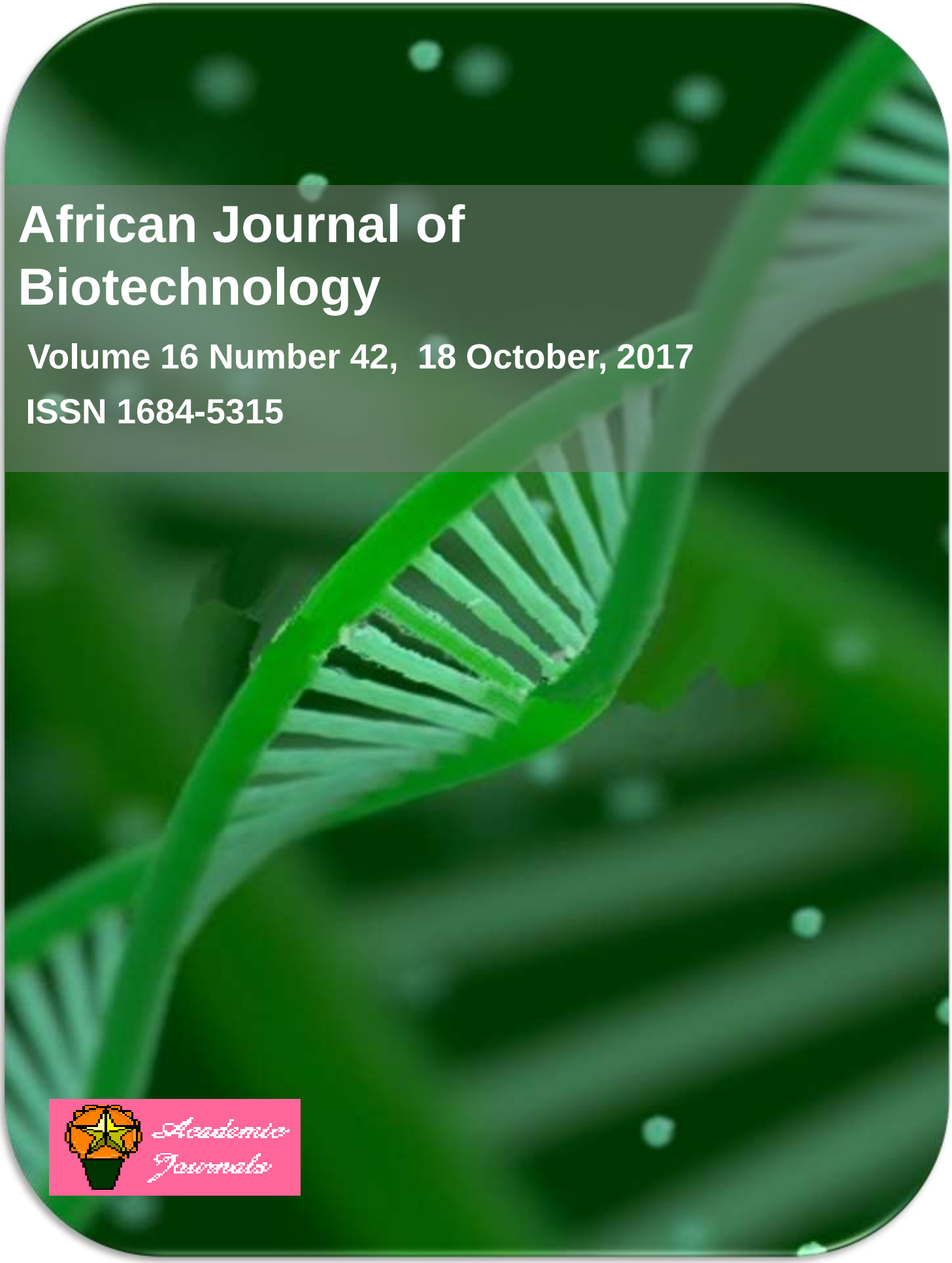

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African Journal of Biotechnology

Full Length Research Paper

Fungi and aflatoxin analysis of processed *ogiri-egusi* and *ogiri-ugba* consumed in Abakaliki metropolis

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This study investigated fungi and total aflatoxins quality of *ogiri* [fermented melon seed (*Citrullus vulgaris*] and fermented African oil bean seed (*Pentaclethra macrophylla*) consumed as soup condiment in Abakaliki, Ebonyi State, Nigeria. The *ogiri* samples were purchased from two major markets in Abakaliki metropolis [meat market (OM and UM) and rice mill market (OR and UR)]. The samples were screened for their pH values, total fungi count and total aflatoxins content. A total of four samples were analzed for six weeks. The total aflatoxins were analyzed using highly sensitive competitive enzyme link immunosorbent assay (ELISA) reader. The *ogiri* samples were also analyzed for total fungal count using a digital colony counting machine (CCM China). The result showed that all the *ogiri* samples analyzed were heavily contaminated by the fungal cells. The total mean fungal count for the *ogiri* processed from melon seed were 2.2×10^7 and 2.2×10^7 cfu/g for OM and OR, respectively while the *ogiri* processed from African oil bean were 1.4×10^7 and 1.6×10^7 cfu/g for UM and UR samples, respectively. The *ogiri* samples also contained unacceptable levels of aflatoxins as the average total aflatoxins for all the samples were above the minimum acceptable limits (10 ppm), according to the National Agency for Food and Drug Administration and Control (NAFDAC) as reported by the Food and Drug Administration (FDA) of USA. The research also revealed that *ogiri* samples have high moisture content. The study recommends that more improved process line be put in place to ensure that all *ogiri* sold in Abakaliki are produced using Standard Operating Procedure (SOP).

Key words: *Ogiri*, aflatoxins, fungi, African oil bean, melon seed, enzyme link immunosorbent assay (ELISA).

INTRODUCTION

Food condiments (seasoning/spices) are substances added to food to impact a particular flavor or to generally improve the sensory/chemical quality of the food/food products (Odibo et al., 1990; Njoku et al., 1990). Fermented food seasonings or local seasoning are those seasonings which undergo traditional foods processing method, that involves biochemical changes brought about by microbes inherent in the food or derived from starter

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culture and their enzymes. These local seasonings can also add extra nutrients such as vitamins B to the food. The use of fermented proteins rich seeds as seasonings is wild spread in Africa and Asia (Sanni, 1993).

The fermented protein rich seeds in addition to the flavor also act as protein supplements and functional ingredients in the processed foods (Achi, 2005). These seasonings are being increasingly marketed throughout the world today. *Ogiri* is a Nigerian fermented condiment produced from various substances, and which when added to soup or yam porridge enhances the flavor. Several research has been carried out on the production of *ogiri* from the fermentation of African oil bean seed (*Pentaclethra macrophylla*) creeping melon (*Colocynthis vulgaris*) (Odunfa, 1985; Jideani and Okeke, 1991; David and Aderibigbe, 2010), *Citrullus lanatus* (David and Aderibigbe, 2010), seeds of castor oil (*Ricinus comminus*) (Odunfa, 1985; Jideani and Okeke, 1991), watermelon seed (*Citrullus vulgaris*) (Odunfa, 1985) and fluted pumpkin (*Telfairia occidentalis Hook*) (Odibo and Umeh, 1989). The choice of substrate for this food condiment, which is popular among many people in Nigeria, depends on the locality (Odibo et al., 1990).

Ogiri ugba is a value added semi-solid fermented product of African oil bean seed (*P. macrophylla*), prepared by the igbos in eastern Nigerian. Fermentation detoxifies the African oil bean seed with subsequent increase in nutrient availability and digestibility. *Ogiri Ugba* is widely used as a soup condiment (flavor enhancing condiment) (Mbata and Orji, 2008) with its production locally done through a mixed wide bacteria fermentation of sliced, boiled and soaked African oil bean seeds. Unprocessed oil bean seeds are bitter and possess anti-nutritional factors among which are pancine, cyanide, oxalates, saponin, phytates and tannins (Achinewhu, 1983; Enuijiugha and Akanbi, 2005; Onwuliri et al., 2004). The microbial population of *Ogiri ugba* is introduced through the air, water, utensil, banana leaves or the handler and no starter culture is used in the traditional method. Microorganisms often involved are predominantly *Bacillus spp., Micrococcus* and *Lactobacillus spp., Pseudomonas spp., Staphyloccoccus spp., Enterobacter spp., Leuconostoc spp, Corynebacterium* and *Alkaligenes spp* (Enuijiugha, 2009; Isu and Njoku, 1997; Isu and Ofuya, 2000; Njoku et al., 1990; Obeta, 1983; Sanni et al., 2000).

Melon seed (*C. vulgaris)* has high protein and low carbohydrate content and belong to the family Cucurbitaceae (Alfred, 1986). *Ogiri-egusi* is characterized by very strong pungent odour which turns into a pleasant aroma upon cooking as soup condiment. Among the consumers, their preferences for either *ogiri-ugba* or *Ogiri egusi* vary among localities.

Production of these condiments (*ogiri-ugba* and *Ogiri-egusi*) by local technology creates room for contamination by diverse microorganisms. The fermentation environment also supports and encourages the growth and multiplication of potentially toxigenic fungi thus leading to the production of diverse forms of mycotoxins. Aflatoxins are secondary metabolite produced by fungi with toxicological properties, that induces a variety of health challenges when foods contaminated with these compounds are ingested. Aflatoxins are stable under most processing conditions and therefore persist to the final products (Oranusi et al., 2013). In this work, the microbes and total aflatoxins analysis of *ogiri-ugba* and *Ogiri-egusi* produced and consumed in Abakaliki metropolis was carried out. The findings of this study will serve the purpose of alerting consumers on the possible danger of consuming poorly processed *Ogiri* (*ogiri-ugba* and *Ogiri-egusi*) on sale in selected markets within Abakaliki Ebonyi State, Nigeria.

MATERIALS AND METHODS

Samples collection

The *Ogiri-egusi* [processed from melon seed (*C. colocynthis*)] and *ogiri-ugba* [processed from African Oil bean seed (*P. macrophylla*)] used in this research were purchased from meat market and rice mill market, Abakaliki, Ebonyi State. *Ogiri*-egusi purchased from meat market was designated as OM while that purchased from rice mill market was designated as OR. Also, *Ogiri*-ugba purchased from meat market was designated as UM whereas that purchased from rice mill market was designated as UR.

Determination of moisture content of the *Ogiri* samples

The moisture content was determined by the gravimetric method. A measured weight of each sample (5 g) was weighed into a cleaned, dried Petri dish. The dish and samples were dried in an oven at 105°C for 3 h at first instance. It was then cooled in a desiccator and reweighed.

The weight was recorded while the samples were returned to the oven for further drying. The drying, cooling and weighing continued repeatedly until a constant weight was obtained. By the difference, the weight of the moisture loss was determined and expressed as a percentage. It was calculated as shown below:

$$\% \text{ Moisture Content} = \frac{W2 - W3}{W2 - W1} \times \frac{100}{1}$$

Where, W_1 = weight of the empty Petri dish; W_2 = weight of the dish and sample before drying; W_3 = weight of the dish and sample after drying to a constant weight

pH determination

The pH of the samples was determined using highly sensitive digital pH meter (Montini 095, Romania). Five grams (5 g) of each sample was weighed and transferred to a clean beaker and 50 ml of distilled water was added to form a slurry.

A standard buffer solution (pH 6.0) was prepared and was used to standardize the pH meter. The electrode of the digital pH meter was dipped in the slurry at a temperature of about 29°C. The pH readings were recorded.

Total viable fungal count

Ten-fold serial dilution and pour plate method were used for the

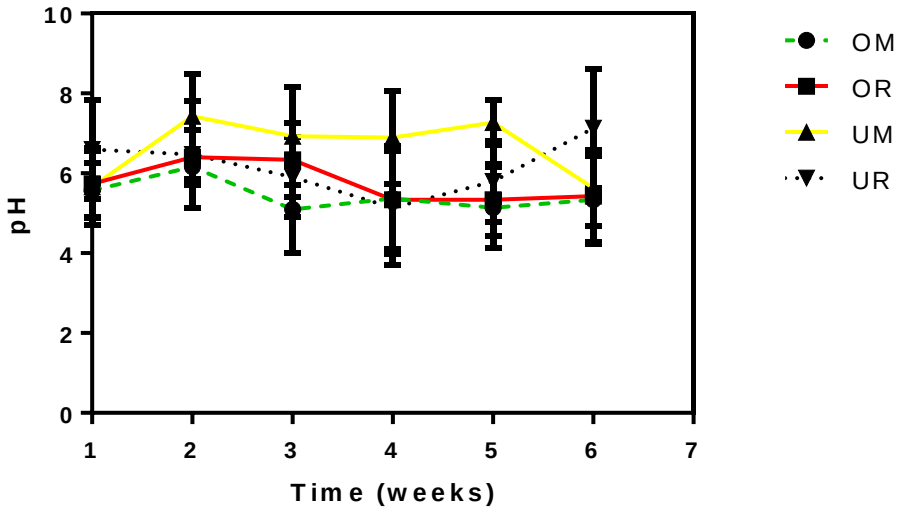

Figure 1. pH values of the *Ogiri-egusi* and *Ogiri-ugba.* OM, *Ogiri-egusi* purchased from meat market; OR, *Ogiri-egusi* purchased from rice mill; UM, *Ogiri-ugba* purchased from meat market; UR, *Ogiri-ugba* purchased from rice mill.

fungal count. The medium used (Saboraud Dextrose Agar) were prepared according to manufacturer's instructions (BioTech India) and autoclave for 15 min at 121°C and 15 psi. The prepared medium was allowed to cool to about 40°C in a water bath and was then poured into sterile Petri-dishes containing 1 ml aliquots of the appropriate dilutions (normal saline as diluents) prepared from the samples.

The samples solutions were prepared by adding 1 g of the sample into 10 ml of normal saline. The plates were incubated for 3 days at room temperature and colonies formed were counted using digital colony counter and expressed in colony forming unit per gram CFU/g.

Total aflatoxins analysis

Determination of total aflatoxins on the *ogiri* samples was done by the use of highly sensitive competitive Enzyme linked immunosorbent assay (ELISA) reader. Extraction of the aflatoxin was done with Tween-ethanol. 25 mm of Tween-ethanol was added to 5 g of the sample and mixed properly. The sample solution was then centrifuged at 250 rpm for 3 min, and filtered using Watman1 filter paper.

Aflatoxin conjugate (200 mL) was dropped in a clean mixing wall and 100 µl of the sample analyte was added. The mixture of the aflatoxin conjugate and the sample was then transferred into antibody incubated micro-walls and incubated under dark cover at room temperature 26 ± 2°C for 15 min. This process allowed the antibody/antigen reaction to take place. After incubation, the solution was then washed off 5 times using deionized water then, 100 µl of the substrate was added and allowed to stand for 5 min. Finally, a stop solution was added and the result was read by ELISA reader.

RESULTS AND DISCUSSION

Ogiri are prone to fungal contamination and spoilage due to poor local processing and packaging method. This study revealed that, the severity of the processing contamination and spoilage varied among the different *ogiri* samples, are reflected in different levels of hygienic processes that were used to produce *ogiri*. Thus, the total fungal populations and the concentrations of total aflatoxins varied among the *ogiri* samples.

pH values

The pH analysis showed that the different *ogiri* samples have different pH that ranges from 6.2 to 7.2 and 6.5 to 8.5 for *ogiri-egusi* and *Ogiri ugba*, respectively (Figure 1). pH is an important factor that influences the microbial content of any food material. The variation in the pH values of the *ogiri* samples could be as a result of the varied fermentation time. According to Achi (2005), the pH of traditionally fermented protein condiments is significantly affected by the fermentation time. The variety of the melon seed and the African oil bean seed could also be a factor, that might have influenced the pH as genetic composition of the seeds, which have been found to determine the chemical composition of the seeds and thus their pH (Achinewu, 1987).

The high pH of fermented legumes (melon seed) and African oil bean seed compared to other materials (cereals) under similar conditions have been attributed to high protein content of the seeds (Zamora and Fields, 1979). According to Achinewhu (1986), unsaturated fatty acids are increased with the hydrolysis of protein in amino acids and peptides. Ammonia is released to the proteolytic activity taking place during fermentation, which therefore raises the pH of the final products and giving the food a strong ammonical odour and flavor (Wang,

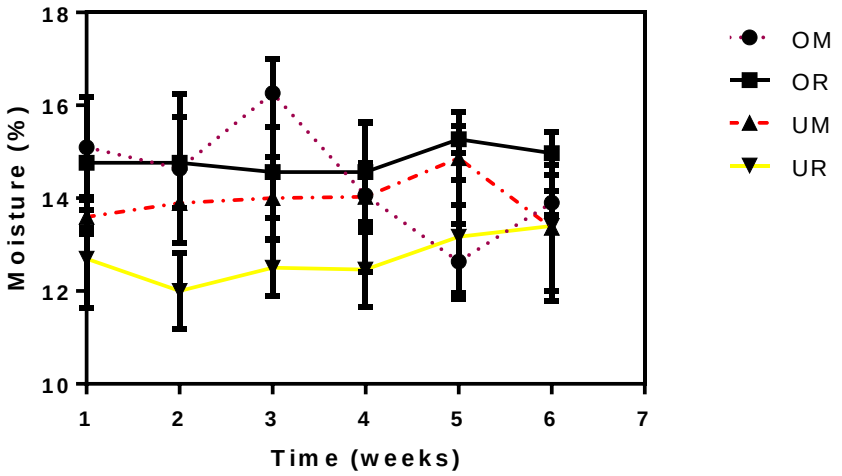

Figure 2. The moisture content of the *Ogiri-egusi* and *Ogiri-ugba.* OM, *Ogiri-egusi* purchased from meat market; OR, *Ogiri-egusi* purchased from rice mill; UM, *Ogiri-ugba* purchased from meat market; UR, *Ogiri-ugba* purchased from rice mill.

Table 1. Total fungi count of *Ogiri-egusi* and *Ogiri-ugba* samples.

Week	Sample (cfu/g)			
	OM	OR	UM	UR
1	$2.3 \pm 0.10 \times 10^{7a}$	$2.0 \pm 0.03 \times 10^{7a}$	$1.6 \pm 0.11 \times 10^{6b}$	$1.3 \pm 0.02 \times 10^{7c}$
2	$2.5 \pm 0.02 \times 10^{7a}$	$2.4 \pm 0.002 \times 10^{7a}$	$1.2 \pm 0.003 \times 10^{7c}$	$1.9 \pm 0.04 \times 10^{7b}$
3	$2.0 \pm 0.003 \times 10^{8a}$	$2.0 \pm 0.003 \times 10^{7a}$	$1.3 \pm 0.10 \times 10^{8c}$	$1.9 \pm 0.04 \times 10^{8b}$
4	$2.1 \pm 0.003 \times 10^{7b}$	$2.5 \pm 0.020 \times 10^{8a}$	$1.5 \pm 0.12 \times 10^{7c}$	$1.6 \pm 0.10 \times 10^{7c}$
5	$2.5 \pm 0.10 \times 10^{7a}$	$2.4 \pm 0.13 \times 10^{7a}$	$1.2 \pm 0.003 \times 10^{8d}$	$1.6 \pm 0.001 \times 10^{7c}$
6	$2.6 \pm 0.001 \times 10^{7a}$	$2.1 \pm 0.001 \times 10^{7b}$	$1.6 \pm 0.001 \times 10^{7c}$	$1.8 \pm 0.001 \times 10^{7c}$

Values are mean of triplicate determination and standard deviation (±SD). Means with different superscript along the row are significantly different ($p<0.05$). AOB, African oil bean; OM, *Ogiri-egusi* purchased from meat market; OR, *Ogiri-egusi* purchased from rice mill; UM, *Ogiri-ugba* purchased from meat market; UR, *Ogiri-ugba* purchased from rice mill.

1996). This we referred to as “Alkaline fermentation” which aids in prolonging the shelf life of such product.

The result of this research is however, in line with the findings of other researchers as observed by David and Aderibigbe (2010) for *ogiri* from different melon seeds. Odunfa (1981) in his work described the fermentation process as essentially putrefactive, noting that the increase in pH was probably due to the formation of ammonia by the deaminase enzymes of *Bacillus* and *Proteus spp.* that often involve in the fermentation process.

Moisture content

The moisture content of the *ogiri* samples was found to be between 12.5 to 15.1% and 11.8 to 13.9% for *ogiri-egusi* and *ogiri-ugba*, respectively (Figure 2). This suggests that the *ogiri* samples were not sufficiently dried after processing.

Moisture content is a key factor that affects the microbial quality of any processed food/food products. Food or food products with high moisture content tend to have higher microbial content and spoil faster than those with lower moisture contents. This result is similar to the finding of David and Aderibigbe (2010), who reported that high moisture content of melon seed significantly affects the overall chemical composition of the *ogiri* made from the melon seed. The high moisture content also affects the microbial content of *ogiri* made from melon seed according to the research done by Odibo and Umeh (1989) and Nwagu et al. (2010).

Total fungi count

The finding of this research shows that the different *ogiri* samples analyzed were heavily contaminated with fungi (Table 1). The *ogiri ugba* has the lowest average fungal populations (1.2×10^7 - 1.9×10^8) while the *ogiri egusi*

Table 2. Total aflatoxin count of *Ogiri-egusi* and *Ogiri-ugba.*

Week	Sample (ppb)			
	OM	OR	UM	UR
1	11.5 ± 0.004^{b}	11.3 ± 0.01^{b}	11.4 ± 0.001^{b}	12.0 ± 0.002^{a}
2	12.3 ± 0.003^{b}	10.0 ± 0.001^{c}	13.4 ± 0.001^{a}	12.6 ± 0.03^{b}
3	10.0 ± 0.001^{d}	11.2 ± 0.02^{b}	14.3 ± 0.002^{a}	13.3 ± 0.03^{c}
4	15.2 ± 0.01^{b}	10.2 ± 0.02^{a}	11.1 ± 0.002^{d}	12.6 ± 0.10^{c}
5	13.0 ± 0.10^{c}	11.0 ± 0.002^{d}	13.4 ± 0.001^{b}	21.3 ± 0.001^{a}
6	11.2 ± 0.002^{d}	12.3 ± 0.02^{b}	15.4 ± 0.01^{a}	11.3 ± 0.001^{c}

Values are mean of triplicate determination and standard deviation (±SD). Means with different superscript along the row are significantly different ($p<0.05$). AOB, African oil bean; OM, *Ogiri-egusi* purchased from meat market; OR, *Ogiri-egusi* purchased from rice mill; UM, *Ogiri-ugba* purchased from meat market; UR, *Ogiri-ugba* purchased from rice mill.

had the highest fungal cells (2.3×10^{7} - 2.5×10^{8}) during the study period. There was a significant difference among the fungal populations of the *ogiri* samples.

