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Table of Content: Volume 16 Number 46 15 November, 2017

ARTICLES

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Full Length Research Paper

Effect of different stablizers on quality characteristics of the set-type yogurt

Emine Macit[1*] and Ihsan Bakirci[2]

[1]Department of Food Engineering, Engineering Faculty, Bayburt University, 69000 Bayburt, Turkey.
[2]Department of Food Engineering, Faculty of Agriculture, Atatürk University, 25240 Erzurum, Turkey.

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The aim of this study was to determine the effects of seven different stabilizers on some properties of set-type yogurt. Stabilizers used were sodium caseinate, gelatin, carrageenan, xanthan gum, guar gum, locust bean gum (LBG), native corn starch. For the control group, no stabilizer was added. During the storage period, while the fat and pH values of the yoghurt samples were reduced, the stabilizers had no effects on the physical and chemical properties. The color values were affected by stabilizers and storage period at p<0.01 level. Stabilizers, except LBG, adversely affected the development of *Streptococcus thermophilus* but did not show the same effect on *Lactobacillus delbrueckii* subsp. *bulgaricus*. Control sample was the most favorite example in sensory evaluations. The microstructural properties of yogurt samples containing gelatine, xanthan gum, guar gum and locust bean gum were quite different as compared to the control sample. The results of this study indicate that sodium caseinate and gelatin were the most suitable stabilizers that could be used in the production of set yoghurt.

Key words: Yogurt, stabilizer, gum, physical and chemical properties, microstructure.

INTRODUCTION

Yogurt, a functional food, is one of the most consumed fermented dairy products in the world (Buttriss, 1997; Mckinley, 2005; Weerathilake et al., 2014). The texture of yogurt is as important as its taste and flavor in terms of consumer preferences. However, the properties of the milk used in yogurt production, the production and storage conditions or the transportation to far sales points can lead to textural defects such as viscosity variations and syneresis (Trachoo, 2002; Hematyar et al., 2012). Various stabilizers are used to prevent these problems and to create desirable textural characteristics (Keogh and O'Kennedy, 1998; Athar et al., 2000; Mohammadifar et al., 2007).

Stabilizers, also called thickeners, gelling agents or hydrocolloids, can be obtained from different sources including animal connective tissues, sea and land plants and microorganisms (Imeson, 2010). They have gelling, thickener and stabilizer properties (Lal et al., 2006; Tamime and Robinson 2007).

Sodium caseinate and gelatin increase the density of the protein network in the gel microstructure (Remeuf et al., 2003; Amatayakul et al., 2006; Supavititpatana et al.,

*Corresponding author. E-mail: emacit@bayburt.edu.tr.

2009). Many researchers reported that yogurt fortification with sodium caseinate or gelatin resulted in a stronger network, higher viscosity and less syneresis (Modler et al., 1983; Athar et al., 2000; Guzmán-González et al., 2000; Schmidt et al., 2001; Remeuf et al., 2003; Ares et al., 2007; Damin et al., 2009).

When starch is heated, it swells by taking water and increases solution viscosity by forming a gel after a certain temperature. Therefore, the addition of the starch into yogurt enhances the viscosity (Schmidt et al., 2001; Williams et al., 2003; Mishra and Rai, 2006; Ares et al., 2007). Athar et al. (2000) ranked stabilizers as cornstarch, gelatin, pectin, guar gum, CMC, carrageenan and sodium alginate according to the reduction rates of syneresis when compared with the control group. The characteristics of yogurt that natural wheat starch (NWS) is added to, are similar to those of yogurt with gelatin; therefore, NWS may be preferred as stabilizer in set-type yogurts (Schmidt et al., 2001).

Guar gum, xanthan gum and locust bean gum (LBG) are used as thickener in food industry (Tamime and Robinson 2007). They enhance the texture by increasing the viscosity of continuous phase and reduce syneresis (Hematyar et al., 2012). Locust bean gum has synergic effects with other stabilizers to reduce serum separation and increase viscosity (Köksoy and Kilic, 2004). Ünal et al. (2003) reported that LBG concentrations above 0.02% decreased water holding capacity (WHC) and viscosity, while it increased syneresis. 0.1% of guar gum in yogurt achieved the best result for low acidity and low pH (Mehmood et al., 2008). El-Sayed et al. (2002) argued that the xanthan gum and its mixture with other stabilizers significantly increased the viscosity of cow milk yogurt and reduced syneresis. They also pointed out that yogurt including 0.01% of xanthan gum was the most favorite in sensorial evaluation.

