorganism	Substrate	Fermentation condition			Fermentation Vol. (ml)	Microb/substrate conc.	Max ethanol yld	References
		pH	Temp (°C)	Duration/day				
<i>Escherichia coli</i> KO11	<i>Laminaria Japonica</i>	-	-	-			0.40 g/g	Kim et al. (2011)
<i>Saccharomyces cerevisiae</i>	Sorghum Pito Mash	pH 6.0	30	4	500	10 ml/50 g	3.03 g/L	Ofosu et al. (2016)
<i>Zymomonas mobilis</i>	Sorghum Pito Mash	pH 5.5	35	3	500	10 ml/50 g	3.63 g/L	
<i>S. cerevisiae</i>	<i>Ziziphus mauritiana</i>	pH 6	30		500	8.0 g/20 g	63.00 g/L	Togarepi et al. (2012)
<i>S. cerevisiae</i> and <i>Pachysolen tannophilus</i> MTCC 1077	Kinnow waste and banana peels	6% 4%	30	-	500	8 g/25 g	0.43 g/g	Sharma et al. (2007)
<i>Saccharomyces cerevisiae</i>	Jackfruit Rind	-	25	4	250	15 ml/50 g	4.64 g/L	Suhas et al. (2013)
<i>Saccharomyces cerevisiae</i>	Pineapple Rind	-	25	4	250	15 ml/50 g	4.38 g/L	
<i>Zymomonas mobilis</i>	Sugarcane juice		-	-	250	0.8 ml/50 g	59.95%	Almeida and Angelis (2016)
<i>Zymomonas mobilis</i>	Sugarcane juice		-	-	250	0.8 ml/50 g	68.53%	
<i>Zymomonas mobilis</i>	Sugarcane juice		-	-	250	0.8 ml/50 g	74.49%	
<i>Saccharomyces cerevisiae</i>	Sugarcane juice		-	-	250	0.8 ml/50 g	70.03%	
<i>Saccharomyces cerevisiae</i>	Sugarcane juice		-	-	250	0.8 ml/50 g	77.10%	
<i>Saccharomyces cerevisiae</i>	Sugarcane juice		-	-	250	0.8 ml/50 g	78.47%	
<i>Zymomonas mobilis/S. cerevisiae</i>	Sugarcane juice		-	-	250	(0.4/0.4) ml/50 g	70.86%	
<i>Zymomonas mobilis/S. cerevisiae</i>	Sugarcane juice		-	-	250	(0.4/0.4) ml/50 g	79.07	
<i>Zymomonas mobilis/S. cerevisiae</i>	Sugarcane juice		-	-	250	0.4 ml each/50 g	80.86%	
<i>Saccharomyces cerevisiae</i>	Pineapples waste	4.5	30	-	2.5	20 ml/1.5 L	3.90%	
<i>Saccharomyces cerevisiae</i>	<i>Saccharina latissima</i>	6.8	30	2	25	1 g/L	0.42 g/g	Lamb et al., 2018

70.86, 79.07 and 80.86% of the theoretical yield respectively.

CONCLUSION

Shea nut pulp could be unique source of valuable monomeric sugars that have significant importance to the bioenergy sector for renewable energy generation by fermentation. The ordinary fermentation processes previously

relied on the use of chemical pretreatments of the substrate under harsh conditions. To maintain a high glucose yielding substrate and to evade chemical use for the pretreatment, a milder and more selective fermentation process is required. Currently, research is focused on the nutritional and mineral composition of the shea nut pulp but several enzymes and microorganisms have also been identified to cause severe deterioration to the fruit skin in its natural environment.

Some studies have covered the use of commercial enzymes or microbial consortium in simultaneous saccharification and fermentation processes. Although commercial enzyme mixtures have generally been developed for terrestrial plant biomass processing, the use of indigenous microbial consortia can be cost effective with equal yield or better. This further allows for reduction in chemicals use in bioethanol production process and thus holds enormous potential for creation of sustainable

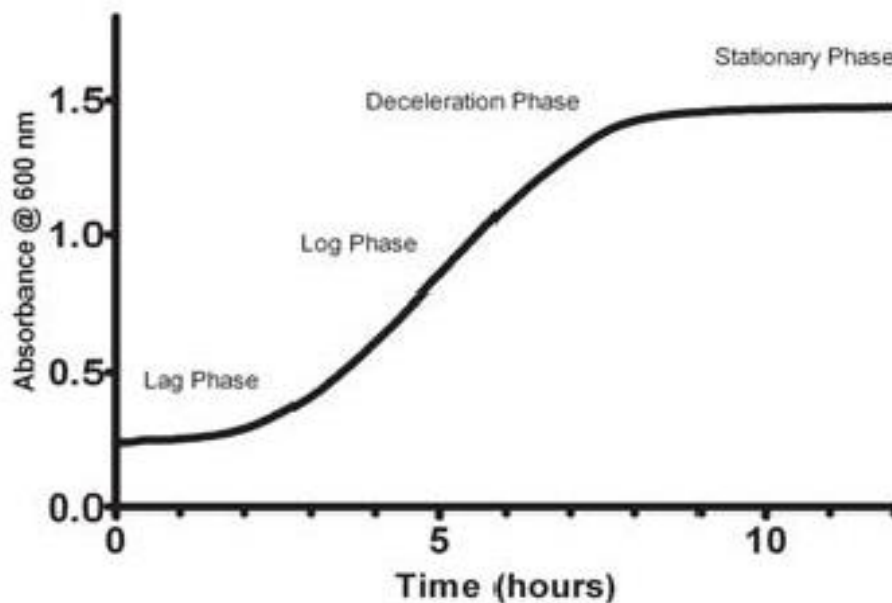


Figure 4. Typical yeast growth curve. *S. cerevisiae* grown in YPD media at 30°C for 12 h with data measurements every 2 min. Source: Paul (2010).

ethanol processing from SNP substrate.

RECOMMENDATIONS

A research conducted into the potentials of the Ghanaian shea nut pulp for use as substrate for the production of fuel ethanol will be of enormous benefit to renewable energy policy targets. This can be done by either using enzymes directly or by microbial consortia to hydrolyze the dry or fresh shea nut pulp at optimum conditions for optimal bioethanol yield. Such a conversion will find more uses for the shea nut waste away from its environmental nuisance at the shea butter processing centers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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