The level of fungal load in the *ogiri* sample revealed the extent of fermentation as well as the storage stability of *ogiri* samples. *Ogiri* that have had longer fermentation time tend to have higher fungal populations and also tend to spoil faster than those with lower fungal populations. This is because with higher fungal population, the rate of metabolic activities of the fungal cells on the *ogiri* becomes faster resulting in production in high proportion of certain undesirable metabolites which subsequently lead to off-flavors and general change in the chemical composition of the flour. This high fungal population seen in these *ogiri* samples could be due to poor processing resulting in cross-contaminations of the *ogiri* from both the traditional processing equipments and the personnel.

The presence of high fungal cells in all the samples could also be attributed to the normal flora of the seeds. Normal microbial flora have been reported to withstand processing procedures and conditions and could be found in final products. Fungi are common environmental contaminants and are known to produce spores that survive unfavourable environmental conditions; this could explain their presence in this food condiments (Oranusi et al., 2013). The levels of contaminants could be associated to contamination from the environment, the food vendors and personnel involved in the production process (De Roever, 1998; Beuchat, 2002). It has been reported that, the microbial contamination of a product is dependent on the environment it passed through and to sanitary quality of the processing water, transportation, storage and processing of the produce (EC-SCF, 2002; Oranusi and Braide, 2012; Buck et al., 2003; WHO, 2008).

The result of this research is similar to the findings of other researchers in which they reported that, *Ogiri* condiments are fermented products often contaminated by diverse species of fungi due to poor local technology (Odunfa, 1981; Adenike and Kehinde, 2008; Nwagu et al., 2010; Oranusi et al., 2013).

Total aflatoxin

The finding of this research showed that the *ogiri* samples were contaminated with different concentrations of aflatoxins ranging from 10.0 to 15.2 ppb and 11.1 to 21.1 ppb for *ogiri-egusi* and *ogiri-ugba,* respectively (Table 2). The level of aflatoxins is one of the key safety and quality indicator parameter of any processed food/food products. Aflatoxin has been reported to be produced under conducive environmental condition of high water activity and high pH (Zuber et al., 1993). Aflatoxins are potent natural carcinogenic substance linked to higher prevalence of hepatocellular cancer in Africa (Strosnider et al., 2006). It has also been revealed that the highest risk occurs with hepatitis B and C carriers in developing cancer, when exposed to aflatoxin (Williams et al., 2003). Other studies also linked aflatoxin to immunosuppression and stunted growth in children (Turner et al., 2003; Wu and Khlangwiset, 2010).

Liver is the principal organ affected by aflatoxin. In the liver, aflatoxin may be transformed by certain P_{450} enzyme to its DNA reactive form aflatoxin-8,9-epoxide. These molecules may bind to liver proteins and lead to their failure, potentially resulting to active aflatoxicosis. Most food products, *ogiri* condiments inclusive with total aflatoxins level beyond 10 ppm are designated as unfit for human consumption according to National Agency for Food and Drug Administration and Control (NAFDAC). This levels of total aflatoxin in the food commodities were generally above the maximum allowable limits (10 ppb for food meant for domestic consumption) specified by the European Commission (AESAN, 2011), which is also currently being used NAFDAC in Nigeria. This is because consumption of foods with such high levels of aflatoxins contaminations has been linked with both acute and chronic heart diseases including cancer (Azi et al., 2016).

It has been established that over 5 billion people,

mostly in developing countries, are at risk of chronic exposure to aflatoxins from contaminated foods; food condiments inclusive (Shuaib et al., 2010). This high concentration of aflatoxins revealed in this study could be as results of high fungal contamination of the *ogiri* condiments. The finding of this research is similar to the investigative report of Food and Agriculture Organization (FAO) and Food and Drug Administration (FDA) risk assessment report on aflatoxins in foods, in which they discovered different concentrations of aflatoxins in the different foods assessed, including food condiments.

Conclusion

The finding of this research showed that processed *ogiri* which is consumed in Abakaliki are heavily contaminated with molds, with potential for aflatoxins production. The aflatoxins analysis also revealed unacceptable levels of aflatoxins in the *ogiri* samples. It is therefore recommended that urgent review of the entire process line for *ogiri* sold in Abakaliki metropolis, is carried out to ensure that all *ogiri* sold in Abakaliki are produced following standard operating procedures (SOP).

Also, the processed *ogiri* should be stored at dry and cool environment (temperature preferably below 20°C and relative humidity below 80%), to reduce the chance of fungal contamination and proliferation during storage. There is also an urgent need to educate producers of food condiments, food vendors and consumers on the dangers poor food handling and storage and the need to apply Good Manufacturing Practices (GMP) and Hazard Analysis Critical Control Point (HACCP) in processing of *ogiri* in Abakaliki metropolis.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Achi OK (2005). Traditional Fermented Protein Condiments in Nigeria. Afr. J. Biotechnol. 4(3):1612-1621.

Achinewhu SC (1983). Protein of the African Oil bean (*Pentaclethra macrophylla*). J. Food Sci. 48:1374-1375.

Achinewhu SC (1986). The effect of fermentation on carbohydrate and fatty acid composition of African oil bean seed (*Pentaclethra macrophylla*). Food Chem. 19: 105-116.

Achinewu SC (1987). Carbohydrate and Fatty Acid Composition of Fermented Melon Seeds (*Citrullus vulgaris*). J. Food Sci. Technol. 24:16-19.

Adenike AO, Kehinde OO (2008). Microbial load and Incidence of Food-Borne Indicator bacteria in most Popular Indigenous Fermented food Condiments from middle-belt and Southwest Nigerian. Afr. J. Microbiol. Res. 2:323-339.

AESAN (2011). Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) in relation to the effect on the Spanish population of the derogation of national regulation on maximum allowed limits for aflatoxins B1, B2, G1 & G2 in food. pp. 1-16.

Alfred BG (1986). Tropical colour encyclopedia of exotic plants and trees, 3rd Edition. New York: Acad Press London, 472p.

Azi F, Ogbo FC, Nwankwegu AS, Odo MO, Anagboso MO (2016). Effect of Pre-treatment Methods on the Quality Characteristics of Stored Irvingia kernel. Br. Microbiol. Res. J. 14(5):1-7.

Beuchat LR (2002). Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. Microbes Infect. 4:413-423.

Buck JW, Walcott RR, Beuchat LR (2003). Recent trends in microbiological safety of fruits and vegetables. Plant Health Progress 10:1094.

David OM, Aderibigbe EY (2010). Microbiology and Proximate Composition of *Ogiri*, Melon Seeds. New York. Sci. J. 3(4):18-27.

De Roever C (1998). Microbiological safety evaluations and recommendations on fresh produce. Food Contr. 9:321-347.

European Commission-Scientific Committee on Food (EC-SCF) (2002). Risk Profile on the Microbiological Contamination of Fruits and vegetables Eaten Raw. Report of the Scientific Committee on Food, European Commission health and Consumer protection Directorate-general.

Enuijiugha VN (2009). Major Fermentative Organisms in some Nigerian Soup Condiments. Pak. J. Nutr. 8:279-283.

Enuijiugha VN, Akanbi CT (2005). Compositional Changes in African Oil Bean (*Pentaclethra macrophylla Benth*) Seed Processing during Thermal Processing. Pak. J. Nutr. 4(1):27-31.

Isu NR, Njoku HO (1997). An evaluation of the microflora associated with fermented African oil Bean (*Pentaclethra macrophylla*) seeds during ugba production. Plant Foods Hum. Nutr. 51: 145-157.

Isu NR, Ofuya CO (2000). Improvement of the traditionally processing and fermentation of African oil bean *(Pentaclethra macrophylla* Bentham) into a food snack-ugba. Int. J. Food Microbiol. 59:235-239.

Jideani IOA, Okeke CR (1991). Comparative Study of Microorganisms and Sensory Attributes of Condiments from the Fermentation of Different Seeds. Plant Food Hum. Nutr. 41:27-34.

Njoku HO, Ogbulie JN, Nnubia C (1990). Microbiological Study of Traditional Processing of African Oil Bean (*Pentaclethra macrophylla Benth*) for Ugba Production. Food Microbiol. 7(1): 13-26.

Mbata T, Orji MU (2008). Process optimization in the production and preservation of Ugba, a Nigerian fermented food. Int. J. Microbiol. 4:2-6.

Nwagu TN, Amadi C, Alakwe J (2010). Role of Bacteria Isolates in the Spoilage of Fermented African Oil Bean Seed Ugba. Pak. J. Biol. Sci. 13:497-503.

Obeta JAN (1983). A note on the microorganisms associated with fermentation of seeds of the African oil bean tree (*Pentaclethra macrophylla*). J. Appl. Bacteriol. 54: 433-433.

Odibo FJC, Nwabunia E, Osuigwe DI (1990). Biochemical changes during fermentation of *Telfairia* Seeds for *Ogiri* production. World J. Microbiol. Boitechnol. 6:425-427.

Odibo FJC, Umeh AI (1989). Microbiology of Melon Seed Fermentation for *Telfairia* seeds for *Ogiri* Production. MIRCEN J. Appl.. Microbiol. Biotech. 5:217-222.

Odunfa SA (1981). Microorganisms associated with fermentation of African locust beans (*Parkia filicoidea*) during iru fermentation. J. Plant Foods 3:245-250.

Odunfa SA (1985). Microbiological and toxicological aspects of fermentation of castor oil seeds for *ogiri* production. J. Food Sci. 50:1758-1764.

Oranusi SU, Oguoma OI, Agusi E (2013). Microbiological quality assessment of foods sold in students cafeterias. Global Res. J. Microbiol. 3(1):1-7.

Oranusi US, Braide W (2012). Microbiological safety assessment of apple fruits (*Malus domestica* Borkh) sold in Owerri Imo State Nigeria. Adv. J. Food Sci. Technol. 4(2):97-102.

Sanni AI (1993). The need for process optimization of African fermented foods and beverages. Int. J. Food Microbiol. 18:85-95.

Sanni AI, Ayernor GS, Sakyi-Dawson E, Sefa-Dedeh S (2000). Aerobic spore-forming bacteria and chemical composition of some Nigerian fermented soup condiments. Plant Foods Human Nutr. 55:111-118.

Shuaib FMB, Ehiri J, Abdullahi A, Williams JH, Jolly, PE (2010). Reproductive health effects of aflatoxins: A review of the literature. Reproductive Toxicolol. 29:262-270.

Strosnider H, Azziz-Baumgartner E, Banziger M, Bhat RV, Breiman R,

Brune MN, DeCock K, Dilley A, Groopman J, Hell K, Henry SH (2006). Workgroup Report: Public Health Strategies for Reducing Aflatoxin exposure in Developing Countries. Environ. Health Perspect. 114:1898-1903.

Turner PC, Moore SE, Hall AJ, Prentice AM, Wild CP (2003). Modification of immune function through exposure to dietary aflatoxin in Gambian children. Environ Health Perspect. 111:217-220.

Wang J, Fung DY (1996). Alkaline fermented foods: A review with emphasis on pidan fermentation. Critical Rev. Microbiol. 22(2):101-138.

World Health Organization (WHO) (2008). WHO Global Strategy for Food Safety: Safer Food for Better Health. Food Safety Issues. Geneva: WHO, 26p.

Williams J, Philips TD, Jolly PE, Stiles JK, Jolly CM, Aggarwal J (2003). Human Aflatoxicosis in Developing Countries. A review of toxicology, exposure, potential health consequences and interventions. Am. J. Clin. Nutr. 80:1106-1122.

Wu F, Khlangwiset P (2010). Health Economic Impact and cost-Effectiveness of Aflatoxin Reduction Strategies in Africa: Case Studies in Biocontrol and Postahrvest Interventions. Food Addit. Contam. 27:496-509.

Zamora AF, Fields MI (1979). Nutrive uality of fermented cowpea (*Virgna sinensis*) and chickpeas (*Cicer arietinum*). J. Food Sci. 44:234.

Zuber P, Nakano MM, Marahiel MA (1993). Peptide antibiotics. In Bacillns snbtilis and Other Gram-positive Bacteria, pp. 897-916. Edited by A. L. Sonenshein, J. A. Hoch & R. Losick. Washington, DC : American Society for Microbiology.

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Full Length Research Paper

Evaluation of crude preparations of *Saccharomyces cerevisiae* (ATCC 52712) pectolytic enzymes in cassava starch extraction: Effects of variety on yield and starch recovery rates

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Application of enzyme preparations has shown great potential in aiding extraction procedures. However, the focus has mostly been on single crop varieties, thereby limiting knowledge on the effects of enzyme technology to those (single) varieties studied. The present work compared the effects of various dosages of crude pectolytic enzymes from *Saccharomyces cerevisiae* (ATCC 52712) on yield and extraction rates of starch from the roots of five indigenous cassava varieties (*Nkabom*, *Afisiafi*, *Doku duade*, *Bankye hemaa* and *Esam bankye*). The study aimed to establish whether varietal differences (with respect to response of variety to the technology) existed and to establish which variety is best suited for the technology. Generally, application of the crude pectolytic enzymes with activity of about 4.91 U significantly (P<0.05) increased starch yield and recovery rates in all selected cassava varieties. However, optimization of both yield and recovery rate was dependent on an interplay of variety, enzyme dosage and holding time for enzyme action. An enzyme dosage of 0.02% was found as significant (P<0.05) for peak of starch yield in the *Esam bankye* and *Nkabom* varieties at 0.5 and 1 h holding time in the *Afisiafi* variety; 0.025% enzyme dosage at 0.5 h holding time was the optimum treatment combination for starch yield in both *Bankye hemaa* and *Doku duade* varieties. The study therefore showed that although application of pectolytic enzymes for starch extraction enhances yield, the technology is affected by varietal differences. Given the heavy dependence of most Ghanaian industries on starch, the technology if made available would greatly boost the productivity of these sectors at relatively lower cost.

Key words: Cassava varieties, crude pectinase, pectolytic enzyme dosage, polygalacturonase, submerged fermentation.

INTRODUCTION

Until recently, Ghana's economy has progressively thrived on the performance of her agricultural sector. An economic survey report indicates that in the year 2016, the sector contributed about 20% of the country's gross domestic product (GDP) (CIA, 2017). Of this, cassava is estimated to have contributed about 22% of agricultural

gross domestic product (AGDP) (Essabrah-Mensah, 2016) with production potential estimated to have increased, from the third quarter, by about 4% at the end of the year (FAO, 2016). The crop, roots and leaves are equally a staple and contributes immensely to dietary caloric intake and to industry. The cassava root, especially, finds application in many domestic and industrial circles. Its starch has many remarkable characteristics including high paste viscosity, high paste clarity and high freeze-thaw stability, which are advantageous to many industries (Chinma et al., 2013). The Food and Agriculture Organization (FAO) estimates that in the year 2015 about 8,405 tons of cassava flour and starch were traded globally and although Africa is touted the highest exporter of cassava tuber (exporting about 153,451 tons of global exports of 281,050 tons), only Nigeria contributed significantly (about 150 tons) to exports of cassava flour and starch from Africa (FAO, 2016). However, they also reported that global demand for cassava starch could increase especially as global and regional demands for alcohol, ethanol, starch and animal feed sectors, as well as their lucrative export markets continue to rise (FAO, 2016). In Ghana, the opening of an export-oriented starch factory near Accra in 2003 led to an "explosion" in farmers' demand for high-yielding, disease-resistant varieties to help feed the factory with raw materials (Business and Financial Times Newspaper, 2013). This factory, the Ayensu Starch Factory, established as part of the Presidential Special Initiative (PSI) on cassava, was projected to work at 70% installed capacity (Business and Financial Times Newspaper, 2013); however, its operations were fraught with challenges, key among which were power cuts, financial challenges and insufficiency of raw materials (Addo, 2013): the factory could therefore only operate at 20% of its installed capacity (Business and Financial Times Newspaper, 2013). An optimization of production parameters and a careful selection of variety could have helped boost production and enhance the revenue outlook for the African starch industry. Some work have been carried out on enzymatic extraction of starch from native crops in many places worldwide (Sit et al., 2015; Pinyo et al., 2016) and results from these studies have been very remarkable.

In Ghana, cassava is commonly used in starch production and attempts at improving extraction processes with enzymes have been with only single varieties, usually with the *Afisiafi* (Dzogbefia et al., 2008a, b), a variety which is also mealy. The aim of this study was therefore to establish if other cassava varieties would produce a similar (improved) response to starch recovery rates and yield when enzyme technology is applied. It was expected that varietal differences in cell wall biochemistry (especially regarding pectin content) would greatly influence the parameters being measured; this will help determine which variety is best suited for the technology. With this, we could also establish what varieties would be better suited for industrial starch production and which would be best for dietary consideration and thereby avoid undue competition between food and other industrial applications.

METHODOLOGY

Cassava, manioc or yucca (*Manihot esculenta* Crantz) is a perennial shrub of the New World which is currently the sixth world food crop for more than 500 million people in tropical and sub-tropical Africa, Asia and Latin America (El-Sharkawy, 2004). Taxonomically, the crop belongs to the family Euphorbiaceae and the genus *Manihot* which is known to have about 100 species among which, the *M. esculenta* Crantz is the only commercially cultivated species (Alves, 2002).

The mature plant grows to an average height of about 1 to 4 m (Alves, 2002). It is a monecious plant that is cross pollinated and seed propagated, leading to genetic segregation among the various species. As a crop, cassava is vegetatively propagated via stem cuttings.

Cassava was first introduced into Ghana by Portuguese slave ships from Brazil in 1750 (Safo-Kantanka, 2004). Since that time, much genetic variability has arisen mainly through accidental hybridization and spontaneous recombination between varieties. The current climax population, however, is a product of farmers' artificial selections (Fregene et al., 2003). Such varieties have been given names by farmers to demonstrate major attributes of the varieties. Hence, names such as 'Bankye-Broni' (DMA-001), 'Tu-gyabi-tuntum' (DMA-003), 'Bokentenma' (DMA-015), 'Nfiemenu-Bankye' (DMA-016), 'Kowoka' (DMA-009), 'Bankye-soja' (WCH-1), 'Ampe nkyere', 'Bankye-Ababaawa' (ASF-010) and many others, now commonly ascribed to local cassava varieties, are available on the Ghanaian market (Safo-Kantanka, 2004).

Morphology and uses of some cassava varieties in Ghana

Cassava cultivars can be distinguished by their morphological characteristics such as leaf size, colour and shape, branching habit, plant heights, colour of stem and petioles, tuber shape, time - to - maturity, yield and level of cyanogenic glycosides in the tuber and leaves (IITA, 1990). For example, the *Afisiafi* clone (*TMS 30572*) was introduced from IITA to Ghana in 1988 under the code *GC/88-07*. Its morphological characteristics include light green petiole, brownish grey mature stem and a light brown outer skin of tuber with cream inner skin. The variety can be grown in both major and minor seasons and is highly tolerant to major pests and diseases. Information on the uses and yield of some commonly grown cassava varieties in Ghana are as shown Table 1.

'Afisiafi' and 'Tek bankye' have peak flour yields of 23 and 22.4%, respectively, at 13 months after planting, while 'Abasafitaa' and 'Gblemoduade' had their peak flour yields increasing uniformly from 11 to 13 months after planting after which it decreased. 'Abasafitaa' and 'Tek bankye' were also reported to have flour yields increasing from 9 through 12 months after planting after

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Table 1. Features and uses of some cassava varieties grown in Ghana.

Variety	Year released	Maturity period (months)	Mean root yield (T/ha)	Total dry matter (%)	Uses	CMD Resistance
Afisiafi	1993	12-15	28-35	32	Starch, flour, gari	Tolerant
Abasafitaa	1993	12-15	29-35	35	Starch, flour, gari	Tolerant
Tekbankye	1997	12-15	30-40	30	fufu, ampesi, gari	Susceptible
Dokuduade	2005	12	35-40	30	Starch, gari	Resistant
Agbelifia	2005	12	40-45	33	Starch, gari	Resistant
Essam bankye	2005	12	40-50	35	Flour, gari	Resistant
Bankyehemaa	2005	9-12	40-50	32	Flour, gari, fufu	Resistant
Capevars bankye	2005	9-12	30-35	30	Flour, gari, fufu, starch	Resistant
Bankye botan	2005	12-15	25-30	28	Flour, gari, starch	Tolerant
Eskamaye	2005	15-18	16-23	25	Tuo, konkonte	Tolerant
Filindiakong	2005	15-18	16-20	28	Tuo, konkonte	Tolerant
Nyerikobga	2005	15-18	17-29	30	Tuo, konkonte	Tolerant
Nkabom	2005	12-15	28-32	32	Starch, fufu	Tolerant
IFAD	2005	12-15	30-35	30	Starch, fufu	Tolerant
Ampong	2010	12	40-50	36	Flour, Starch, fufu	Resistant
Broni Bankye	2010	12	40-45	33	Flour, bakery products	Resistant
Sika bankye	2010	12	40-45	36	Flour, Starch	Tolerant
Otuhia	2010	12	35-40	39	Flour, Starch	Resistant

RTIMP Ghana, 2014.

which flour yield fell. The starch yield of flour, its solubility, ash content and pasting characteristics were all reported (Apea-Bah et al., 2007) to be significantly affected by variety.

With regards to physicochemical dynamics of the extracted starch with age, Sriroth et al. (1999) reported that age of the root and environmental conditions at harvest influence granule structure and hydration properties and that starches extracted from cassava roots harvested at different times were characterized by unique starch granule structure and function.

Cassava cultivation

Cassava is propagated using stem cuttings. The cuttings, usually cut into 20 to 25 cm long, can be planted in a slanting or angular position (45°) by burying in the soil with one-third of the cuttings above the soil surface and ensuring that lateral buds point upwards. This is where the cuttings sprout. Conventionally, it is recommended that the cuttings are planted at a spacing of 1 × 1 m on the crest of ridges or mounds. This will give a plant population of 10,000 stands/ha. Vertical or angular planting is recommended in areas of high rainfall. Horizontal planting is better in dry areas. Generally, farmers plant by hand and it takes 8 to 10 persons to plant 1 ha in 1 day.