Carrageenan is used as gelling and has different forms. In the presence of calcium, κ-carrageenan forms a stiff and brittle gel, whereas ι-carrageenan forms a soft gel and λ-carrageenan will not form a gel, but acts as a thickener (Glicksman, 1987). Sağdıç et al. (2004) stated that 0.01 and 0.03% of κ-carragenan could be used in yogurt production.

The aim of this study was to investigate the effects of different stabilizers such as sodium caseinate, gelatin, carrageenan, xanthan gum, guar gum, locust bean gum and native corn starch on some characteristics of plain set-type yogurt.

MATERIALS AND METHODS

Raw cow's milk, starter culture and skim milk powder were obtained from the dairy farm of Agriculture Faculty. Stabilizers were supplied from ORKİM (Chem. Subst. Inc.), Turkey.

Preparation of yogurt

The milk was homogenized at a pressure of 20 Mpa (ALFA LAVAL, separations technique GMBH) after being passed through the clarifier (Model ALFA-LAVAL model 313 T, centrifugal clarifier) and divided into eight parts. In each portion, 3% skimmed milk powder and a different stabilizer were added. Stabilizers were added to the milk, mixing with skimmed milk powder. Prepared mixes were heat treated at 80°C for 20 min, then cooled to 44±1°C and inoculated with 20 g/100 L yogurt culture (*Streptococcus thermophilus*, *Lactobacillus bulgaricus*; DVS culture, Valiren, Mayasan). They were filled into glass jars of 300 mL and incubated until it reached pH 4.7±0.1 at 44±1°C. The yogurts that completed incubation were stored in cold storage at 4±1°C. Analyzes were performed on the 1st, 7th, 14th and 21th days of the storage.

Sample codes for yogurt samples were as follows: C (the control group, without stabilizer), SC (0.5% sodium caseinate), G (0.3% gelatin), CR (0.025% carrageenan), XG (0.015% xanthan gum), GG (0.02% guar gum), LBG (0.02% locust bean gum) and NCS (1.25% native corn starch). The ration of stabilizer used was determined by pretesting and regarding the ratios used in various studies (El-Sayed et al., 2002; Ünal et al., 2003; Ares et al., 2007; Soukoulis et al., 2007; Hematyar et al., 2012).

Physical and chemical analyses

Yogurt samples were stored at 4°C for 21 days and all measurements were performed on days 1, 7, 14 and 21. The total solids and ash were determined by gravimetric method and the fat by the Gerber method. Yogurt samples were analyzed for protein by the mikro Kjeldahl method (IDF, 1993). Syneresis and WHC were determined respectively by the methods described by Sahan et al. (2008) and Sodini et al. (2004). Viscosity was determined using Poulten RV-8 model viscometer at 20 rpm. The pH values were measured using a digital pH meter (WTW 340-1, Germany). The research was conducted in two repetitions and the analyses were done in parallel.

Color

The color of yogurt was measured using Minolta Colorimetre (CR-200 Minolta Colorimeter, Osaka, Japan). The colorimeter used L (lightness), a (redness) and b (yellowness) scales (Sert et al., 2010).

Microbiological analysis

M17 agar (Merck) was used in the *S. thermophilus* count. Plates were incubated for 48 h at 37°C under aerobic condition. The enumeration of *Lactobacilli* was performed with MRS agar (Merck) for 72 h at 37°C under anaerobic conditions.

Sensory analysis

The sensory evaluations of the yogurt samples were carried out with a panelist group of 6 staff on the 1st, 7th, 14th and 21st days of the storage period. Odor, appearance, consistency, taste and general acceptability of yogurt samples were assessed by sensory evaluation. The highest score in the evaluation was 9 points and the lowest score was 1 point (9-8: very good, 7-6: good, 5-4: medium, 3-2-1: bad) (Bodyfelt et al., 1988).

Microstructure

About 30 g of the yogurt sample was weighed in the aluminum caps, and dried in the vacuum oven at 70°C and at 3.3 kPa for

Table 1. The means and standard deviations of some physical and chemical analysis results of yogurt samples.