The optimum age when the starch and dry matter yields are highest is 9 to 12 months after planting, depending on the variety and the climate. Some varieties mature in 15 to 18 months. Extended cold season may delay the maturity of cassava. Harvesting too early results in a low yield, while delayed harvesting could reduce yield.

Cassava roots are harvested when they are mature to have accumulated enough starch but have not yet become fibrous. The root is harvested by cutting the plant stem at about 30 to 50 cm above the ground and then gently pulling the residual stem to lift the roots out of the soil. This is to avoid bruising the tubers as this could hasten deterioration of the root especially during storage. Harvesting is done when the soil is wet and loose.

Plant materials

Fresh local cassava (*M. esculenta* Cranz) varieties *Afisiafi*, *Esam bankye*, *Bankyehemaa* and *Nkabom* and *Doku duade* harvested at nine months after planting (MAP) were obtained under a running project at the Department of Agriculture Engineering, K.N.U.S.T. All varieties were planted on the same field of the Agriculture Research Station (at *Anwomaso-Domeabra*, Kumasi) and had been subjected to similar edaphic and climatic conditions.

Cell culture and enzyme production

Prior to enzyme production, *S. cerevisiae* (ATCC 52712) cells were propagated and subcultured in malt extract broth (M.E.B.) and agar (M.E.A.) slants to obtain pure cultures. A loop full of pure culture from M.E.A. slant was inoculated in 100 ml malt extract broth and incubated for 3 days at 28°C. During the period, light absorbance (at 540 nm) of the culture in MEB and cell enumeration (on malt extract agar using pour plate technique) were taken at 12 h intervals. The values obtained were used to derive a standard calibration curve for cell density. 4 ml of M.E.B. culture (density 6.32 × 10^{12} per 100 ml) was then transferred into 100 ml of 1% pectin medium– formulated based on a modification of the method used by Ranganna (1986) and cultured for 8 days at 28°C. During this period, the concentration of crude protein (enzyme) was monitored and cell density was estimated daily at 540 nm using spectrophotometry vis-à-vis the standard (calibration) plots for cells density obtained; crude protein (enzymes) were obtained by centrifugation at a speed of 3600 g for 10 min at a temperature held

at 4°C by careful aseptic recovery of the supernatant with 1000µL micropipette into sterile boiling tubes.

Pectolytic enzyme assay

Assaying for polygalacturonase (pectolytic enzyme) activity of the crude extract was carried out using the method described by Ranganna (1986). One unit of polygalacturonase (PGase) activity was as defined by Jayani et al. (2005). They defined one unit (U) of PGase activity as the amount of enzyme that liberates 1 µmol/ml/min of D-galacturonic acid from pectic substances under standard assay conditions.

Amylase enzyme assay

This was based on modification of the method described by Bernfield (1955). A 1% starch solution was slightly warmed in 100 ml sodium acetate buffer (0.1 M, pH 4.7). The extraction buffer was 1 M K_2HPO_4, pH 6.5. 1 ml of 1% starch and 1 ml of the crude enzyme extract were incubated at 27°C for 15 min. At the end of the incubation period, the reaction was stopped by the addition of 2 ml of dinitrosalicylic acid reagent and the resulting solution heated in a boiling water bath for 5min. While the test tubes and its content were warm, 1 ml of 40% potassium sodium tartrate solution was added and the content cooled under running tap water. The volume was then made up to 10 ml by the addition of 6 ml water. Spectrophotometric measurement (absorbance) was read at 560 nm. However, for the control, the reaction between the 1% starch and crude enzyme extracted was terminated at zero time. The amount of the reducing sugars formed was calculated from a standard graph prepared from known concentrations (10 to 100 mg) of maltose.

Determination of moisture content in the cassava pulp

Moisture content was determined using gravimetric methods described by Association of Official Analytical Chemists (AOAC, 1990).

Preparation of cassava mash for starch extraction

The procedure used for starch extraction from each variety is as shown in the flow chart (Figure 1). The methodology was to simulate starch extraction processes of traditional starch producers; however the method was slightly improved by submerged fermentation of the cassava mash with the crude enzymes.

Determination of starch (suspension) flow rate

Immediately the holding time for each mash was up, the enzyme-cassava mash mixture was emptied on to a cotton (cheese) cloth lining a funnel with a diameter of about 9 cm. The open end of the funnel (from which the starch milk was to drain) was inserted into a 250 ml measuring cylinder prior to pouring the mash unto the cheese cloth. Volume of each starch suspension obtained at 15 s intervals was recorded for 3 min (180 s).

Statistical analysis

Data were analysed using the completely randomized design (CRD). All data were subjected to ANOVA and significant differences were tested using the Duncan's new multiple range test. For all parameters measured, the statistical software used was the *SigmaPlot* for Windows Ver. 11 by Systat Software Inc.® (2008).

RESULTS AND DISCUSSION

Moisture content of cassava pulp

Cassava pulp from the various varieties had moisture contents that were significantly different. Moisture contents recorded were similar to values reported in some literature (Morgan and Choct, 2016). The *Esam bankye* variety had the least moisture content and the *Afisiafi* had the highest amount of moisture in its pulp. This information could prove helpful in the selection of variety as low moisture varieties have high dry matter content (which includes starch).

Enzyme activities of the crude enzyme extract

A polygalacturonase (pectolytic enzyme) activity of 4.91 U with specific activity (calculated per minute) of 4.291 U/mg protein was recorded in the crude enzyme preparation. Bali (2003) however reported a pectinase activity of 210.37 nmoles/ml/60 min suggesting that the crude enzyme preparation had a rather high activity. Endogenous amylase activity of 0.293 U/ml (with a specific activity of 0.257 U/mg) was also recorded from the extract. This value is also higher than the 0.16 and 0.09 U/mg (for amylase activity and specific activity, respectively) reported by Dzogbefia et al. (2008a). This also suggests a high possibility of amylolysis occurring during stages of cassava mash incubation with the enzyme.

Effects of enzyme dosage on starch yield and reaction time

Starch content from each mash was generally lower than those reported in most literature (Aldana and Quintero, 2013; Roslimi et al., 2016). This is probably due to differences in methodology used in estimating starch content. However, since all the varieties were exposed to the same experimental and edaphic conditions, it is possible to compare and appreciate the contributions of variety to the process.

At very low enzyme dosages (between 10 and 20 mg/200 g of mash) improvement in yield was not significantly different from that of the control ($P>0.001$) (Figures 2 to 6). Significant yields were however recorded at higher dosages (between 30 and 50 mg/200 g of mash) in all varieties with optimum yields being recorded in the 40 and 50 mg dosages. The *Doku duade* and *Bankyehemaa* varieties recorded their highest yields with the 50 mg/ml crude protein per 200 g mash (0.025%)

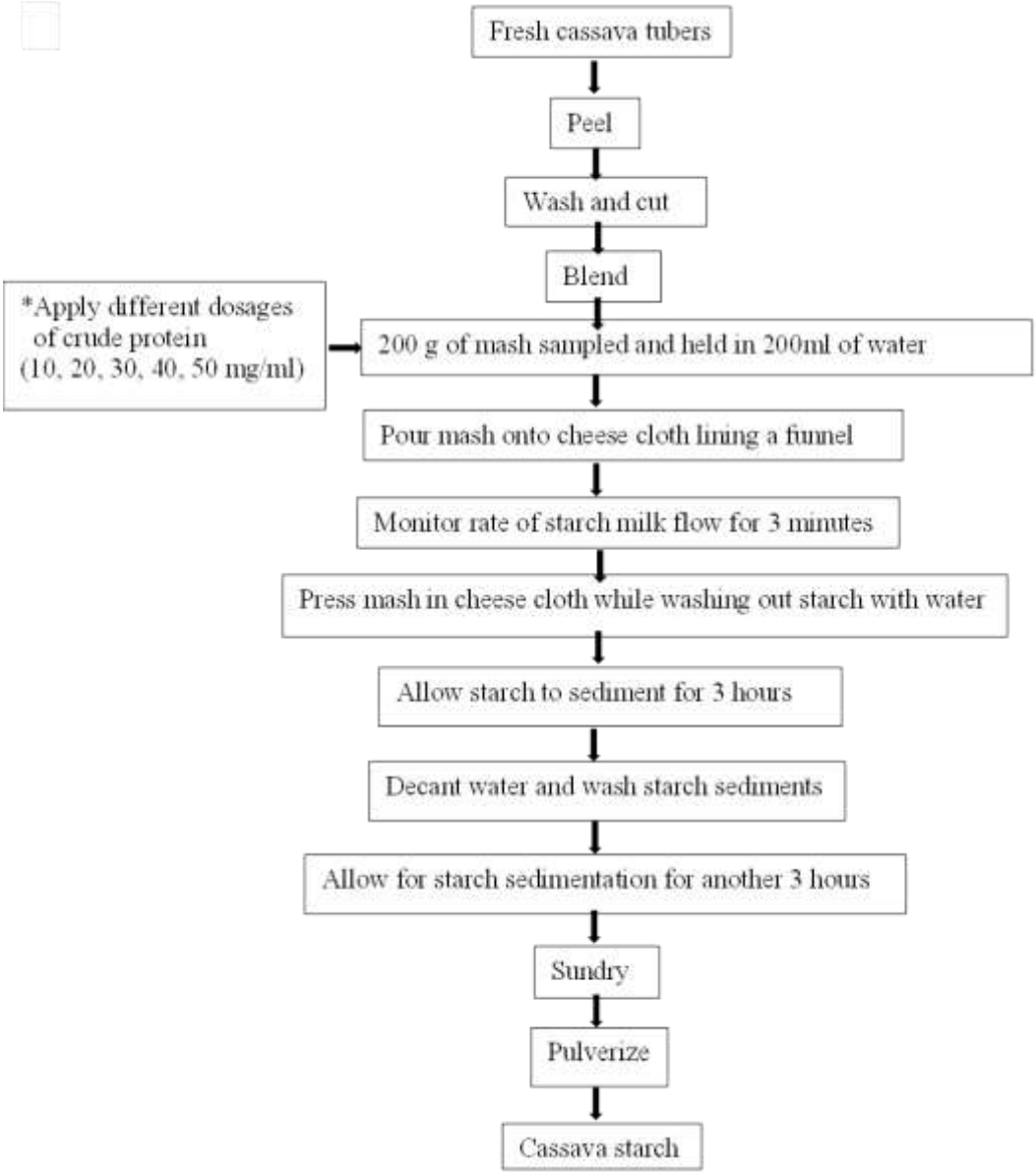

Figure 1. Flow chart of procedure used in enzyme-assisted starch extraction. * Control samples did not require this step.

dosage; all the other varieties (*Afisiafi*, *Nkabom* and *Esam bankye*) gave their (most significant) highest starch yield with the 40 mg/ml per 200 g mash (0.02%) dosage. Gummadi and Panda (2003) mentioned substrate inhibition kinetics in PGase activity. The enzyme has an alternative (allosteric) site to which the same substrate binds to inhibit substrate binding at the active site and enzyme activity only increases at higher enzyme dosages.

Although, quantitative data on the pectin content of cassava root is rather scanty, pectin content of some root tubers have been estimated to range from 0.2 to 2.5 g per 100 g of mash (Schoeninger et al., 2000). At high PGase dosages, varieties with high pectin content would present high PGase activity. Additionally, differences in the amounts of mineral ions in the pectin framework could also influence the results observed. Pectin, a polyanionic molecule, naturally binds divalent ions like Ca^{2+}, Mg^{2+}, Cu^{2+} and Zn^{2+} as part of its framework. These ions, at high concentrations have been reported to be rather inhibitory to PGase activity (Vázquez et al., 1986). This suggests that varieties that sequester high mineral ions would subject PGase to higher levels of inhibition at long holding times as more of these ions are released. However, pectate lyase (PL), whose isoforms have been reported (O'Neill et al., 2001) to require Ca^{2+} in its activity could take over pectolysis. Thus, any further pectolytic activity to release granular starch will depend on what

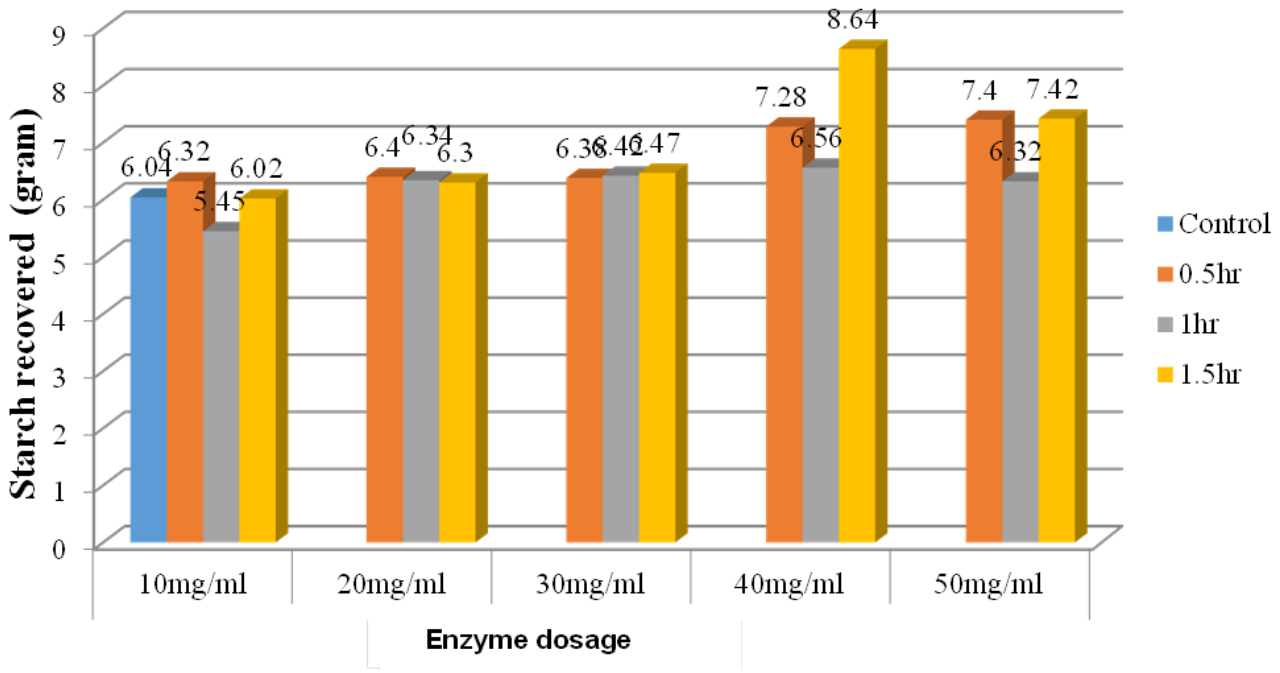

Figure 2. Effects of enzyme dosages on yield in the *Esam Bankye* variety.

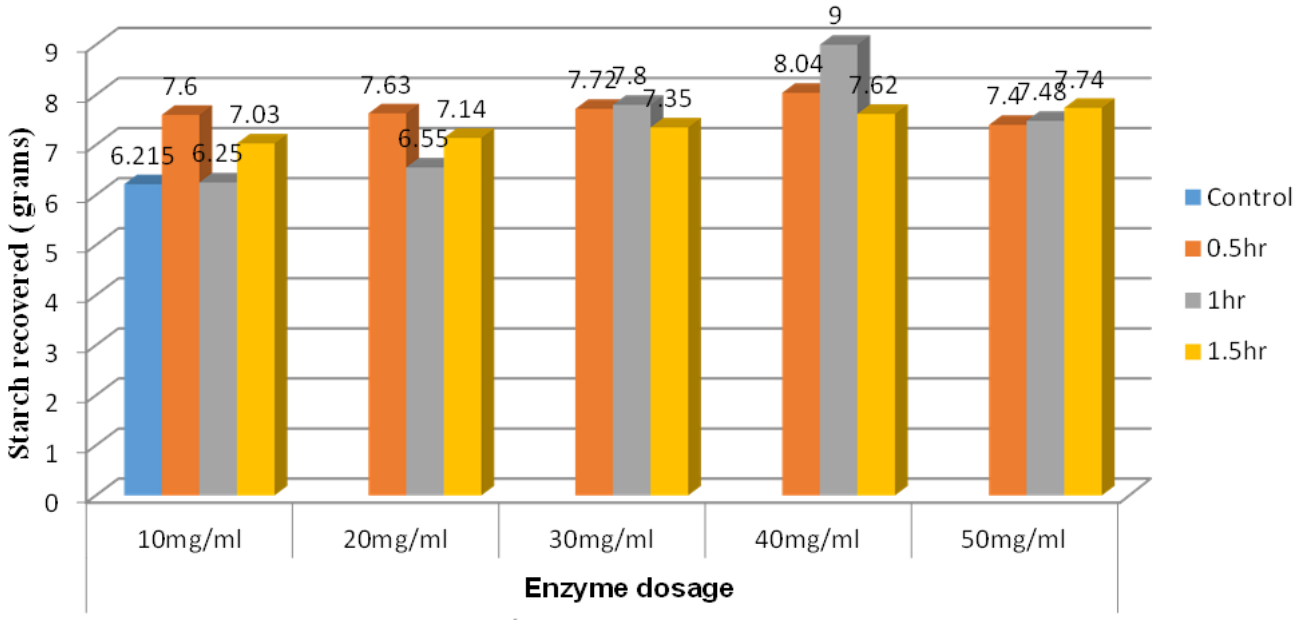

Figure 3. Effects of enzmye Dosages on Yield in the *Afisiafi* variety.

fraction of calcium ions are available to PL. Apparently, the use of PGase activity (in crude protein preparations)alone to estimate enzymatic response of variety to starch extraction presents a rather complex situation. It is however shown that longer holding times, due to the presence of endogenous amylase in the enzyme preparation, could have deleterious effects on the starch by converting it into limit dextrins (which includes glucose) (Miguel et al., 2013), maltose and maltotriose (Li et al., 2017); this affects the original functional properties of the starch, especially those related to starch granule structure and amylose content

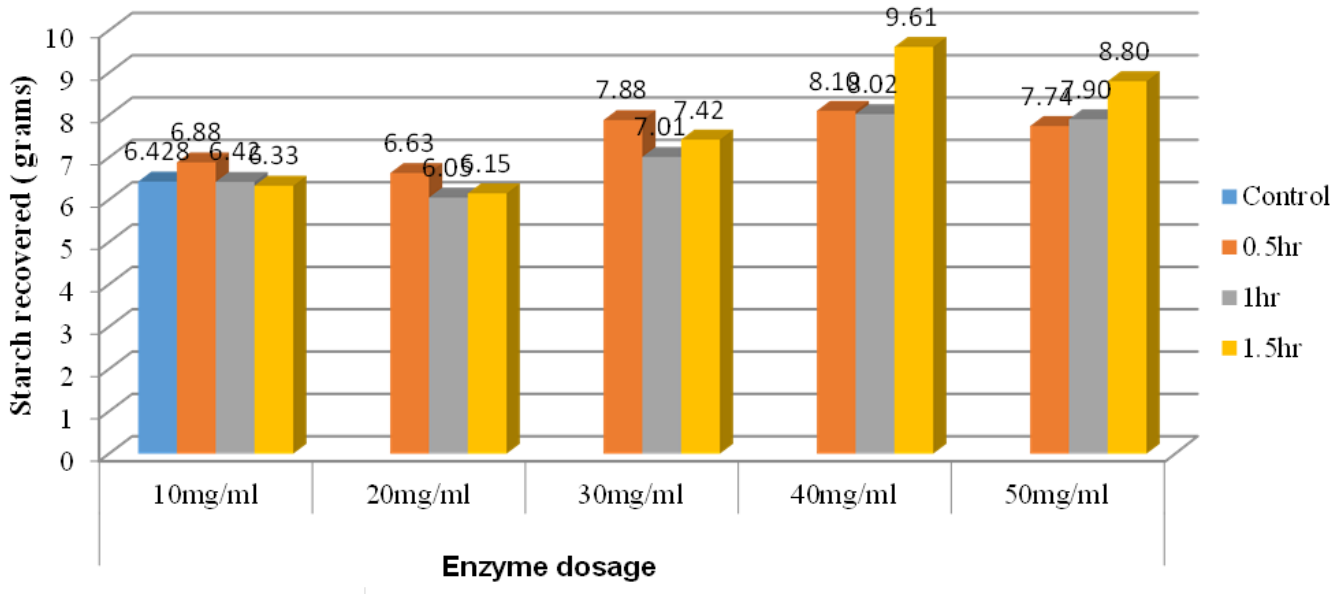

Figure 4. Effects of enzyme dosages on yield in the *Nkabom* variety.

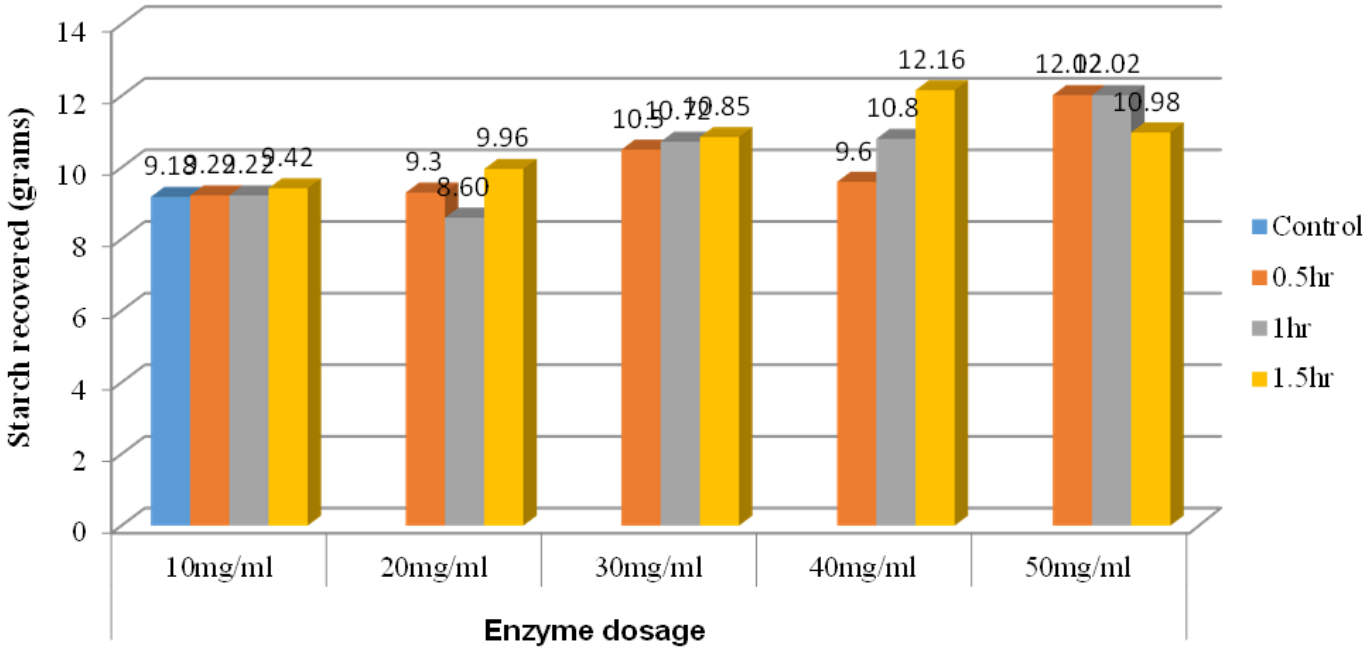

Figure 5. Effects of enzyme dosages on yield in the *Bankye hemaa* variety.

which ultimately determine the swelling, solubility and pasting properties of starch (Wang et al., 2015). Additionally, longer holding times affect the stability of pectinase enzymes (Vatanparast et al., 2014) and impact profits negatively as turnover time is increased. Hence if apparent (improved) yields at a given dosage and longer times are not so different from those obtained at higher dosages with shorter time requirements, then it will be economically prudent to adopt the latter. For these reasons, although, the *Esam bankye* recorded highest starch yield (about 43%) with the 0.02% enzyme dosage vs. 1.5 h holding time combination (Figure 2 and Table 2), the 0.02% vs. 0.5 h dosage combination could be adopted (starch yield of about 20.53% over the control).

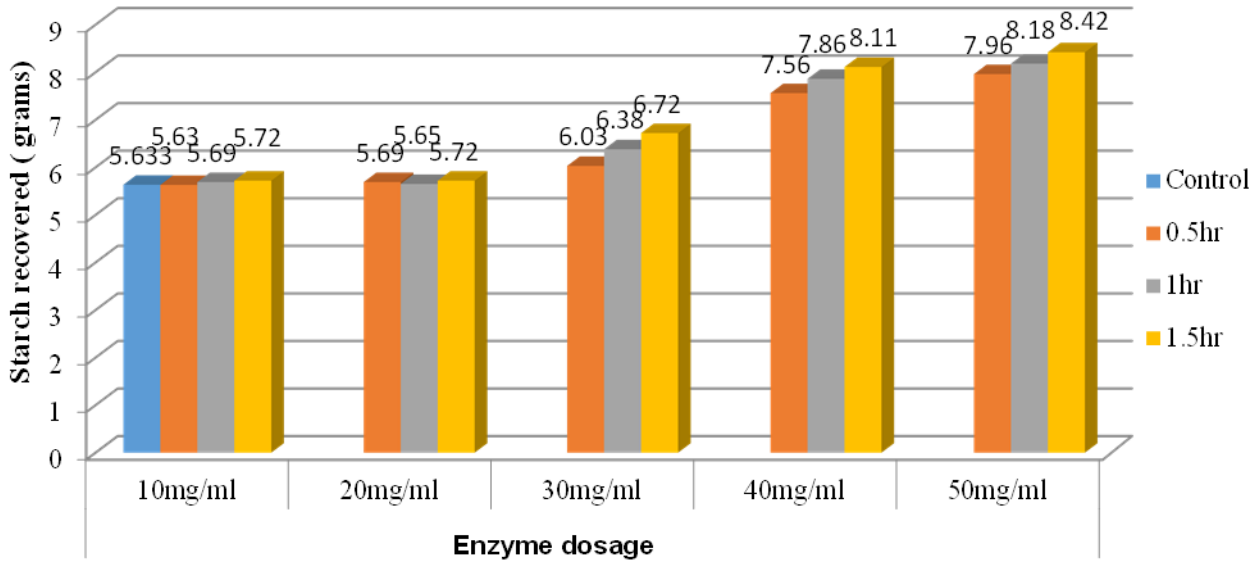

Figure 6. Effects of enzyme dosages on yield in the *Doku duade* variety.

Table 2. Moisture content of cassava root pulp from the various cassava varieties.

Cassava variety	Moisture content (%)
Esam bankye	55. 25 ± 0.589[a]
Doku duade	57.414 ± 1.360[b]
Afisiafi	65.857 ± 0.862[c]
Nkabom	61.197 ± 1.817[d]
Bankye hemaa	58.492 ± 0.699[b]

Different letters in parenthesis show significant (P<0.05) differences.

An optimal treatment combination (0.02% vs. 0.5 h) in the *Nkabom* gave an enhanced yield of 26.15%, while in the *Afisiafi* variety, an optimal holding time of 1 h at the 0.02% enzyme dosage gave an enhanced yield of about 45% which was significantly (P<0.001) higher than that obtained (about 29.36%) at the 0.5 h holding time. Dzogbefia et al. (2008a) gave similar reports of the 0.02% enzyme treatment being the most effective for starch extraction in this variety. However, they obtained significantly higher yields at the 0.5 h holding time with this dosage. These differences could be attributed to differences in activity of the enzyme (necessitating a longer holding time for optimal activity), difference in the age of the variety as well as varying environmental conditions under which the variety was cultivated (which affect the total biomass composition). The *Doku duade* and *Bankye hemaa*, however, required a treatment combination of 0.025% (50 mg/ml of crude protein in 200 g mash) dosage at 0.5 h (holding time) to optimize yield (about 41.46 and 30.91%, respectively).

Quantitative measurements of polygalacturonase (PGase) activity on samples using 0.5 g of mash from each variety gave an activity of 0.121 U/ml on the *Bankye hemaa* variety; 0.137 U/ml on the *Doku duade* variety; 0.181 U/ml on the *Afisiafi* variety; 0.067 U/ml on the *Esam bankye* variety and 0.094 U/ml on the *Nkabom* variety. This pattern of activities seems to agree with the trend for starch yield obtained due to the enzyme treatment (Table 3). Hence, the responses observed are largely due to the different amounts and/or type of fiber materials (including pectic substances) present (Moelants et al., 2014). Apparently, pectic substances from especially, the *Nkabom* and the *Esam bankye* varieties were low in their polygalacturonan (PG) contents which probably explain why these varieties required relatively lower dosages (0.02%) and shorter holding times for maximum activity.

It is however noteworthy that despite improvements in the yield due to enzyme treatment, a key determinant of choosing a variety would be the variety's innate starch content and physico-chemical properties of its starches. Comparing the controls (Figures 2 to 6), the *Bankye hemaa* variety produced the highest starch yield from its mashes and with enzyme treatment, seconded the *Doku duade* variety in terms of yield improvements (Table 3). This suggests that the variety will be the most suitable choice for enzyme-assisted starch extraction.

Effects of enzyme dosage on flow pattern and starch milk recovery

Generally, all the varieties displayed typical parabolic flow

Table 3. Summary of percentage increase in starch yield due to enzyme treatment at optimal dosage and holding times.

Cassava variety	Optimum treatment combination (dosage/holding time)	Increase in yield (%)
Nkabom	0.020% vs. 0.5 h	26.15 ± 7.367
Afisiafi	0.020% vs. 1.0 h	45.10 ± 11.877
Esam bankye	0.020% vs. 0.5 h	20.53 ± 11.409
Doku duade	0.025% vs. 0.5 h	41.46 ± 5.165
Bankye hemaa	0.025% vs. 0.5 h	30.91 ± 4.648

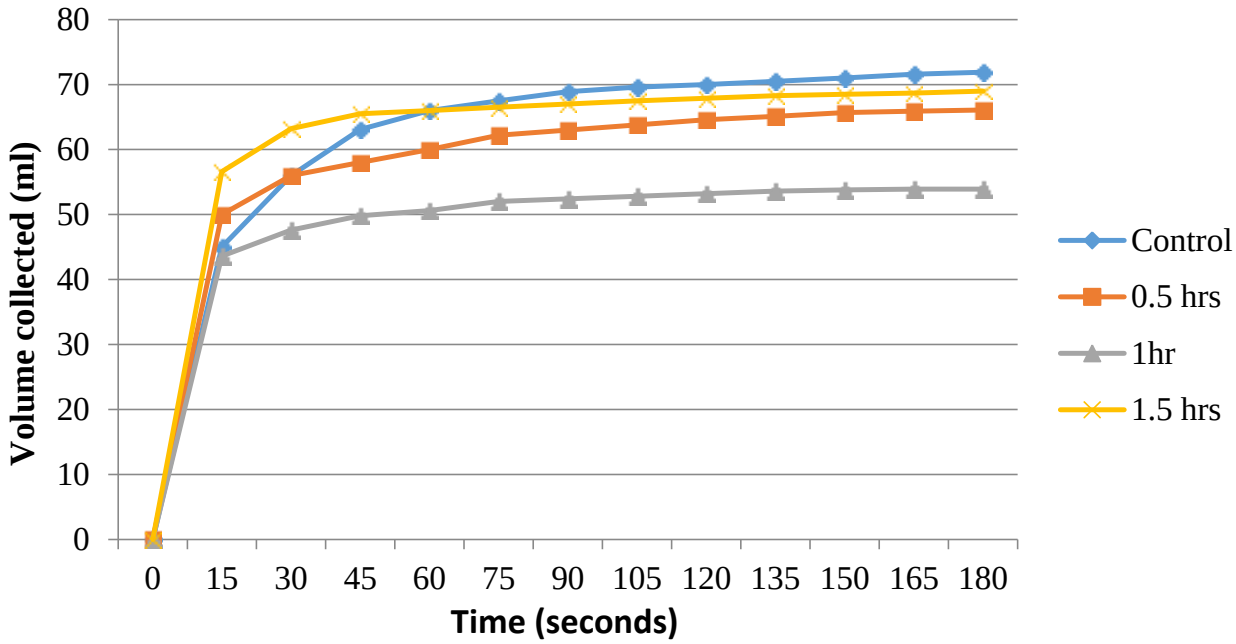

Figure 7. *Esam bankye* starch milk flow pattern at all holding times of its optimum enzyme dosage (40 mg/ml crude protein in 200 g mash, 0.02%).

patterns at all dosages and holding times with rates of starch milk recovery, after enzyme treatment, generally being higher when compared with their controls within the first 15 s of flow (Figures 7 to 11). This suggests that technology allows the pressing (Figure 1) of the mashes to be carried out earlier. The only exception to this general trend however was the *Esam bankye* variety where milk recovery for the control (as compared to enzyme treated samples) was faster.

Mashes from the *Bankye hemaa* and *Doku duade* varieties had their viscosities greatly reduced by the technology as these recorded the highest improvement in flow (Table 4). This observation pattern agrees with the trend of pectolytic activities on the mash (cited earlier).

CONCLUSION AND RECOMMENDATION

Optimization of starch extraction from cassava with crude pectolytic enzymes from *S. cerevisiae* was found to be dependent on variety as well as the dosage-holding time treatment combinations adopted. Generally, enzyme dosages of 0.02% (for the *Nkabom*, *Afisiafi* and *Esam bankye*) and 0.025% (for the *Doku duade* and *Bankyehemaa*) were required to optimize yield for starch recovery. This was related to the varying composition of pectic materials in the root mashes as activity of the crude enzyme preparation on the mashes varied. However, application of the technology generally enhanced starch yield and recovery in all varieties with the highest starch yield (due to enzyme treatment) being recorded in the *Afisiafi* and the greatest recovery rates being recorded in the *Bankyehemaa* variety.

However, the high (dry) biomass, susceptibility to pectolytic (enzyme) activity, high starch content (even without enzyme treatment) and low holding time requirements for enzyme-assisted yield optimization

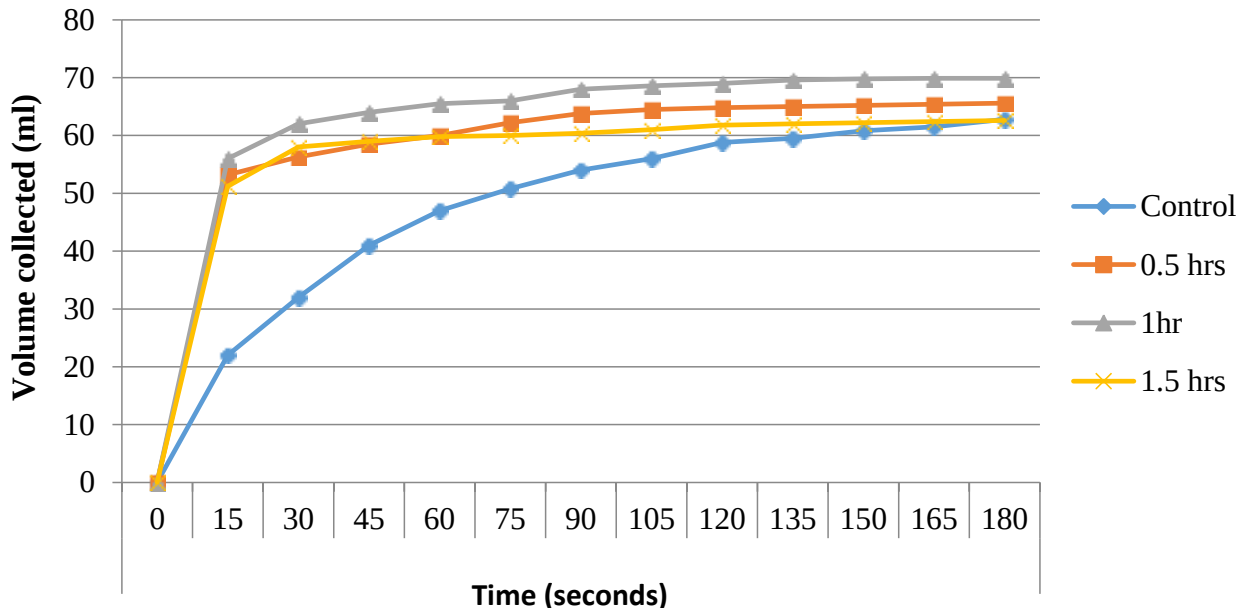

Figure 8. *Afisiafi* starch milk flow pattern at all holding times of its enzyme dosage optimum (40 mg/ml crude protein in 200 g mash, 0.02%).

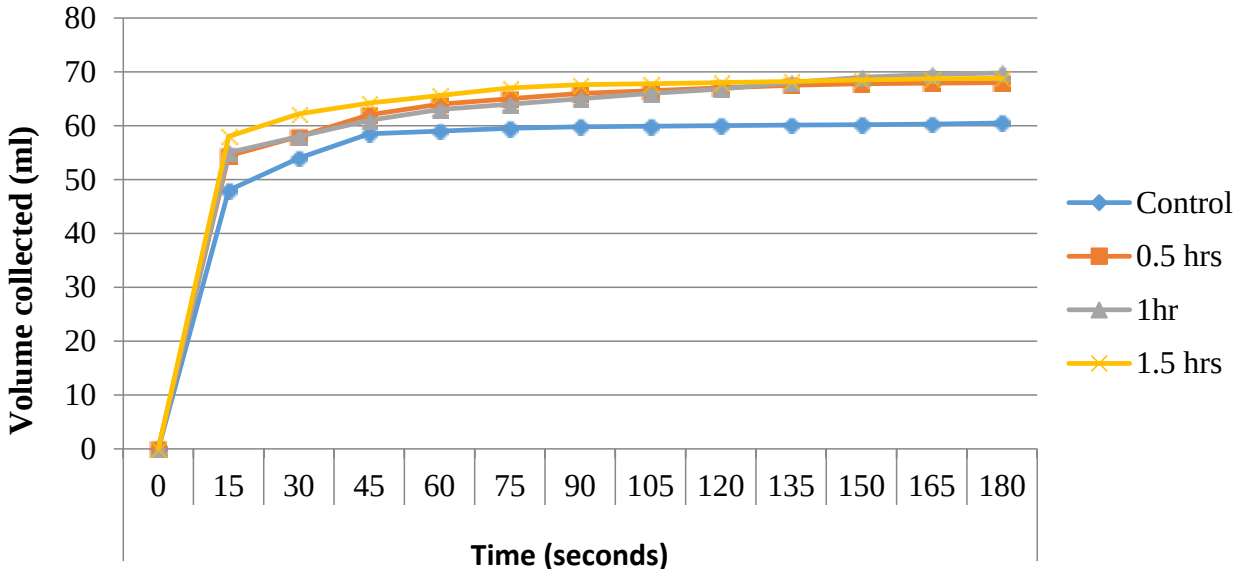

Figure 9. *Nkabom* variety's starch milk flow pattern at all holding times of its enzyme dosage optimum (40 mg/ml crude protein in 200 g mash, 0.02%).

altogether confirm that the *Bankyehemaa* variety is best suited for enzyme-assisted starch extraction. Its low root (pulp) moisture content also suggest that farmers would be transporting a lot more (dry) biomass from their farms and their pulp will probably dry faster for storage; results of both yield and starch milk recovery rates in the *Esam bankye* variety was rather inconsistent hence utilization of the variety for crude (pectolytic) enzyme-based cassava starch extraction might be discouraging.

Although, high dosages (as with the 25 mg/ml per 100 g mash in this work) of crude pectolytic enzyme and longer retention time (1hour and 1.5 hours) have been reported to significantly affect starch biochemistry by causing extensive amylolysis, the very high starch yields obtained from these dosages cannot be overlooked. Such dosages could be employed in the production of

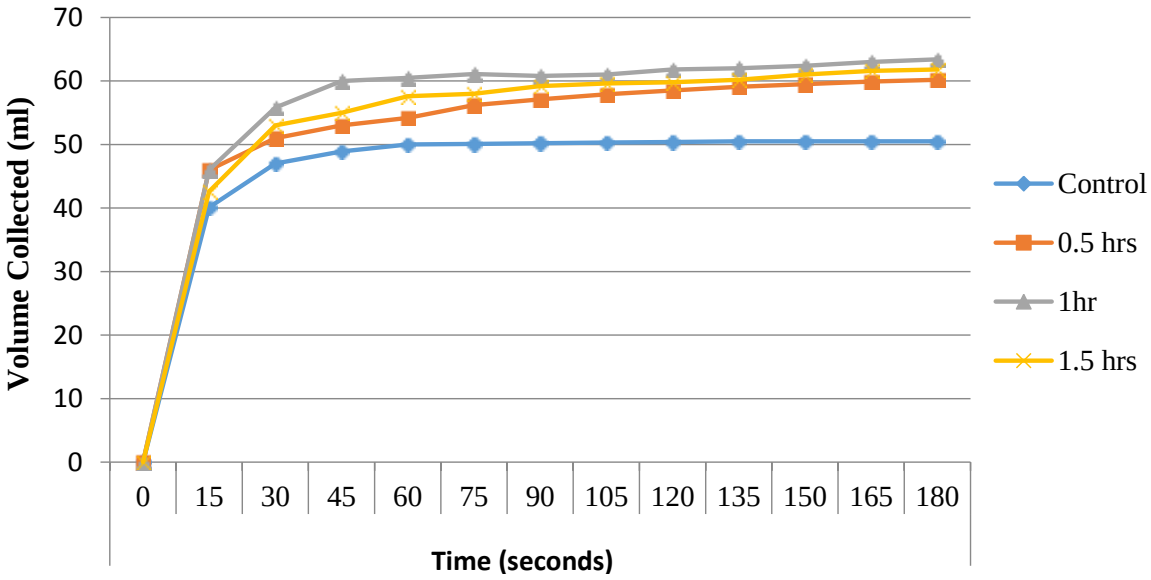

Figure 10. *Bankye hemaa*'s variety starch milk flow pattern at all holding times of its enzyme dosage optimum (50 mg/ml crude protein in 200 g mash, 0.025%).

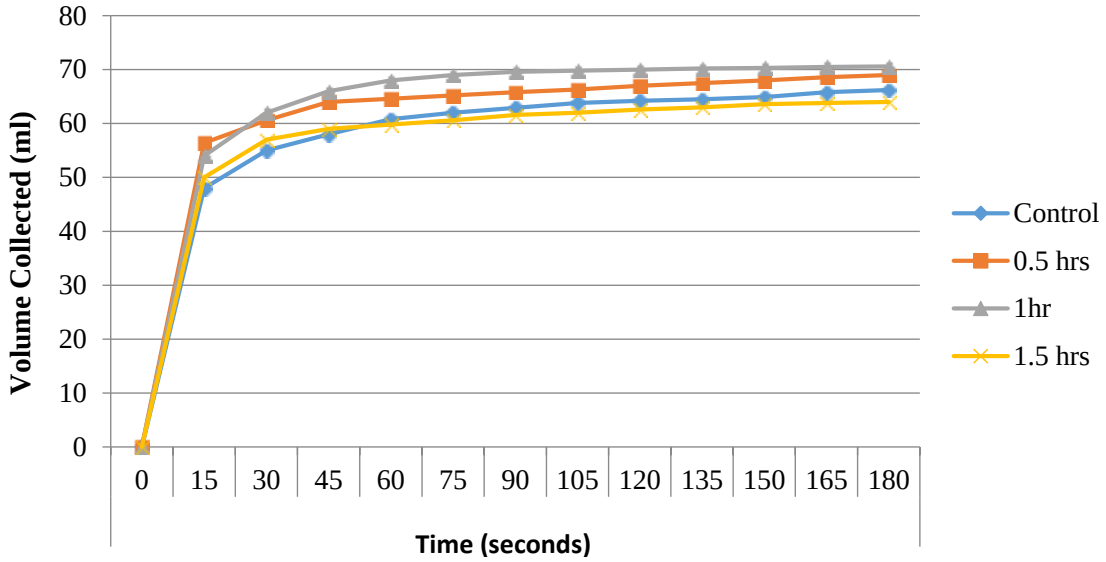

Figure 11. *Doku duade* variety's starch milk flow pattern at all holding times of its enzyme dosage optimum (50 mg/ml crude protein in 200 g mash, 0.025%).

Table 4. Rates of starch milk flow from treated and untreated starch mashes of the cassava varieties.