Sample code	Total solids (%)	Ash (%)	Protein (%)	Fat (%)	pH	Viscosity (cp)	WHC (g/kg)
C	12.84±0.72	0.886±0.030	4.01±0.30	2.74±0.41	4.14±0.14	5516±990	60.10±4.66
SC	13.13±0.58	0.897±0.038	4.23±0.39	2.69±0.35	4.09±0.05	6432±1344	60.82±5.48
G	13.04±0.55	0.880±0.041	4.06±0.38	2.56±0.41	4.15±0.17	4877±1145	58.58±6.08
CR	12.73±0.71	0.883±0.044	3.94±0.40	2.73±0.31	4.08±0.11	4887±1331	56.26±4.91
XG	12.87±0.68	0.898±0.058	3.94±0.44	2.84±0.34	4.09±0.05	5444±1067	54.28±5.49
GG	12.73±0.59	0.881±0.050	3.90±0.37	2.66±0.36	4.11±0.11	4753±1279	53.15±4.08
LBG	13.47±0.35	0.897±0.028	4.08±0.22	2.70±0.72	4.16±0.09	4983±777	56.44±3.59
NCS	13.44±0.63	0.897±0.050	3.92±0.30	2.75±0.41	4.09±0.08	5080±728	60.92±6.82
Σ	13.03	0.89	4.01	2.71	4.11	5246	57.56
Storage period (days)							
1	13.16±0.77	0.884±0.042	3.99±0.36	2.89±0.34[a]	4.22±0.11[a]	5245±1058	55.04±4.31
7	12.99±0.67	0.900±0.048	4.04±0.39	2.92±0.27[a]	4.10±0.07[b]	5200±950	57.43±4.78
14	12.99±0.59	0.895±0.048	3.99±0.33	2.74±0.23[a]	4.08±0.05[b]	5575±1462	58.11±7.27
21	12.96±0.55	0.880±0.026	4.01±0.35	2.28±0.44[b]	4.05±0.09[b]	4966±1155	59.70±5.42
P	0.23	0.484	0.03	7.820**	8.61**	0.58	1.46

Letters a and b indicates means that are significantly different at $P < 0.01$ level.

about 16 h. The samples taken out from the oven were stored until it reached a constant weight in airtight desiccant (containing silica gel as the desiccant) before analysis. Thus, the samples were fully dried. Lentil-sized pieces were cut from dried samples and were analyzed with SEM (Field Emission Scanning Electron Microscope, JEOL JSM- 7001F) after the process of gold plating in the vacuum cabin (Jaya, 2009).

Statistical analysis

The SPSS statistical software program version 13 (SPSS Inc., Chicago, IL, USA) was employed to analyze experimental data and Duncan's multiple range tests were employed to determine differences between results (SPSS, 2004).

RESULTS AND DISCUSSION

Raw cow's milk had 11.93% total solid, 3.5% milk fat, 3.31% protein, 0.71% ash, 6.52 pH and 1.031 specific gravity.

Physical and chemical analyses

According to the statistical analysis results, stabilizer and storage period had no effects on the physical and chemical properties of yogurt samples. Only the storage period had a significant effect ($p<0.01$) on the fat and pH values (Table 1).

The dry matter ratio of milk is one of the important factors that determine the physical properties of yogurt. The average dry matter ratio of yogurt samples was found as 13.03%. Smit (2003) stated that the dry matter ratio in commercial yogurts ranged from 13 to 17%. The ash content of yoghurt samples varied between 0.880 and 0.898%. Proteins have water binding properties and reduce syneresis by increasing the water holding capacity of the yogurt (Smit, 2003). The average protein content of yoghurt samples was determined to be 4.01%. Sodium caseinate, gelatin and LBG were found to increase protein values, but this increase was not statistically significant.

The mean values of fat and pH regarding the yoghurt samples were 2.71% and 4.11, respectively. While the stabilizers had no effect on these values, the storage period was effective on fat ratio and pH at $p <0.01$ level. The lowest fat content (2.28%) was determined on the 21st day of storage. This decrease could be a result of the lipolytic activities of yogurt bacteria during storage. The highest pH value (4.22) was obtained on the 1st day of storage (Figure 1). It was similar in 7, 14 and 21 days. This change in pH was due to the increase in acidity of the yogurt samples during the storage period.

The viscosity values of the yogurt samples varied between 4753 (sample GG) and 6432 cp (SC); WHC values were between 53.15 (GG) and 60.92 g/kg (NCS). Although sodium caseinate increased viscosity and WHC values, an increase in WHC value was also observed via natural corn starch; these increases were not statistically significant.