Cassava variety	Average flow rate (ml/s) within the first 15 s		Percentage increase starch milk flow
	Control	Treated	
Afisiafi	2.5 ± 0.047	4.3 ±0.095	73.3 ± 0.555
Nkabom	2.7±0.094	3.2±0.000	17.1± 4.030
Bankye hemaa	2.2±0.047	3.9±0.047	78.5±6.091
Doku duade	1.8 ± 0.047	3.1± 0.000	73.7 ± 4.658
Esam bankye	1.8±0.047	1.7±0.047	-10.9±0.282

starches that find application in the food industry as this will enhance sweetness and digestibility of the starches.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Addo E (2013). Logistical Constraints collapse Ayensu Starch again. Available at https://www.ghanaweb.com/GhanaHomePage/business/Logistical-Constraints-Collapse-Ayensu-Starch-AGAIN-263892.

Aldana S, Quintero F (2013). Physicochemical Characterization of two cassava (*Manihot esculenta* Crantz) starches and flours. Scientia Agroalimentaria 1 (2013):19-25.

Alves AAC (2002). Cassava: Biology, production and utilization, CAB International, Brazil. pp. 63-89.

Association of Official Analytical Chemists (AOAC) (1990). Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists. Washington DC.

Apea-Bah FB, Oduro I, Ellis WO, and Safo-Kantanka O. (2007). Time of harvesting and its effects on quality of flour from four cassava varieties, Proceeedings of the 13th ISTRC Symposium, Pp. 248-259.

Business and Financial Times Newspaper (BFT) (2013). Ayensu Starch Factory resumes production. Available at https://www.ghanaweb.com/GhanaHomePage/business/Ayensu-starch-factory-resumes-production-267048

Bali R (2003). Isolation of extracellular fungal pectinolytic enzymes and extraction of pectin using kinnow waste as substrate. A Thesis submitted in Partial Fulfillment of the requirement for the Award of Master of Science (Biotechnology), Deemed University,Patiala,Punjab, India. In: Food Innovation Asia Conference 2010: Indigenous Food Research and Development to Global Market, June 17-18, 2010, BITEC, Bangkok, Thailand. Available at http://www.agri.ubu.ac.th/news/fiac/data/oral_session7.pdf.

Bernfield P (1955). Amylases, Alpha and Beta. Methods Enzymol. 1:149-158.

Central Intelligence Agency (CIA) (2017). World Fact Book. Available at https://www.cia.gov/library/publications/the-world-factbook/geos/print_gh.html.

Chinma CE, Ariahu CC, Abu JO (2013). Chemical composition, functional and pasting properties of cassava starch and soy protein concentrate blends. J. Food Sci. Technol. 50(6):1179-1185.

Dzogbefia VP, Ofosu GA, Oldham JH (2008a). Evaluation of locally produced *Saccharomyces cerevisiae* pectinase enzyme for industrial extraction of starch from cassava in Ghana. Sci. Res. Essays 3(8):365-369.

Dzogbefia VP, Ofosu GA, Oldham JH (2008b). Physicochemical and pasting properties of cassava starch extracted with the aid of pectinase enzymes produced from *Saccharomyces cerevisiae* ATCC52712, Sci. Res. Essays 3(9):406-409.

El-Sharkawy MA (2004). Cassava Biology and Physiology. Plant Mol. Biol. 56 (4):481-501.

Essabrah-Mensah E (2016). Cassava – Crop of the Decade. Business and Financial Times Available at http://thebftonline.com/business/agribusiness/18923/cassava-crop-of-the-decade-.html.

Food and Agricultural Organization (FAO) of the United Nations (2016), Food Outlook Biannual Report on Global Food Markets. P 36. http://www.fao.org/3/a-i7343e.pdf

Fregene MA, Suraez M, Mkumbira J, Kulembeka H, Ndedya E, Kulaya A, Mitchel S, Gullberg U, Rosling H, Dixon AG, Dean R, Kresovich S (2003). Simple sequence repeat marker diversity in cassava landraces: genetic diversity and differentiation in an asexually propagated crop. Theor. Appl. Genet. 107(6):1083-1093.

Gummadi SN, Panda T. (2003). Analysis of Kinetic Data of Pectinases with Substrate Inhibition. J. Microbiol. Biotechnol. 13(3):332-337

Jayani RS, Saxena S, Gupta R. (2005). Microbial pectinolytic enzymes: A review. Process Biochem. 40(9):2931-2944.

Li J, Zhou W, Francisco P, Wong R, Zhang D, Smith SM (2017). Inhibition of *Arabidopsis* chloroplast β-amylase BAM3 by maltotriose suggests a mechanism for the control of transitory leaf starch mobilization. PLoS One 12(2):e0172504.

Miguel ÂSM, Martins-Meyer TS, Costa Figueiredo ÉVD, Lobo BWP, Dellamora-Ortiz GM (2013). Enzymes in Bakery: Current and Future Trends. Food Ind. 14:288-321.

Moelants KRN, Cardinaels R, Buggenhout SV, Loey AMV, Moldenaers P, Hendrickx ME (2014). A Review on the Relationships between Processing, Food Structure, and Rheological Properties of Plant-Tissue-Based Food Suspensions. Compr. Rev. Food Sci. Food Saf. 13(3):241-260.

Morgan NK, Choct M (2016). Review Article Cassava: Nutrient composition and nutritive value in poultry diets. Anim. Nutr. 2(4):253-261.

O'Neill MA, Darvill AG, Albersheim P (2001). Pectic substances. In: Encyclopedia of Life Sciences. Web pub: www.els.net . Macmillan Reference Ltd., London.

Pinyo J, Luangpituksa P, Suphantharika M, Hansawasdi C, Wongsagonsup R (2016). Effect of enzymatic pretreatment on the extraction yield and physicochemical properties of sago starch**.** Starch - Stärke 68(1-2):47-56.

Ranganna S (1986). Handbook of analysis and quality control for fruit and vegetable products. Tata Me Graw- Hill Publishing Company, New Delhi. P 1112.

Roslimi DI, Herman, Sofyanti N, Chaniago M, Restiani R, Novita L. (2016). Characteristics of 22 cassava (*Manihot esculenta* Crantz) genotypes from Riau Province, Indonesia. Sabrao J. Breed Genet. 48(2):110-119.

Safo-Kantanka O (2004). Development of cassava varieties through the exploitation of variability in local germplasm: A Presentation at the 6th Scientific Meeting of the Cassava Biotechnology Network (CBN VI), CIAT, Cali, Columbia, 8th-14th March, 2004.

Schoeninger MJ, Bunn HT, Murray SS, Marlett JA (2000). Composition of Tubers Used by Hadza Foragers of Tanzania. J. Food Composit. Anal. 13:1-11.

Sit N, Deka SC, Misra S (2015). Optimization of starch isolation from taro using combination of enzymes and comparison of properties of starches isolated by enzymatic and conventional methods. J. Food Sci. Technol. 52(7):4324-4332.

Sriroth K, Santisopasri V, Petchalanuwat C, Kurotjanawong K, Piyachomkwan K, Oates CG (1999). Cassava starch granule structure–functional properties: influence of time and conditions at harvest on four cultivars of cassava starch, Carbohydr Polym., 38(2):161-170.

Vatanparast M, Hosseininaveh V, Ghadamyari M, Sajjadian SM (2014). Plant Cell Wall Degrading Enzymes, Pectinase and Cellulase, in the Digestive System of the Red Palm Weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae). Plant Protect. Sci. 50(4):190-198.

Vázquez C, Martínez MJ, Lahoz R, Reyes F (1986). Effect of calcium and other metal ions on pectic activities from autolysed cultures of *Alternaria alternate.* FEMS Microbiol Lett. 37(2):227-230.

Wang S, Li C, Copeland L, Niu Q, Wang S (2015). Starch Retrogradation: A comprehensive review. Compr. Rev. Food Sci. Food Saf. 14(5):568-585.

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Full Length Research Paper

Fermentation of deproteinized cheese whey by *Saccharomyces fragilis* IZ 275 for ethanol production on pilot scale

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Cheese whey is presented as an alternative for the production of ethanol to be a major source for growth of microorganisms, which catalyze lactose directly to ethanol and other products. Thus the aims of this study were to analyze the influence of nutrients in the cheese whey (15%w/v) fermentation by *Saccharomyces fragilis* IZ 275, to estimate the ethanol production and verify the repetition of the results of fermentation on a laboratory and pilot scale. Based on the results the nutrients, ammonium sulphate and yeast extract showed no significant difference at 5%, however, a positive ethanol productioin of 5.07% (w/v) and 5.43% (w/v), in laboratory and pilot scale, was respectively observed. In both kinetics, the ethanol yields were 5.6% (v/v), demonstrating that the use of deproteinized cheese whey for industrial fermentations is possible due to repetition of the results from laboratory to pilot scale, presenting as a way to reduce the pollution potential of this by-product, and at the same time to obtain value-added product.

Key words: Microorganism biotechnology, industrial whey, bioethanol fermentation, nutrients sources, hydrolysis.

INTRODUCTION

The production of dairy products, especially cheese, increases about 11.4% per year in Brazil (Wissmann et al., 2012). Cheese Whey is a by-product of cheese and casein production in the dairy industry, and represents about 85% of the total milk used in the process (Panesar and Kennedy, 2012). This by-product retains about 55% of milk nutrients, especially lactose (4.5 to 5% w/v), soluble protein (0.6 to 0.8% w/v), such as β-lactoglobulin, α-lactalbumin, lipids (0.4 to 0.5% w/v) and mineral salts (8 to 10% w/v, dry weight). The principa minerals are

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Table 1. Effect of nutrient sources added to the cheese whey.

Treatments	Nutrient sources	(g/100 mL)
T1	Control	0.0
T2	Yeast extract	1.0
T3	Potassium phosphate	0.5
T4	Peptone	1.0
T5	Ammonium sulfate	0.6
T6	Magnesium Sulfate	0.06
T7	T2 + T3 + T4 + T5 + T6	0.5 + 1.0 + 0.6 + 0.06

sodium chloride, potassium chloride and calcium salts (Siso, 1996; Panesar et al., 2007).

Not very many yeast strains are capable of fermenting lactose to ethanol. Most of the *Saccharomyces* species cannot ferment lactose to ethanol because of the lack of lactose fermenting enzymes, such beta-galactosidase (Ozmihci and Kargi, 2007). *Saccharomyces fragilis* is described as a homothallic, hemiascomycetous yeast and production of several enzymes among them beta-galactosidase in which it has the ability to hydrolyze the lactose in whey (Llorente et al., 2000; Dagbagli and Goksungur, 2008). The major common feature of *S. fragilis* is the capacity to assimilate lactose and to use this sugar as a carbon source. Lactose can be converted into bioethanol through fermentation process using yeast, especially species of *S. fragilis*. The long history of safe association with food products helped *S. fragilis* achieve GRAS (Generally Regarded As Safe) and QPS (Qualified Presumption of Safety) in the United States and European Union, respectively. This designation means that there are few restrictions on application and largely enhances their potential in the biotechnology sector (Schaffrath and Breunig, 2000; Fukuhara, 2006; Domingues et al., 2010).

Several value-added products obtained from micro-organism fermentation of cheese whey include single cell protein, ethanol, organic acids, enzymes, biopolymers, biogas and biodegradable plastics. Cheese whey has been used as an inexpensive and nutritionally rich raw material for ethanol production (Marwaha and Kennedy, 1988; Ozmihci and Kargi, 2008; Spalatelu, 2012).

Considering the optimal adaptation of the yeast *S.fragilis* IZ 275 in the fermentation of cheese whey, the aim of this work was to analyze the influence of the addition of nutrients sources in the fermentation of cheese whey, check the growth of the yeast and the production of ethanol and evaluating the growth and development of the yeast in laboratory and pilot scale in order to make the production of ethanol using cheese whey as nutrient source possible.

MATERIALS AND METHODS

The steps employed in the treatment of cheese whey and fermentations were carried out based on the results of preliminary experiments. It was used a factorial design 3^3 for ethanol production optimization using concentrated deproteinized cheese whey, which evaluated the concentration of deproteinized cheese whey, initial pH and inoculum in ethanol production by *S. fragilis* IZ 275 (SF IZ 275).

Pre-treatment of the cheese whey

The cheese whey powder (Confepar®, Brazil) was solubilized in distilled water at a concentration of 15% (w/v). The solution was pretreated by adding lactic acid (85%) to pH 4.6 and followed by a heat treatment of 90°C for 30 min. After precipitation, the protein fraction was removed by filtration and the pH adjusted to 5.0. Thus, Deproteinized Cheese Whey – DCW was used in the experiments.

Inoculum preparation

The yeast used was SF IZ 275, maintained in test tubes containing Potato Dextrose Agar (PDA) inclined and stored at 6ºC. In preparing the inoculum was used a loop of microorganisms under sterile conditions in DCW sterilized at 121ºC for 15 min. The inoculum was incubated in an orbital shaker (Tecnal®, Brazil) at 35ºC, 100 rpm for 24 h.

Effect of nutrients in cheese whey for ethanol production

To evaluate the effect of nutrients sources on the production of ethanol by SF IZ 275, an experimental design was carried out with two replications and seven treatments (Table 1). Fermentations were conducted in 250 mL Erlenmeyer flasks with 100 ml DCW inoculated with 5% (v/v) inoculum. The flasks were incubated on an orbital shaker (Tecnal®, Brazil) at 100 rpm, 35ºC for 24 h.

Kinetics fermentation

Kinetics of fermentation to produce ethanol with SF IZ 275 were performed in laboratory scale in 250 mL Erlenmeyer flasks with 100 mL of DCW in an orbital shaker (Tecnal®, Brazil). The kinetic fermentation in laboratory scale it was repeated twice.

To assess the reproducibility on pilot scale fermentation it was performed with the same conditions in an orbital fermenter (Suck Milk®, Brazil) with 50 L of cheese whey pretreated. The kinetic fermentation in pilot scale it was repeated twice.

The media was pasteurized at 90ºC for 30 min and cooled to 35ºC. Fermentations were performed for 30 h, using as substrate 15% (w/v) deproteinized cheese whey, pH 5.0 and 5% (v/v) inoculum, at 35ºC and 100 rpm for laboratory scale, and 70 rpm for the pilot scale. Every two hours, samples were collected for the

Table 2. Effect of nutrients in the whey for ethanol production.

Nutrients	Ethanol (%) ± standard deviation
T1. Control	5.52 ± 0.049[c]
T2. Yeast extract	5.80 ± 0.014[ab]
T3. Potassium phosphate	5.40 ± 0.049[d]
T4. Peptone	2.57 ± 0.035[f]
T5. Ammonium sulfate	5.82 ± 0.028[a]
T6. Magnesium Sulfate	4.80 ± 0.007[e]
T7. All nutrients (T2+T3+T4+T5+T6)	5.67 ± 0.042[b]

[a,b,c,d]Different lower case superscript within the same column indicate significant differences ($p < 0.05$) between the nutrients added to the fermentation.

determination of ethanol, glucose, residual lactose and total number of yeast cells.

Analytical determinations

The ethanol obtained from the fermentation was distilled and quantified by the method described by Kaye and Haag (1954). The residual glucose fermentations were determined by an enzymatic colorimetric method of glucose oxidase (Analisa®, Brazil). The initial and residual lactose was determined by the method described by Nickerson et al. (1975). The determination of the total number of yeast cells was performed according to the methodology described by International Dairy Federation (IDF) n. 94B (1990) and the results expressed as colony forming units per milliliter (CFU/mL).

Statistical analysis

The data were statistically analyzed by Statistica software (version 8.0) of StatSoft. The differences between treatment means were determined by Tukey test at a significance level of 5% ($p < 0.05$).

RESULTS AND DISCUSSION

Effect of nutrients in cheese whey for ethanol production

Ethanol production by SF IZ 275 in DCW with the addition of nutrients ranged from 2.57 to 5.82% (v/v). The maximum production of ethanol was obtained in T2 and T5 treatments with yeast extract and ammonium sulfate respectively, with no significant differences at a significance level of 5% ($p < 0.05$) (Table 2).

The lower ethanol production was obtained with the addition of peptone in whey, treatment T4, with only 2.57% (v/v), demonstrating not is a suitable supplement for the production of ethanol. However, Santos et al. (2013) obtained in synthetic glucose media supplemented with sucrose and 1% (w/v) peptone, favorable results for the production of ethanol.

Fermentations T2 and T5 showed a production of 5.07 and 5.43% more ethanol compared to the control, whose showed 5.52% (w/v). This demonstrates that the cheese whey nutrients are sufficient to ferment, and can be used as unsupplemented culture medium in fermentation and ethanol production by yeasts of the genus *Saccharomyces* (Santiago et al., 2004).

The nutrients of the environment are fundamental as they influence the development of yeast, affecting the speed of growth and multiplication, but the right concentration is important because it can inhibit the growth and sugar negatively influence the transformation efficiency of ethanol, when present in excess or insufficient amounts (Camili and Cabello, 2007; Silva, 2007; Sousa and Monteiro, 2011).

Kinetics fermentation

In fermentation kinetics at the laboratory scale, the production of ethanol, lactose consumption, cell growth and glucose content in the culture medium during 30 h of the fermentation were evaluated (Figure 1).

Ethanol production increased linearly from 8 to 18 h of fermentation at a rate of 0.50% h^{-1} ethanol ($R^2 = 0.994$), when it reached the highest concentration with 5.57% (v/v) ethanol. During this period all the lactose has been consumed at the rate of 0.80% lactose h^{-1} ($R^2 = 0.962$) (Figure 1). This indicates that, in laboratory scale the fermentation could be terminated at 18 h. No significant differences ($p < 0.05$) in ethanol concentration between 14 and 30 h fermentation with 3.48 to 4.12% (v/v), respectively was observed (Table 3).

The concentration of residual glucose remained constant during fermentation from 0.02 to 0.09% (v/v), indicating that the yeast hydrolyzes the amount of lactose required for metabolism to transform it into ethanol and carbon dioxide.

In cell multiplication, yeast had a logarithmic growth up to 10 h of fermentation, when reached 1.49×10^8 CFU/mL. The growth rate between 12 and 24 h were lower, reaching 2.1×10^8 CFU/mL. From 26 h of fermentation, the yeast began to decline phase reaching the end, after 30 h of fermentation 9×10^7 CFU/mL. Cell death was probably due to the absence of lactose in the

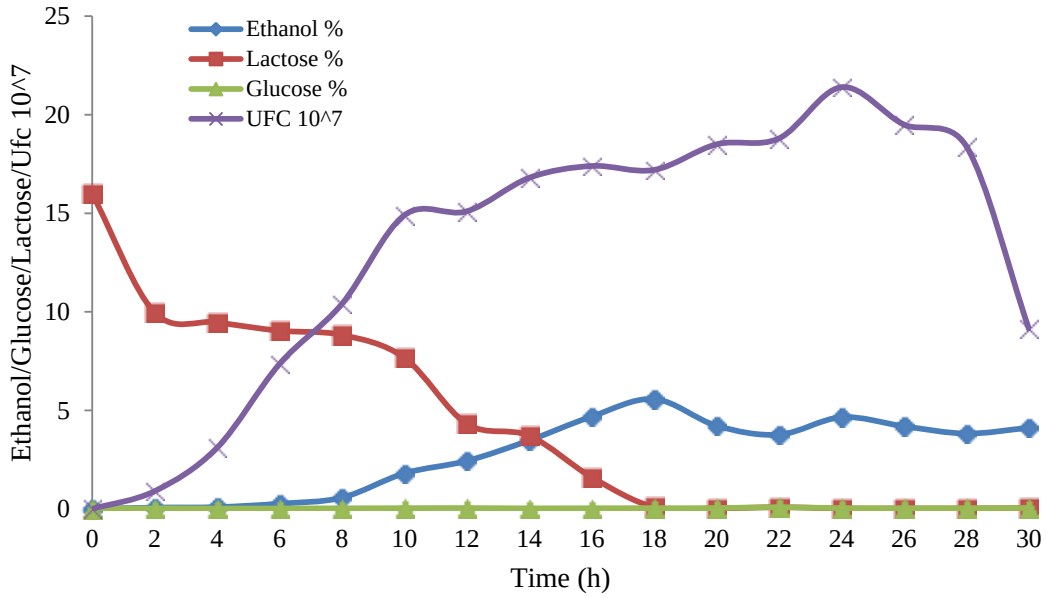

Figure 1. Kinetics of fermentation in cheese whey by SF IZ 275 in laboratory scale.

Table 3. Kinetic parameters of fermentation by SF IZ 275 in laboratory scale.

Time (h)	Ethanol (%)	Final lactose (%)	Final glicose (%)	UFC/mL 10^7
2	0.07±0.005^{e}	12.9±2.496^{a}	0.03±0.002^{abc}	0.9±0.000^{d}
4	0.09±0.014^{e}	9.47±0.460^{b}	0.02±0.001^{c}	3.1±0.071^{d}
6	0.26±0.030^{de}	9.04±1.104^{ab}	0.02±0.003^{bc}	7.35±0.354^{cd}
8	0.57±0013de	8.83±0.184^{ab}	0.03±0.000^{abc}	10.4±0.849^{bc}
10	1.81±0.106^{cd}	7.67±0.002^{b}	0.04±0.001^{abc}	14.9±1.768^{bc}
12	2.44±0.088^{bc}	4.32±0.505^{b}	0.04±0.023^{abc}	15.1±0.495^{bc}
14	3.48±0.307^{abc}	3.71^{c}±0.001	0.03±0.014^{abc}	16.8±1.697^{ab}
16	4.69±1.063^{ab}	1.61^{cd}±0.323	0.04±0.008^{abc}	17.4±1.273^{ab}
18	5.57±0.684^{a}	0.09^{d}±0.001	0.04±0.003^{abc}	17.2±4.101^{bc}
20	4.22±0.138^{a}	0.03^{d}±0.002	0.04±0.003^{abc}	18.5±0.884^{ab}
22	3.77±0.546^{a}	0.09^{d}±0.002	0.09±0.000^{a}	18.8±1.061^{ab}
24	4.65±0.146^{a}	0.02^{d}±0.003	0.04±0.002^{abc}	21.4±4.101^{a}
26	4.19±0.481^{ab}	0.02^{d}±0.001	0.04±0.002^{abc}	19.5±2.475^{ab}
28	3.82±0.592^{a}	0.02^{d}±0.002	0.04±0.001^{abc}	18.4±1.556^{ab}
30	4.12±0.085^{a}	0.05^{d}±0.034	0.05±0.006^{ab}	9.15±3.960^{bc}

^{a,b,c,D}Different lower case superscript within the same column indicate significant differences ($p < 0.05$) during the fermentation kinetics.

fermentation media, since after 18 h of fermentation, all lactose had been consumed by the yeast.

The same behavior was observed by Murari et al. (2013), which reported that in their fermentation ethanol production presents a decline as lactose will be consumed, and may yeast tolerate a concentration of 10.67% (v/v) ethanol. Thus, in most cases, reduction of cell proliferation is not only associated with higher ethanol concentration but the lack of nutrients.

In the fermentation kinetics in pilot scale, with 50 L of DCW, ethanol production is increased and linear up to 28 h of fermentation at a rate of 0.20% h^{-1} ethanol (R^2 = 0.984) when the concentration reached 5.66% (v/v) (Figure 2). There were no significant differences at 5% ($p < 0.05$), between 26, 28 and 30 h with 5.19, 5.66 and 5.40% (v/v) ethanol respectively (Table 3).

Although the ethanol production on a laboratory (5.57% - v/v) and pilot scale (5.66% - v/v) were similar, the

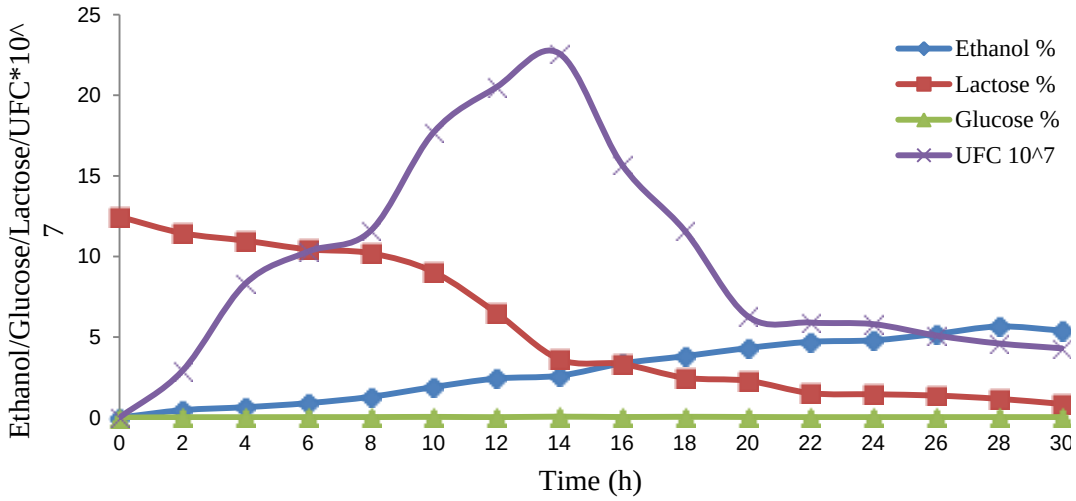

Figure 2. Kinetics of fermentation in cheese whey by SF IZ 275 in pilot scale.

Table 4. Kinetic parameters of fermentation by SF IZ 275 in laboratory scale.

Time (h)	Ethanol (%)	Final lactose (%)	Final glicose (%)	UFC/mL10^7
2	0.46±0.008^{k}	11.45±0.078^{a}	0.026±0.003^{d}	2.9±0.636^{e}
4	0.65±0.010^{jk}	10.98±0.092^{b}	0.028±0.001^{d}	8.3±0.071^{cde}
6	0.90±0.016^{jk}	10.44±0.071^{c}	0.032±0.004^{d}	10.3±1.41^{bcde}4
8	1.29±0.177^{ij}	10.20±0.064^{d}	0.033±0.001^{cd}	11.6±3.960^{bcd}
10	1.90±0.027^{hi}	9.04±0.078^{e}	0.047±0.004^{abc}	17.7±2.828^{ab}
12	2.43±0.010^{gh}	6.50±0.028^{f}	0.030±0.003^{d}	20.5±2.828^{a}
14	2.60±0.261^{g}	3.63±0.035^{g}	0.061±0.004^{a}	22.6±4.243^{a}
16	3.36^{f}±0.035	3.33±0.021^{h}	0.038±0.004^{bcd}	15.7±1.414^{abc}
18	3.81±0.088^{ef}	2.48±0.028^{i}	0.052±0.003^{ab}	11.6±1.273^{bcd}
20	4.32±0.097^{de}	2.29±0.049^{i}	0.040±0.005^{bcd}	6.3±0.849^{de}
22	4.70±0.146^{cd}	1.52±0.042^{j}	0.030±0.004^{d}	5.9±1.697^{de}
24	4.80±0.129^{cde}	1.45±0.007^{j}	0.031±0.003^{d}	5.8±2.121^{de}
26	5.19±0.330^{abc}	1.37±0.127^{jk}	0.032±0.004^{d}	5.1±1.061^{de}
28	5.66±0.052^{a}	1.17±0.007^{k}	0.032±0.005^{d}	4.6±1.414^{de}
30	5.40±0.432^{ab}	0.85±0.071^{l}	0.032±0.006^{d}	4.3±0.707^{e}

^{a,b,c,d}Different lower case superscript within the same column indicate significant differences ($p < 0.05$) during the fermentation kinetics.

fermentation time was significantly greater in pilot scale, with 28 h compared to 18 h laboratory scale.

The results obtained for ethanol production were higher in relation to the work performed by Murari et al. (2013), with the production of ethanol of 1.87% (v/v) obtained from 5.76% (w/v) of lactose after 10 h fermentation with *Kluyveromyces marxianus* 229. Dahiya and Vij (2012), obtained a yield of only 2.0 and 2.5% (v/v) and immobilized in the free state, respectively, after 72 h fermentation with the same yeast.

Lactose curve was descending at a rate of 0.27% h^{-1} (R^2 = 0.945) between 0 and 8 h. Between 8 and 14 h, the consumption of lactose was higher at a speed of 1.11% h^{-1} (R^2 = 0.969). This rapid consumption in this period is related to the increased cell growth. Between 14 and 30 h, the consumption of lactose was lower, with a speed of 0.17% h^{-1} (R^2 = 0.926). After 30 h of fermentation there was a residue of only 0.85% (v/v) of lactose. The glucose concentration was constant throughout fermentation, ranging from 0.026 to 0.061% (v/v) (Table 4), reproducing what has been observed in laboratory kinetics.

Cell growth was linear with 1.64 CFU/mL.h^{-1} (R^2 = 0.983) to 14 h of fermentation when reached 2.2 × 10^8 CFU/mL. After 16 h there was a decrease in yeast count 6.3 × 10^7 CFU/mL in 20 h of fermentation. At 22 h fermentation was smaller and the decline after 30 h of

incubation, counting was 4.3×10^7 CFU/mL (Table 4).

The fermentation process yields a series of compounds that may act as potential inhibitors. Among them is ethanol, a metabolite produced in greater amounts, which may be toxic after a certain concentration causing reduction in cell viability in yeast, due to stress caused by exposure thereof to ethanol (Silva et al., 2008), it causes changes in the composition of the lipid layer of the membrane, acting synergistically intoxicating cell yeast, leading to death, with a reduction in cell viability (Oliva Neto, 2006).

However, tolerance to different ethanol concentrations is not yet fully understood. Thus, the catalytic process of converting lactose in ethanol can be influenced by the concentration of lactose in the fermentation broth or ethanol produced can change the metabolism of the microorganism. However, the yeast *K. marxianus* has been employed successfully in research, proving to be potentially viable in the process of converting lactose to ethanol (Silveira et al., 2005).

Severe conditions during fermentation, such as high alcohol content, high osmotic pressure of the substrate and strong inhibition of ethanol for the production phase, may cause the loss of cell viability with consequent cell death, and increased the fermentation period (Christensen et al., 2011; Li et al., 2009; Stanley et al., 2010).

Over 10% of ethanol may result in a decrease in efficiency in yeast fermentations, the denaturing, reducing ethanol production due to the dissolution of the cell plasma membrane, while a content of below 7% impairs the fermentation yield (Sousa and Monteiro, 2011; Silva et al., 2008; Silva et al., 2003).

Fermentation is affected by factors other than the high ethanol concentration, such as substrate inhibition and salt concentration being reduced by an increase of dead cells, shortage of nutrients, low water activity, and accumulation of polysaccharides and other macro-molecules, or undesirable loss of oxygen in the fermenter (Ezeji et al., 2004; Kumar and Gayen, 2011; Maddox, 1989).

Conclusion

Supplementation with yeast extract or ammonium sulfate in the fermentation of cheese whey with *S. fragilis* IZ 275 provided the largest ethanol production, with 5.80 and 5.82% respectively. However, due to the high availability of nutrients in the whey, supplementation with all the nutrients sources tested together have not shown up before the advantageous results compared with the control. The kinetics on laboratory and pilot scale showed similarities in ethanol production, however, the fermentation in a pilot scale required a longer fermentation. Thus, it can be seen that yeasts of the genus *Saccharomyces* are capable of fermenting deproteinized cheese whey in a pilot scale, showing possibilities of use on an industrial scale, presenting as a way to reduce the pollution potential of this byproduct and at the same time to obtain value-added product.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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REFERENCES

Camili EA, Cabello C (2007). Produção de etanol de manipueira tratada com processo de flotação. São Paulo.

Christensen AD, Kádár Z, Oleskowicz-Popiel P, Thomsen MH (2011). Production of bioethanol from organic whey using *Kluyveromyces marxianus*. J. Ind. Microbiol. Biotechnol. 2(38):283-289.

Dagbagli S, Goksungur Y (2008). Optimization of β-galactosidase production using *Kluyveromyces lactis* NRRL Y-8279 by response surface methodology. Electron. J. Biotechnol. 11:11-12.

Dahiya M, Vij S (2012). Comparative analysis of bioethanol production from whey by diferente strains of immobilized thermotolerant yeast. Int. J. Sci. Res. Pub. 2(3):1-5.

Domingues L, Guimarães PMR, Oliveira C (2010). Metabolic engineering of *Saccharomyces cerevisiae* for lactose/whey fermentation. Bioeng. Bugs 1(3):164-171.

Ezeji TC, Qureshi N, Blaschek HP (2004). Acetone butanol ethanol (ABE) production from concentrated substrate: reduction in substrate inhibition by fed-batch technique and product inhibition by gas stripping. Appl. Microbiol. Biotechnol. 63(6):653-658.

Fukuhara H (2006). *Kluyveromyces lactis*: a retrospective. FEMS Yeast Res. 6(3):323-324.

International Dairy Federation (1990). Milk and Milk products: Enumeration of yeast e moulds. FIL-IDF Standard, n. 94B, Brussels, Belgium.

Kaye S, Haag HB (1954). Determination of ethyl alcohol in blood. J. Forensic Sci. 1(6):373-381.

Kumar M, Gayen K (2011). Developments in biobutanol production: New insights. Appl. Energy 88(6):1999-2012.

Li F, Zhao XQ, Ge XM, Bai FW(2009). An innovative consecutive batch fermentation process for very high gravity ethanol fermentation with self-flocculating yeast. Appl. Microbiol. Biotechnol. 84(6):1079-1086.

Llorente B, Malpertuy A, Blandin G, Artiguenave F, Wincker P, Dujon B (2000). Genomic exploration of the hemiascomycetous yeasts: 12. *Kluyveromyces marxianus* var. *marxianus*. FEBS Lett. 487:71-75.

Maddox IS (1989). The acetone–butanol–ethanol fermentation: recent progress in technology. Biotechnol. Genet. Eng. Rev. 7(1):189-220.

Marwaha SS, Kennedy JF (1988). Review: Whey pollution problem and potential utilization. Int. J. Food Sci. Technol. 23:323-336.

Murari CS, Moraes DC, Bueno GF, Del Bianchi VL (2013). Avaliação da redução na poluição dos laticínios, a partir da fermentação do soro de leite em etanol pela levedura K*luyveromyces marxianus* 229. Rev. Inst. Latic. Cândido Tostes. 68(393):42-50.

Nickerson TA, Vujicic IF, Lin AY (1975). Colorimetric estimation of lactose and hydrolytic products. Department of Food Science and Technology. J. Dairy Sci. 59(3):386-390.

Oliva Neto P (2006). Efeito de Fatores Inibidores na Fermentação Alcoólica. Produção de Etanol: qualidade da matéria-prima. Faculdade de Ciências e Letras de Assis – UNESP, São Paulo.

Ozmihci S, Kargi F (2007). Kinetics of batch ethanol fermentation of

cheese-whey powder (CWP) solution as function of substrate and yeast concentrations. Bioresour. Technol. 98:2978-2984.
Ozmihci S, Kargi F (2008). Ethanol production from cheese whey powder solution in a packed column bioreactor at different hydraulic residence times. Biochem. Eng. J. 42:180-185.
Panesar PS, Kennedy JF (2012). Biotechnological approaches for the value addition of whey. Crit. Rev. Biotechnol. 32:327-348.
Panesar PS, Kennedy JF, Gandhi DN, Bunko K (2007). Bio-utilisation of whey for lactic acid production. Food Chem. 105(1):1-14.
Santiago PA, Marquez LDS, Cardoso VL, Ribeiro EJ (2004). Estudo da produção de β-galactosidase por fermentação de soro de queijo com *Kluyveromyces marxianus*. Ciênc. Tecnol. Aliment. 24(4):567-572.
Santos EFS, Schautz LCA, Cardoso CAL, Ernandes JR, Batistote M (2013). O efeito da complexidade estrutural da fonte de carbono e nitrogênio no desempenho fermentativo de leveduras industriais. Ciênc. Nat. 35(2):9-14.
Schaffrath R, Breunig KD (2000). Genetics and molecular physiology of the yeast *Kluyveromyces lactis*. Fungal Genet. Biol. 30:173-190.
Silva GC, Mata CMREM, Braga DEM, Queiroz SV (2003). Extração e fermentação do caldo da algaroba (*Prosopis juliflora*) para produção de aguardente. Revista brasileira de produtos agroindustriais 5(1):51-56.
Silva JA, Damasceno BPGL, Silva FLH, Madruga MS, Santana DP (2008). Aplicação da metodologia de planejamento fatorial e análise de superfícies de resposta para otimização da fermentação alcoólica. Quim. Nova 31(5):1073-1077.
Silva JS (2007). Produção de álcool combustível na fazenda e em sistema cooperativo. Viçosa.
Silveira WB, Passos FJV, Mantovani HC, Passos FML (2005). Ethanol production from cheese whey permeate by *Kluyveromyces marxianus* UFV-3: A flux analysis of oxido-reductive metabolismo as a function of lactose concentration and oxygen levels. Enzyme Microb. Technol. 36(7):930-936.
Siso MIG (1996). The biotechnological utilization of cheese whey: a review. Bioresour. Technol. 57:1-11.
Sousa JLU, Monteiro RAB (2011). Fatores interferentes na fermentação alcoólica para produção de etanol. Cadernos de Pós-Graduação da Fazu. 2.
Spalatelu C (2012). Biotechnological valorisation of whey. Innov. Rom. Food Biotechnol. 10:1-8.
Stanley D, Bandara A, Fraser S, Chambers PJ, Stanley GA (2010). The ethanol stress response and ethanol tolerance of *Saccharomyces cerevisiae*. J. Appl. Microbiol. 109(1):13-24.
Wissmann MA, Hein AF, Follmann J, Rachow NIP (2012). Environmental costs: analysis of its impact and importance in the pursuit of eco-efficiency in an industry of cheese. Cust. Agronegocio 8(3):2-23.

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Full Length Research Paper

Adoption of pelleted *Digitalia iburua* grain as carrier for heat stable Newcastle disease vaccine virus for village poultry

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The development of pelleted food–based vaccine against Newcastle disease (ND) using a thermostable $NDVI_2$ strain in Acha (*Digitalia iburua)* is reported. The Acha cereal was subjected to rigorous processing of washing, soaking, boiling, roasting and grinding into fine powder prior to virus incorporation. The mixture which was passed through a locally fabricated pelleting machine resulted in fine pellets. The virus content in the pellets determined by inoculation of susceptible embryonated chicken eggs showed appreciable titres above 10^8 Egg Infective Dose 50% end points (EID_{50}) per g of feed. These titres appear to be higher than the recommended minimum immunizing dose of $10^{5.5}$ EID_{50} per ml of vaccine. Further *in-vitro* and *in-vivo* assessment of the pelleted vaccine is advocated.

Key words: Pelleted, $NDVI_2$, food vaccine, *Digitalia iburua*, high virus titres.

INTRODUCTION

Newcastle disease (ND) is endemic in poultry in Nigeria (Abdu et al., 1992; Sa'idu et al., 1994; Halle et al., 1999). The maintenance of the causative virus in the country is largely by the scavenging poultry in villages, which act as reservoirs for themselves and the more susceptible exotic flocks in commercial farms (Gomwalk et al., 1985).

The most cost effective control strategy for ND is by vaccination (Lurthu Reetha et al., 2016). Current conventional vaccines in use are formulated in multiple dose units targeted towards commercial flocks. Such vaccines have little relevance in village poultry which are often small, scattered, multi-aged, and free- roaming with

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Table 1. Proximate analysis of untreated Acha (*Digitaria iburua*).

Moisture	Crude protein (g/100 g of sample)	Crude fibre (g/100 g of sample)	Crude fat (g/100 g of sample)	Ash (g/100 g of sample)	NFE (g/100 g of sample)	Calcium (mg/100 g of sample)	Phosphorous (g/100 g of sample)
Raw Acha	4.60	10.49	7.23	2.88	14.15	65.25	0.14

NFE, Nitrogen – free extract.

Table 2. Phytochemical analysis of untreated Acha (*Digitaria iburua*).

Phytochemical analysis	Phytic acid (mg/ 100 g of sample)	Oxalate (mg/100 g of sample)	Tanins (mg/100 g of sample)
Raw Acha	49.34	20.00	0.827

minimal control.

A viable solution to this problem is the formulation of pelleted feed – based vaccine using a thermostable virus strain in small doses. The search for a suitable feed material is a continuous one. This has been attempted in several countries including Nigeria with limited successes (Iroegbu and Nchinda, 1999; Wambura et al., 2007; Echeonwu et al., 2008).

In the present study, Acha (*Digitalia iburua*), a protein–rich cereal obtained from Jos plateau in central Nigeria, was processed and used as a carrier of heat stable $NDVI_2$ vaccine virus. The vaccine-incorporated carrier was pelleted and the virus content assessed for virus viability in susceptible embryonated chicken eggs.

MATERIAL AND METHODS

Proximate and phytochemical analysis of Acha (*D. iburua*) grains

The untreated Acha grains were subjected to proximate and phytochemical analysis using 100 g of sample according to the method of the Association of official analytical chemists (AOAC, 1990).

Treatment of Acha grains

Approximately, 1 kg of Acha grains was weighed out, washed in clean water, sieved of sand particles and soaked for 24 h. Thereafter, the soaked Acha was re-washed and per-boiled for 10 min with continues stirring during the boiling process. Per- boiled Acha was then air – dried for 10 min, at room temperature in aluminum pans.

The air- dried grains were spread in an oven (100°C) to roast for 4 h. The roasted grains were grinded to a fine powder using a manual hand blender. Once this process was completed, the Acha was ready for vaccine incorporation.

Preparation of pelleted vaccine

The thermostable $NDVI_2$ vaccine stock was obtained from the virus vaccine production division of the National Veterinary Research Institute, Vom, Nigeria. The vaccine virus was propagated and titrated in 10 days – old embryonated chicken eggs, according to standard methods (OIE, 2013).The egg infective dose 50% end point (EID_{50}) was calculated using the Karber formular (Muthannan, 2016).

The prepared $NDVI_2$ vaccine, consisting of a minimum titre of $10^{8.5}$ EID_{50} per ml was taken unto a sterile conical flask. To this was added 100 ml peptone consisting of 2,180 ml wet virus harvest water, 100 ml antibiotics made up to five times the normal working strength of penicillin, streptomycin, gentamycin, and amphotericin B (5xPSGA). This virus / antibiotic / antifungal mixture was added unto 2000 g of processed Acha powder in a glass trough. The mixture was allowed to stand for 8 min for adsorption to take place. The adsorbed mixture was placed in a pelleting chamber of a locally fabricated pelleting machine. The resultant pellets were stored at -80°C.

Titration of pelleted feed vaccine virus

Each batch of the pelleted feed vaccine virus was titrated as follows: 1 g of the pellets was weighed out and placed in each of three sterile universal bottles containing 9 ml of 5xPSGA and votexed. The votexing was repeated at 15 min intervals for 1 h to dissolve the pellets.

Similarly, 1 ml of wet $NDVI_2$ virus only was added to 9 ml of 5xPSGA as control. Thereafter, 0.5 ml of the feed pellet suspension was added to 4.5 ml phosphate buffered saline (PBS) to make a 1:10 feed/ virus suspension. The virus suspension was titrated in embryonated chicken eggs as earlier described (OIE, 2013).

RESULTS

The result of proximate analysis of untreated Acha (*D. iburua*) showed that it contained the following per 100 g of grains (Table 1): Crude protein 4.6 g; crude fiber 10.49 g; crude fat 7.23 g; ash 2.88 g; NFE 14.15 g; Ca 65.25 mg; and P 0.14 g. The following phytochemical compounds were also detected per 100 g Acha grains (Table 2): Phytic acid 49.34 mg; Oxalate 20.00 mg; and Tannins 0.827 mg.

The results of virus concentrations in pelleted feed

Table 3. Results of virus concentrations in $NDVI_2$ pelleted feed vaccine batches.

Vaccine batch	Pellet set	End point titres (log_{10}EID50)	Mean titres
1	A	8.45	-
1	B	8.50	8.48
2	A	8.10	-
2	B	8.50	8.30

showed a range between $10^{8.1}$ EID_{50} and $10^{8.5}$ EID_{50} per g of feed (Table 3). The positive $NDVI_2$ (only) controls for batches A and B were $10^{8.8}$ EID_{50} per ml and $10^{8.30}$ EID_{50} per ml, respectively.

DISCUSSION

The development of a pelleted $NDVI_2$ vaccine is to provide a viable alternative for the immunization and protection of the scavenging (village) poultry flocks against the most dreaded disease, the ND. This need arises from the fact that, village poultry accounts for approximately 84% of the entire poultry population in Nigeria (Sonaiya, 2007), contributing between 68.5 and 72.7% national poultry meat and egg supply, respectively (David-West, 1972). These birds serve as economic, social, ritual, pest control, and waste disposal functions as well as sources of organic fertilizer for rural farmers.

Village poultry has the potential of providing bulk of the much needed animal protein, for majority of Nigerians who live in rural areas. The productivity of village poultry is constrained by annual outbreaks of ND among others. ND is the most important infectious disease affecting village poultry with mortality rates of up to 90% in susceptible flocks (Janviriyasopak et al., 1989; Cumming, 1992; Echeonwu et al., 1993).

Immunization of this group of birds using feed - based thermostable virus vaccine has been advocated as a viable alternative for their protection. The selection and use of $NDVI_2$ virus vaccine in this study is based on its thermostability profile reported previously (Ibu et al., 2009; Guoyuan et al., 2016).

The formulation and maintenance of viable virus content in infected feed pellets for easy vaccine administration has been the greatest challenge to this immunization alternative. This challenge occurs due to the presence of anti-viral elements inherent in the grains as a natural defensive strategy (Egbuna and Ifemeje, 2015). Some of these antiviral elements as contained in the phytochemical analysis of *D. iburua* (Acha) grain elicited the rigorous processing of the grains, to reduce their concentrations prior to incorporation of the vaccine virus.

The determination of virus content in the treated Acha grain is an effective way of assessing the concentration and viable virus retention. The Egg Infective Dose 50% end point (EID_{50}) virus concentration calculated for the pellets showed appreciable viral titre retention in the feed.

A mean virus titre of $10^{8.10}$ to $10^{8.50}$ EID_{50} per g of feed obtained in batches 1 and 2 pelleted vaccine obtained herewith, seems to exceed the minimum immunizing dose threshold of $10^{5.5}$$EID_{50}$ per ml required to vaccinate birds to achieve protection (OIE, 2013).

Conclusion

Further work is required for quality assessment of this pelleted vaccine *in-vitro* and *in-vivo* to determine potency, safety, shelve life, as well as challenge studies among other requirements prior to recommendation for its use.

It should also be noted that the use of a simple locally fabricated easily reproducible pelleting machine used in this work, enables this process to be assessible to less sophisticated laboratories in the third world.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Abdu PA, Mera UM, Saidu L (1992). A study of chicken mortality in Zaria,Nigeria. Proceedings of National Workshop on Livestock and Veterinary Services, Vom, Nigeria, Pp. 51-55.

AOAC (1990). Association of official analytical chemists, Official methods of analysis of the Association of Official Analytical Chemists, 15th edition. Washinton DC. Suite 400 2200 Wilson Boulevard Arlington, Virginia 22201 USA.

Cumming RB (1992). Newcastle disease research at the University of New England. Newcastle Disease in Village chickens. ACIAR. Proc. No. 39, Canberra, Australia 84-85.

David-West KB (1972). Newcastle Disease in Nigeria: Retrospection

and Anticipation. Bull. Epiz. Dis. Afr. 20:291.
Echeonwu BC, Ngele MB, Echeonwu GON, Joannis TM, Onovoh EM (2008). Response of chickens to oral vaccination with Newcastle disease virus vaccine strain I2 coated on maize offal. Afr. J. Biotechnol. 7:1594-1599.
Echeonwu GON, Iroegbu GB, Amerua AC (1993). Recovery of velogenic Newcastle disease virus fromdead and healthy free roaming birds in Nigeria. Avian Pathol. 22:383-387.
Egbuna C, Ifemeje JC (2015). Biological Functions and Anti-nutritional Effects of Phytochemicals in Living System. J. Pharm. Biol. Sci. 10(2):10-19.
Gomwalk NE, Adesiyun JT, Bishu G, Adesiyun AA (1985). A serological survey of Newcastle disease virus in domestic poultry around Zaria. Nig. Vet. J. 14(1):70.
Guoyuan W, Xiao H, Kang Z, Hongling W, Zhenyu Z, Tengfei Z, Jinlong Y, Qingping L, Rongrong Z, Zishu P, Huabin S, and Qingzhong Y (2016). Molecular basis for the thermostability of Newcastle disease virus. Sci. Rep. 6:22492.
Halle PD, Umoh JU, Saidu L, Abdu PA (1999). Prevalence and seasonality of Newcastle disease in Zaria Nigeria. Trop. Vet. 17:53-62.
Ibu JO, Okoye JOA, Baba SS, Soyinka SVO, Chah KF, Antiabong J, Eze D, Salihu E, Oladele SB (2009). Thermostability profile of Newcastle disease viruses isolated from wild birds in central Nigeria and the selection of a thermostable clone from the subpopulation. Inter. J. Poult. Sci. 9(8):791-794.
Iroegbu CU, Nchinda GW (1999). Evaluation of cassava feed for oral delivery of Newcastle disease V4 vaccine. Bull. Anim. Prod. Afr. 47:155-161.
Janviriyasopak O, Thitisak W, Thepkraiwan L, Jongsathlen KM, Ekapratheep M, Kruedener R, Morris RS (1989). A health and productivity study of village poultry. In. Proceedings, International Seminar on Animal Health and Production Services for Village Livestock, KhonKaen,Thailand, 2-9 August 1989. pp. 163-171.
Muthannan AR (2016). Determination of 50% endpoint titre using a simple formular. World J. Virol. 5(2):85-86.
OIE (2013). Office of International Epizootics – Terrestrial Manual of Standards.
Sa'idu L, Abdu PA, Umoh JU, Abdullahi US (1994). Diseases of Nigerian indigenous chickens. Bull. Anim. Health Prod. Afr. 42:19-23.
Sonaiya EB (2007). Family poultry, food security and the impact of HPAI. Worlds Poult. Sci. J. 63:132-138
Wambura PN, Meers J, Spradbrow P (2007). Survival of avirulent thermostable Newcastle disease virus (strain I-2) in raw, baked, oiled, and cooked white rice at ambient temperatures. J. Vet. Sci. 8:303-308.
Lurthu Reetha T, Johnson Rajeswar J, Harikrishnan TJ, Sukumar K, Srinivasan P, John Kirubakaran J (2016). Studies on the effectiveness of oral pellet vaccine in improving egg production and egg quality in desi chicken. Vet World 9(8):900-903.

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Full Length Research Paper

Aroma characterization of ripe date fruits (*Phoenix dactylifera* L.) from Algeria

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The headspace of eight Algerian date varieties with low market value were analyzed for their aroma compounds using solid phase micro extraction and gas chromatography combined with mass spectrometry. In this study, 61 identified compounds were categorized in various chemical classes on the basis of their functional groups, alcohols, esters, aldehydes, terpenoids, ketones, hydrocarbons, and ethers. Twenty specific volatiles were found to be representative of a single variety and four shared molecules were exclusively observed in all the studied dates. Some dates such as Bent Qbala, Litima, and Timjouhart were statistically different from the other varieties which presented on the contrary a significant similarity between them. In the present study, forty eight new volatile compounds were identified which could be useful for the characterization of the Algerian dates.

Key words: Date fruit, Algerian varieties, aroma, solid phase microextraction.

INTRODUCTION

The date (*Phoenix dactylifera* L.) is the most important agricultural product in arid regions, such as Southern Algeria, seeing its socio-economic value for the populations of oases. Several studies have shown the antioxidant activity of date (Benmeddour et al., 2013; Mansouri et al., 2005) and its technological aptitude for the manufacture of different products such as syrup, vinegar, etc. (Belguedj et al., 2015; Benamara et al., 2008). Date fruit has higher sensory quality which is due to its wonderful flavour. This characteristic is one of the critical point for consumer's acceptability and it draws the scientist and the investor attention. Consequently, it is important to determine molecules that constitute it (Biniecka and Caroli, 2011). About 360 compounds were detected in strawberry flavour, more than 600 in coffee and around 850 in wine (Crouzet, 1998). These

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compounds belong to many chemical classes (Crouzet, 1998). Moreover, the climatic conditions such as sunlight and agricultural practices can influence the harvested product flavour (El Hadi et al., 2013). Likewise, some researchers showed the environment effect on raspberry aroma (Moore et al., 2002).

Only a few workers reported the volatile compounds of dates. Jaddou et al. (1984) studied the variety of Iraki Zahdi. Reynes et al. (1996) analyzed the volatiles of Tunisian varieties: Alligh, Deglet Nour, and Kentichi. Harrak et al. (2005) identified the volatiles of some Moroccan varieties such as Aziza, Boufeggous, Bouskri, Bousthammi Noire, Iklane, Jihel, Mejhoul, and Najda, and they detected 47 volatile compounds. These studies showed a great difference in the volatile composition of dates.

In Algeria, there are many varieties of dates which differ in colour, morphology, flavor, and geographical distribution which made them different from the dates of other countries.

The majority of date varieties suffer each season from very important crop loss, because of limited marketing of these dates. Their crop is destined for animal feeds with low prices causing huge economic losses for the farmers. These conditions push the scientist to search new opportunities to use and transform these date varieties into high-value products to valorize them. Fruit aroma is in much demand in food industry for aromatized products. Dates aroma can add to new flavour for the dairy product processors, because in Algeria the dates are often eaten with milk or fermented milk.

To the best of our knowledge, all the researches carried out on the dates from Algeria studied their physico-chemical composition and their potential for transformation into various products; no previous research was performed on the volatile compounds of Algerian dates which are different from others in the geographical location and organoleptic characteristics. Therefore, the aim of this study is to identify, for the first time, the aroma compounds of eight varieties of Algerian date palm fruit that have a low market value so as to characterize their flavour. This work can be of interest to processors to produce processed products based on dates or flavour extracts from low market varieties to valorize them. The determination of these chemicals was carried out using a recent method, solid phase microextraction (SPME) to sample the headspace around the fruits, and gas chromatography/mass spectrometry (GC/MS) for separation and identification of the sampled compounds.

MATERIALS AND METHODS

Collection and storage of date samples

Eight varieties of date palm fruit (*Phoenix dactylifera* L.) were collected according to their availability during the 2013 harvest season in three regions of Southern Algeria as follows: Biskra region: Ghars, Litima, Houbales and Hamraia; El Oued region: Tinicine and Tantbouchet; Ghardaia region: Timjouhart and Bent Qbala.

These regions are characterized by arid climate. The mean temperature is 21.8, 21.0 and 21.8°C in Biskra, Ghardaia and El Oued, respectively. The rainfall remains always limited; 141 mm in Biskra, 68 mm in Ghardaia, and 74 mm in El Oued.

Ripe fruits free of defects and without any disinfestation or other treatment were stored at -20°C in glass bottles, immediately after harvesting until analysis. The maturation of dates is empirically determined by date palm farmers based on date characteristics such as: size and shape, skin colour, flesh colour, and flesh firmness.

Aroma compounds analysis

A small quantity of date flesh (5 g) was put into a 25-ml glass vial. After the equilibration time for 30 min, the headspace of date flesh was sampled by Supelco (Bellefonte, PA, USA) SPME devices which were coated with polydimethylsiloxane (PDMS, 100 µm). SPME sampling was performed using the same new fibre, preconditioned according to the manufacturer instructions, for all the analyses. Sampling was accomplished in an air-conditioned room (22±1°C) to guarantee a stable temperature.

After the equilibration time, the fibre was exposed to the headspace for 50 min. Once sampling was finished, the fibre was withdrawn into the needle and transferred to the injection port of the GC-MS system. All the SPME sampling and desorption conditions were identical for all the samples. Furthermore, blanks were performed before each first SPME extraction and randomly repeated during each series. Quantitative comparisons of relative peaks areas were performed between the same chemicals in the different samples.

GC-Electron Impact Mass Spectrometry analyses were performed with a Varian (Palo Alto, CA, USA) CP 3800 gas chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm × 0.25 µm; Agilent, Santa Clara, CA, USA) and a Varian Saturn 2 000 ion trap mass detector.

The analytical conditions were as follows: injector and transfer line temperatures were 220 and 240°C, respectively; oven temperature was programmed from 60 to 240°C at 3°C min^{-1}; helium at 1 mL min^{-1} was used as the carrier gas; splitless injection.

Aroma compounds identification

The identification of the constituents was based on a comparison of the retention times with those of authentic samples, comparing their linear retention indices (LRI) relative to a series of *n*-hydrocarbons, and on computer matching against commercial (NIST, 2000; Adams, 2007) and home-made library mass spectra and MS literature data (Stenhagen et al., 1974; Adams, 2007). The home-made library was implemented using the function of the NIST software using both measurements from pure compounds or known mixtures subjected to GC-MS analysis.

Statistical analysis of data

The results were analyzed using Minitab software (Minitab® version 16, Minitab Ltd, United Kingdom). To study the similarities between the date varieties, a hierarchical clustering of date varieties was performed on the basis of the presence or absence of number of aroma compound in each variety of date. A factorial correspondence analysis (FCA) was executed to reveal relationships that would not be detected in the varieties of pairs of hierarchical clustering.

RESULTS AND DISCUSSION

Aromatic profile of date samples

The headspace analyses of the eight Algerian date varieties permitted to characterize 61 volatile compounds (Table 1) among them 48 specific compounds were identified only in these Algerian date varieties and could characterize them. The volatiles can be sorted into eight chemical classes: alcohols, esters, aldehydes, terpenoids, ketones, saturated hydrocarbons, unsaturated hydrocarbons, and ethers. Comparison with previous studies of Jaddou et al. (1984) and Reynes et al. (1996) revealed that some similarities were observed with our date samples (Table 1; footnotes c,d, and e): four aldehydes, two saturated hydrocarbons, three alcohols, two ketones, one terpenoid and one ester, that is, hexanal, octanal, nonanal, decanal, *n*-hexadecane, *n*-heptadecane, 1-hexanol, 1-octen-3-ol, 1-octanol, 6-methyl-5-hepten-2-one, 2-undecanone, limonene, and ethyl acetate. None of unsaturated hydrocarbons and ethers which were identified in the volatile profile of Algerian dates were found in the precedent studies.

Analysis of aroma compounds of Algerian date (Table 2) showed that there were only four shared compounds in all the studied varieties: ethyl acetate, nonanal, decanal, and (*E*)-geranyl acetone. Other shared volatiles by seven varieties (Table 1) were isopentyl alcohol, 2,3-butandiol, 6-methyl-5-hepten-2-one, *n*-tetradecane, *n*-pentadecane and *n*-hexadecane. If we consider only six varieties, the shared compounds extend also to octanal, (*E*)-2-nonen-1-ol, and dodecanal. Some volatiles were only identified in two varieties, such as 2-pentyl furan, ethyl hexanoate, limonene, phenylethyl alcohol, 1-nonanol, 1-decanol, ethyl nonanoate, (*Z*)-2-tridecene, 1-pentadecene, methyl dodecanoate, methyl tetradecanoate and ethyl tetradecanoate. The difference in volatile composition of varieties may be due to the presence or absence of precursors and their content as well as to maturation conditions, which controls the biosynthesis of aroma compounds. Yu et al. (2017) found the candidate genes in the biosynthesis of fruit aroma, notably terpenoid molecules. Volatile compounds of fruit aroma were derived from various molecules including phytonutrients such as carotenoids, phenols, fatty acids, terpenoids and amino acids (Goff and Klee, 2006). β-Ionone and β-cyclocitral resulting from the degradation of β-carotene and lycopene during maturation stage (Crouzet, 1998). Saturated hydrocarbons may be produced from lipids. The mechanism of alcohols formation may involve the decomposition of hydroperoxides of the unsaturated fatty acids and some may also form the reduction of carbonyl compounds which are present in date flavour (Jaddou et al., 1984). Volatile esters are formed by esterification of alcohols by alcohol acetyltransferase, normally using a CoA moiety or CoA-ester as the acyl donor during the ripening of many fruits (Beaulieu, 2006). Ethyl acetate was also formed from carbohydrate fermentation by microorganisms. Aldehydes arise from the enzymatic degradation of lipid and/or are produced from free fatty acids, such as linoleic and linolenic acids, via the lipoxygenase activity of amino acids such as acetaldehydes from alanine (Grechkin et al., 2006). Furthermore, some of these compounds may be produced by the plant as a response to different biotic and abiotic stress in the growth habitat. A number of shared compounds were noted between Tinicine and Hamraia and between Tinicine and Litima (19 in both cases).

Moreover, twenty specific aroma compounds (Table 2) were identified; 1-butanol in Tantbouchet, 2-octen-1-ol, 1-octanol, methyl octanoate, 2-ethyl-3-hydroxyethyl 2-methylpropanoate, (*Z*)-2-octenal, (*E*)-2-octenal, 2-undecanone and (*E*)-β-ionone in Litima, isopentyl acetate, ethyl heptanoate, ethyl dodecanoate, 2-phenylethyl acetate and styrene in Bent Qbala, (*Z*)-4-heptenal in Timjouhart, (*E*)-2-nonenal and 2-methyl tetradecane in Hamraia, (*E*)-2-decenal and *n*-dodecane in Ghars, *cis-threo*-davanafuran in Tinicine. It was clear that Litima and Bent Qbala were the richest varieties in specific volatile compounds (8 and 5, respectively). These results may be due to the presence of enzymes and precursors responsible for the biosynthesis of specific aroma compounds in these varieties. This explains the clear separation of these varieties observed in the dendrogram (Figure 3).

Chemical classes of volatile compounds

The molecules responsible for the flavour consist of a hydrocarbon skeleton which can be linear, cyclic or aromatic. Almost all the chemical functions carried by these chains are represented: alcohols, aldehydes, esters, ethers, phenols, sulphur derivatives, and heterocycles (Fernandez and Cabrol-Bass, 2007).

The total amounts of the different chemical classes of volatile compounds identified in the eight varieties of Algerian dates are as shown in Figure 1. It is apparent that the Bent Qbala variety had the high amount of alcohols and esters, while the Tantbouchet variety was distinguished by the high amount of aldehydes. Important levels of terpenoids and ketones were evidenced in the Ghars variety, while hydrocarbons were essentially observed in Hamraia one. Finally, the Tinicine variety was characterized by ethers.

The Hierarchical Cluster Analysis (Figure 2), revealed the existence of two varieties clusters (Distance = 0.53). The first one was formed by Ghars, Hamraia, Houbales, Tinicine and Tantbouchet, whereas Litima, Timjouhart and Bent Qbala contribute to the second cluster. A great similarity was noted between Ghars and Hamraia varieties, which was rich in alcohols, aldehydes and saturated hydrocarbons. Analogously, Litima and

Table 1. Major and minor aroma compounds[a] of Algerian date varieties and its linear retention indices and detection threshold of some compounds.

Compound	l.r.i.[b]	BQ	GH	HA	HO	TAN	LI	TIN	TIM	Average	Detection threshold (µg/kg)[f]
Major volatiles											
(E)-Geranylacetone	1455	1.0	40.1	31.6	27.2	18.4	13.1	30.1	14.9	22.05	-
Ethyl acetate[e]	614	22.7	10.8	7.3	14.2	6.3	7.8	4.6	18.2	11.49	8.5
Isopentyl alcohol	763	29.3	-	3.3	6.3	5.8	11.6	12.0	9.8	9.76	-
Decanal[d]	1206	0.3	11.3	12.0	10	11.9	4.4	9.8	10.8	8.81	-
2-Propanol	516	27.1	-	-	3.3	-	14.4	-	19.3	8.01	-
Minor volatiles											
6-Methyl-5-hepten-2-one [d.e]	987	-	10.3	2.5	6.5	5.7	2.6	5.5	3.2	4.54	-
Nonanal[c.d.e]	1102	0.2	4.9	3.7	6.0	9.8	1.8	5.4	4.3	4.51	-
2,3-Butandiol	789	0.2	2.7	3.5	4.2	2.7	0.8	2.8	-	2.11	-
n-Tetradecane	1400	-	1.0	4.4	1.6	3.3	1.4	2.4	1.2	1.91	-
Undecanal	1308	-	2.1	1.5	-	5.3	-	1.9	1.0	1.48	-
n-Pentadecane	1500	-	0.5	4.0	1.7	2.1	1.8	0.6	0.7	1.43	-
Octanal[c.d.e]	1003	0.3	1.0	0.7	0.6	5.6	-	-	0.8	1.13	-
n-Hexadecane [c]	1600	-	0.8	0.9	1.1	2.2	0.8	2.3	0.5	1.08	-
Ethyl decanoate	1395	3.7	-	-	-	-	2.1	0.7	1.5	1.00	-
Ethyl octanoate	1195	3.6	-	-	-	-	1.7	-	2.6	0.99	-
(E)-2-nonen-1-ol	1171	-	1.3	1.3	1.0	1.5	-	1.1	1.4	0.95	-
1-Octen-3-ol[d]	980	-	0.7	-	-	1.6	2.5	2.1	-	0.86	1.4 - 10
1-Hexanol[d.e]	873	0.4	-	0.5	-	-	3.9	-	1.5	0.79	2.5
n-Heptadecane[c]	1700	-	0.9	1.6	0.8	-	1.3	1.7	-	0.79	-
3-Ethyl-1-hexanol	1033	-	-	0.5	0.8	3.9	-	0.6	-	0.73	-
Phenylethyl alcohol	1110	2.9	-	-	2.9	-	-	-	-	0.73	-
Dodecanal	1409	-	0.8	1.9	1.0	-	0.5	1.1	0.4	0.71	-
Hexanal[c.d.e]	804	-	-	1.2	-	2.0	0.6	1.3	0.5	0.70	4.5×10^{-3}
1-Pentadecene	1492	-	-	3.7	-	-	-	1.5	-	0.65	-
Methyl decanoate	1327	0.4	-	0.9	-	-	3.4	-	-	0.59	-
1,3-Butandiol	788	-	0.7	1.5	1.1	0.2	-	0.6	-	0.51	-
(Z)-2-Octenal	1048	-	-	-	-	-	4.0	-	-	0.50	-
β-Cyclocitral	1222	0.2	-	0.4	0.5	-	1.8	1.0	-	0.49	-
n-Tridecane	1300	-	1.5	-	1.1	-	-	-	0.7	0.41	-
Limonene[e]	1032	-	0.6	-	-	2.6	-	-	-	0.40	0.01
(E,E)-Farnesyl acetate	1843	-	-	-	-	-	1.1	1.4	0.7	0.40	-
2-Methyltetradecane	1462	-	-	2.9	-	-	-	-	-	0.36	-
1-Butanol	659	-	-	-	-	2.6	-	-	-	0.33	0.5
Isobornyl acetate	1287	0.2	-	-	-	-	0.5	1.3	-	0.25	-
Methyl dodecanoate	1526	0.2	-	-	-	-	1.8	-	-	0.25	-
n-Octadecane	1800	-	-	-	0.7	-	0.7	0.6	-	0.25	-
(Z)-2-Tridecene	1304	-	-	-	1.3	-	0.6	-	-	0.24	-
Methyl tetradecanoate	1727	-	-	1.3	-	-	0.6	-	-	0.24	-
1-Decanol	1273	-	-	-	-	-	-	0.8	0.8	0.20	-
Ethyl nonanoate	1297	1.1	-	-	-	-	-	-	0.5	0.20	-
Ethyl tetradecanoate	1796	1.1	-	-	-	-	-	-	0.5	0.20	-
Ethyl hexanoate	998	0.5	-	-	-	-	-	-	0.8	0.16	-
Methyl octanoate	1127	-	-	-	-	-	1.1	-	-	0.14	-
(E)-β-ionone	1486	-	-	-	-	-	1.0	-	-	0.13	7×10^{-6}
Isopentyl acetate	878	0.9	-	-	-	-	-	-	-	0.11	-
(E)-2-Octenal	1063	-	-	-	-	-	0.9	-	-	0.11	-
2-Ethyl-3-hydroxyethyl-2-methylpropanoate	1372	-	-	-	-	-	0.9	-	-	0.11	-

Table 1. Contd.

1-Octanol[c.d.e]	1072	-	-	-	-	-	0.8	-	-	0.10	-
2-Pentyl furan	993	0.2	-	-	-	-	0.5	0.7	-	0.09	-
2-Octen-1-ol	1071	-	-	-	-	-	0.7	-	-	0.09	-
1-Nonanol	1174	0.3	0.4	-	-	-	-	-	-	0.09	-
2-Undecanone[c]	1293	-	-	-	-	-	0.7	-	-	0.09	-
cis-threo-Davanafuran	1415	-	-	-	-	-	-	0.7	-	0.09	-
n-Dodecane	1200	-	0.6	-	-	-	-	-	-	0.08	-
(E)-2-Nonenal	1162	-	-	0.5	-	-	-	-	-	0.06	8×10^{-5}
2-Phenylethyl acetate	1258	0.5	-	-	-	-	-	-	-	0.06	-
(E)-2-Decenal	1266	-	0.5	-	-	-	-	-	-	0.06	-
Ethyl dodecanoate	1596	0.5	-	-	-	-	-	-	-	0.06	-
Styrene	898	0.4	-	-	-	-	-	-	-	0.05	-
(Z)-4-Heptenal	902	-	-	-	-	-	-	-	0.4	0.05	-
Ethyl heptanoate	1097	0.2	-	-	-	-	-	-	-	0.03	-
Total	-	98.4	93.5	93.6	93.9	93.5	94.2	93.9	97.0	-	-

BQ: BentQbala; GH: Ghars; HA: Hamraia; HO : Houbales; TAN: Tantbouchet; LI: Litima; TIN: Tinicine; TIM: Timjouhart. -Not identified, [a]Percentages obtained by FID peak area normalization (HP-5 column). [b]Linear retention indices (DB-5 column). [c]Found previously in Zahdi variety. [d]Found previously in Alligh. Deglet Nour and Kentichi varieties. [e]Found previously in Aziza, Boufeggous, Bouskri, Bousthammi noire, Iklane, Jihel, Mejhoul and Najda varieties. [F]threshold detection determined in water at 20°C.

Table 2. Aroma compounds availability in Algerian date varieties.

Aroma compound	Variety number
Ethyl acetate; nonanal; decanal; (*E*)-geranylacetone	8
Isopentyl alcohol; 2,3-butandiol, 6-methyl-5-hepten-2-one; *n*-tetradecane, *n*-pentadecane; *n*-hexadecane	7
Octanal; (*E*)-2-nonen-1-ol; dodecanal	6
1,3-Butandiol; hexanal; β-cyclocitral; undecanal; *n*-heptadecane	5
2-Propanol; 1-hexanol; 1-octen-3-ol; 3-ethyl-1-hexanol; ethyl-decanoate	4
Ethyl octanoate; isobornyl acetate; *n*-tridecane; methyl decanoate; *n*-octadecane; (*E,E*)-farnesyl acetate	3
2-Pentyl furan; ethyl hexanoate; limonene; phenylethyl alcohol; 1-nonanol; 1-decanol; ethyl nonanoate, (*Z*)-2-tridecene; 1-pentadecene; methyl dodecanoate; methyl tetradecanoate; ethyl tetradecanoate	2
1-Butanol; isopentyl acetate; styrene; (*Z*)-4-heptenal; (*Z*)-2-octenal; (*E*)-2-octenal; 2-octen-1-ol,1-octanol; ethyl heptanoate; methyl octanoate; (*E*)-2-nonenal; *n*-dodecane; 2-phenylethyl acetate; (*E*)-2-decenal; 2-undecanone; 2-ethyl-3-hydroxyethyl 2-methylpropanoate; *cis-threo*-davanafuran; 2-methyltetradecane; (*E*)-β-ionone; ethyl dodecanoate	1

Timjouhart mainly produced alcohols and esters. On the contrary, a great distance was evidenced between Bent Qbala and Tantbouchet varieties, probably because of their different content of esters.

The aroma compounds identified in this study were obtained through several mechanisms. The volatile compounds of food were formed by four different pathways: biosynthesis, direct enzymatic, indirect enzymatic (oxidative), and pyrolytic pathway (Crouzet, 1998). Esters are generally produced by the enzymatic way, starting from acyl-CoA and alcohols. The aldehydes reduction leads to primary alcohols and the acids derive by an enzymatic oxidation. Biosynthesis of monoterpenes was localized in the plastids, whereas that of sesquiterpenes takes place in the cytosol (Bouvier et al., 2005). However, both were synthesized starting from isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), which were condensed into immediate precursors of terpenes, geranyl pyrophosphate (GPP) and farnesyl pyrophosphate (FPP), by a group of enzymes collectively known as short-chain isoprenyl diphosphate synthases (IDSs) (Wang and Ohnuma, 2000). Thermal degradation of β-carotene also leads to apocarotenes. Heating in aqueous medium of lycopene leads to the formation 6-methyl-5-hepten-2-one (Crouzet, 1998).

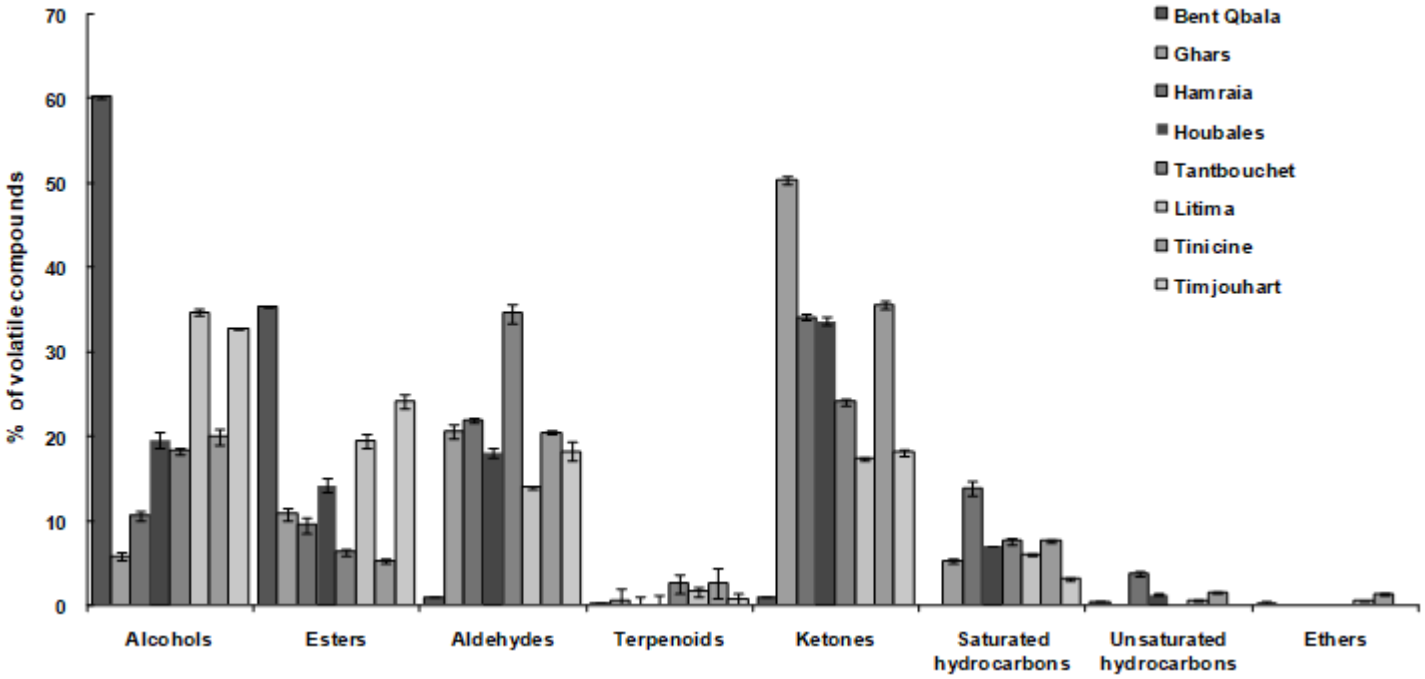

Figure 1. Evolution of each group of volatile compounds in Algerian date variety.

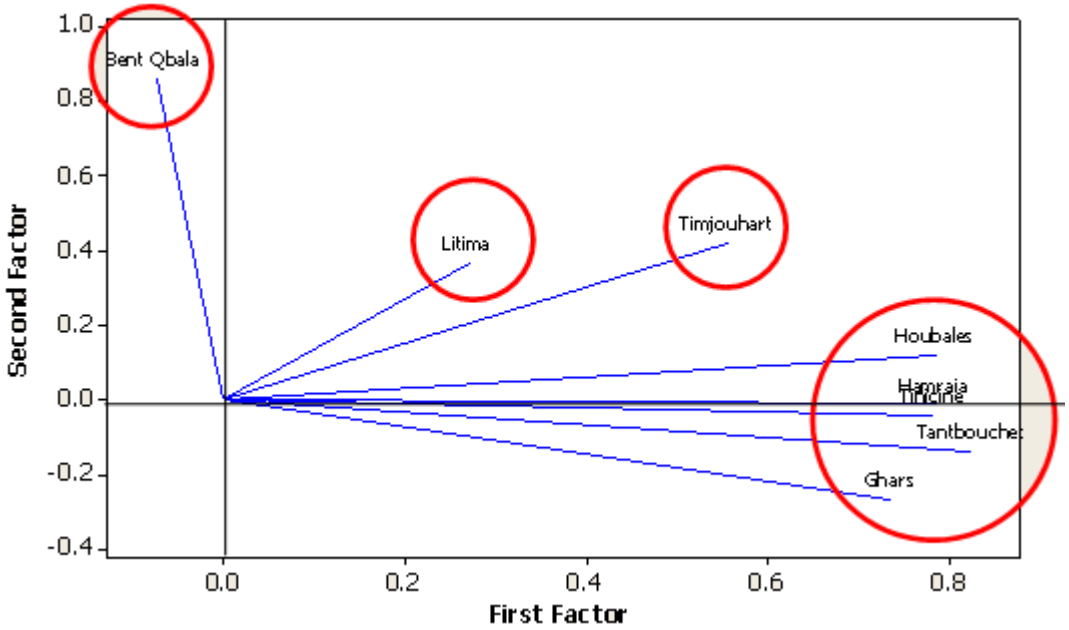

Figure 2. Factorial correspondence analysis based on the presence-absence of volatile compounds in Algerian date varieties.

Moreover, the volatile compounds do not participate in the same degree to the fruit aroma; there are some key compounds which strongly contribute to the final aroma. Alcohols, alkanes, acids and aldehydes were responsible for the *Dialium guineense* characteristic aroma note (Pélissier et al., 2001). However, linalool, limonene, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, nonanal, and *(Z)*-3-hexenal caused the significant differences in odour

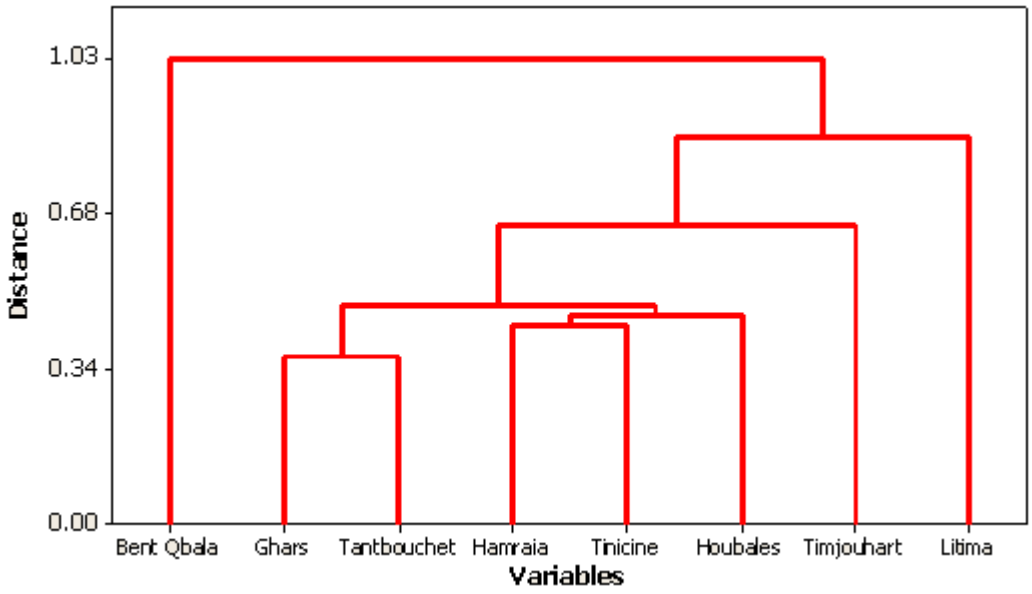

Figure 3. Hierarchical ascendant clustering dendrogram based on presence-absence of volatile compounds in Algerian date varieties.

profiles of different tamarinds (Lasekan and See, 2015). According to Kesen et al. (2013), aldehydes were found as the major aroma active compounds in olive oil, followed by alcohols such as: hexanal, octanal and guaiacol. The aliphatic hydrocarbons identified among the volatiles emitted by dates are probably only of secondary importance for their flavour (Jaddou et al., 1984). Furthermore, alcohols and carbonyl compounds such as, aldehydes and ketones are extremely important compounds involved in many odours such as fruity, floral, and lemon scents (Harrak et al., 2005).

Qualitative composition of Algerian date aroma

The factorial correspondence analysis based on the presence or absence of identified aroma compound in Algerian date varieties (Figure 2) separated the studied varieties into four groups. Three of them were formed by one variety only, that is, Bent Qbala, Litima, Timjouhart, while the fourth one grouped Houbales, Tinicine, Hamraia, Tantbouchet and Ghars. It is apparent that Bent Qbala was clearly separated from all the other varieties. The clustering analysis (Figure 3) tallied with the varieties' distribution in Figure 2 and showed that Bent Qbala variety was separated from other date varieties. This behavior is due to the presence of several esters, such as ethyl acetate, isopentyl acetate, ethyl hexanoate, ethyl heptanoate, ethyl octanoate, 2-phenylethyl acetate, ethyl nonanoate, methyl decanoate, ethyl decanoate, methyl dodecanoate, ethyl dodecanoate, ethyl tetradecanoate and to the lack of ketones and saturated hydrocarbons. The same observation was noted for Litima and Timjouhart varieties which were separated at a lower level, compared to Bent Qbala, from the other date varieties. Litima was characterized by the highest number of volatile compounds (35 out of 61; Table 1) and many esters (ethyl acetate, methyl octanoate, ethyl octanoate, methyl decanoate, 2-ethyl-3-hydroxyethyl 2-methylpropanoate, ethyl decanoate, methyl dodecanoate, methyl tetradecanoate and all the identified ketones (6-methyl-5-hepten-2-one, 2-undecanone, *(E)*-geranylacetone and *(E)*-β-ionone)). Furthermore, Timjouhart also emitted aldehydes (hexanal, *(Z)*-4-heptenal, octanal, nonanal, decanal, undecanal, dodecanal) and esters (ethyl acetate, ethyl hexanoate, ethyl octanoate, ethyl nonanoate, ethyl decanoate, ethyl tetradecanoate and did not produce ethers and unsaturated hydrocarbons.

The use of fruit aroma is frequent in the food, cosmetics and pharmaceutical industries to satisfy the different consumer requirements and improve the taste or smell of the product (Öğütcü et al., 2015; Janiaski et al., 2016) or else masking the undesirable impression such as the bitter taste of most drug in pharmaceutical industry. This study highlighted the aroma composition of some date varieties of low market value and may attract processors attention to exploit its flavour in different products. This field raises undoubtedly the market value of these dates and improves the economic yield of their farmers and encourages them to continue the planting of these varieties. The conservation of date fruit diversity is an important challenge to face the growing of commercial varieties planting which leads to extinction of other dates.

Conclusion

This study showed the aromatic compounds that are the origin of the superior sensory quality of some Algerian date varieties that have a low commercial value. Thirteen molecules were found in preceding studied dates and forty eight volatiles were identified for the first time and could distinguish the Algerian date from others previously studied. These data can aid processors to develop a new aroma and produce processed products from lower market varieties to valorize them. Further investigation is required to describe the key volatile compounds responsible for date characteristic aroma and to study the aromatic composition changes during maturation stages and storage time.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Adams RP (2007). Identification of essential oil components by gas chromatography mass spectroscopy. 4th Ed. Allured Publishing Corporation. 804p.

Beaulieu J (2006). Volatile changes in cantaloupe during growth, maturation and in stored flesh-cuts prepared from fruit harvested at various maturities. J. Am. Soc. Hortic. Sci. 131(1):127-139.

Belguedj N, Bassi N, Fadlaoui S, Agli A (2015). Contribution to industrialization through the improvement of the traditional process of local drink manufacturing based on dates « Rob ». J. New Sci. Agric. Biotechnol. (in French). 20(7):818-829.

Benamara S, Gougam H, Amellal H, Djouab A, Benahmed A, Noui Y (2008). Some technologic proprieties of common date (*Phoenix dactylifera* L.) Fruits. Am. J. Food Technol. 3(2):79-88.

Benmeddour Z, Mehinagic E, Le Meurlay D, Louaileche H (2013). Phenolic composition and antioxidant capacities of ten Algerian date (*Phoenix dactylifera* L.) cultivars: a comparative study. J. Funct. Foods 5(1):346-354.

Biniecka M, Caroli S (2011). Analytical methods for the quantification of volatile aromatic compounds. Trends Anal. Chem. 30(11):1756-1770.

Bouvier F, Rahier A, Camara B (2005). Biogenesis, molecular regulation and function of plant isoprenoids. Prog. Lipid Res. 44(6):357-429.

Crouzet J (1998). Food flavour. Techniques de l'ingénieur. F4100 :01-18. (in French).

El Hadi MA, Zhang FJ, Wu FF, Zhou CH, Tao J (2013). Advances in fruit aroma volatile research. Molecules 18(7):8200-8229.

Fernandez X, Cabrol-Bass D (2007). Flavour analysis.Techniques de l'ingénieur 3233:3201-3222. (in French).

Goff SA, Klee HJ (2006). Plant volatile compounds: sensory cues for health and nutritional value? Science 311(5762):815-819.

Grechkin AN, Bruhlmann F, Mukhtarova LS, Gogolev YV, Hamberg M (2006). Hydroperoxide lyases (CYP74C and CYP74B) catalyze the emolytic isomerization of fatty acid hydroperoxides into hemiacetals. Biochim. Biophys. Acta 1761(12):1419-1428.

Harrak H, Reynes M, Lebrun M, Hamouda A, Brat P (2005). Identification and comparison of volatile components of fruits of eight Moroccan date varieties. Fruits 60(4): 267-278. (in French).

Jaddou H, Mhaisen MT, Al-Hakim M (1984). Flavour volatile analysis of Zahdi dates by gas liquid chromatography. Date Palm J. 3(2):367-379.

Janiaski DR, Pimentel TC, Cruz AG, Prudencio SH (2016). Strawberry-flavored yogurts and whey beverages: What is the sensory profile of the ideal product? J. Dairy Sci. 99(7):5273-5283.

Kesen S, Kelebek H, Sen K, Ulas M, Selli S (2013). GC–MS–olfactometric characterization of the key aroma compounds in Turkish olive oils by application of the aroma extract dilution analysis. Food Res. Int. 54(2):1987-1994.

Lasekan O, See NS (2015). Key volatile aroma compounds of three black velvet tamarind (*Dialium*) fruit species. Food Chem. 168:561-565.

Mansouri A, Embarek G, Kokkalou E, Kefalas P (2005). Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (*Phoenix dactylifera*). Food Chem. 89(3):411-420.

Moore PP, Burrows C, Fellman J, Mattinson DS (2002). Genotye x environment variation in raspberry fruit aroma volatiles. Acta Hortic. 585:511-516.

National Institute of Standards and Technology (NIST) (2000). NIST mass spectral search program for use with Microsoft windows. http://chemdata.nist.gov/mass-spc/ms-search/docs/Ver20Man.pdf

Öğütcü M, Yılmaz E, Güneşer O (2015). Influence of storage on physicochemical and volatile features of enriched and aromatized wax organogels. J. Am. Oil Chem. Soc. 92:1429-1443.

Pélissier Y, Haddad C, Marion C, Milhau M, Bessière J (2001).Volatile constituents of fruit pulp of *Dialium guineense* Wild.(*Cesalpinaceae*). J. Essent. Oil Res.13(2):103-104.

Reynes M, Lebrun M, Shaw PE (1996). Identification of volatile date components and use for multivariate analysis to distinguish date varieties. J. Food Qual. 19(6):505-514.

Stenhagen E, Abrahamsson S, Lafferty MCFW (1974). Registry of Mass Spectral Data. Ed. Wiley. 4:3358.

Wang KC, Ohnuma S (2000). Isoprenyl diphosphate synthases. Biochim. Biophys. Acta 1529(1-3):33-48.

Yu Y, Bai J, Chen C, Plotto A, Yu Q, Baldwin E, Gmitter F (2017). Identification of QTLs controlling aroma volatiles using a Fortune x Murcott (*citrus reticulata*) population. BMC Genomics 18:646.

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