Sample G with the addition of gelatin was different from other samples in terms of various parameters. Syneresis values of sample G decreased rapidly after day 7 and were lower than other samples (Figure 2). The protein and pH levels of sample G quickly decreased (protein from 4.13 to 3.91% and pH from 4.37 to 3.98)

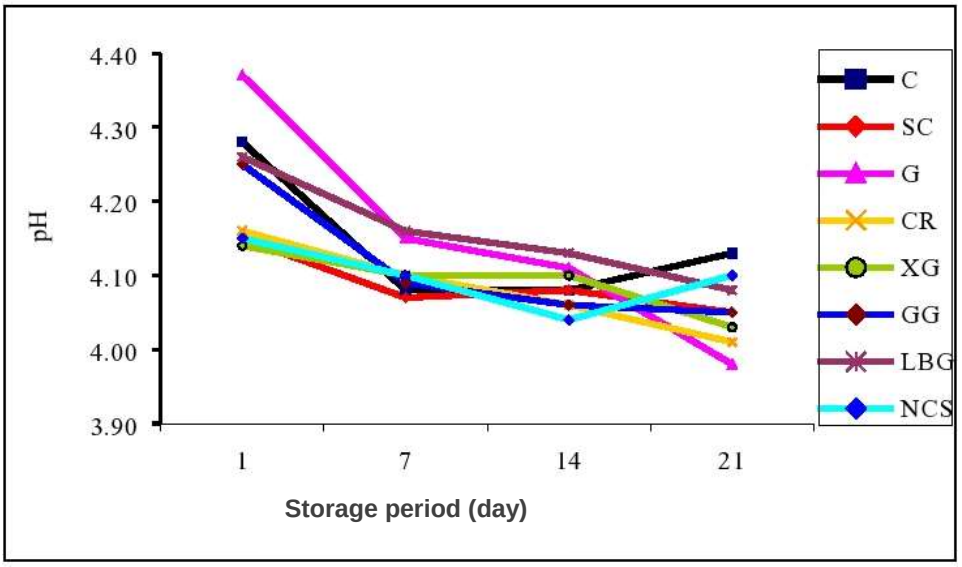

Figure 1. The change in pH values of yogurt samples during the storage period.

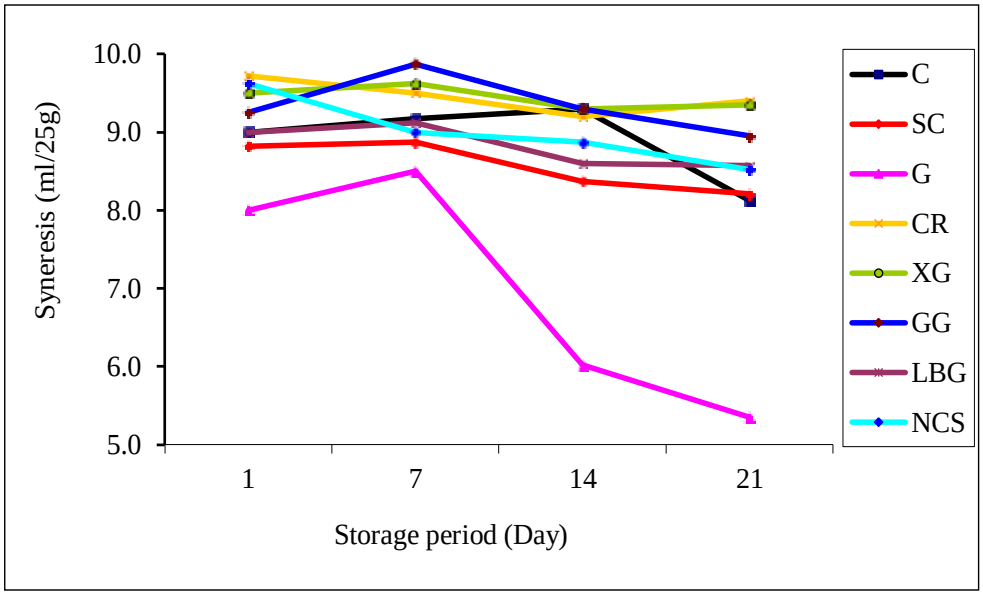

Figure 2. The change in syneresis values of yogurt samples during the storage period.

during the storage period. It was accordingly considered that components with acidic characteristics that were formed as a result of the breakup of gelatin and lactose by yogurt bacteria decreased syneresis with an increase of water holding capacity. These changes reduced the viscosity of sample G. Supavititpatana (2008) found that as the gelatin ratio in yogurt increased, syneresis values decreased. In addition, Fiszman et al. (1999) reported that gelatin formed double network structure in yogurt and this structure reduced the syneresis values of yogurt.

Color

The results of variance analysis indicate that the effect of stabilizer type and storage time on the color values of yogurt samples was found to be significant at the level of $p<0.01$. The changes in color values of yogurt samples during the storage period are shown in Figure 3a, b and c. The highest L and a values were observed in SC sample. Sodium caseinate created an increase in density of the protein network by increasing protein content

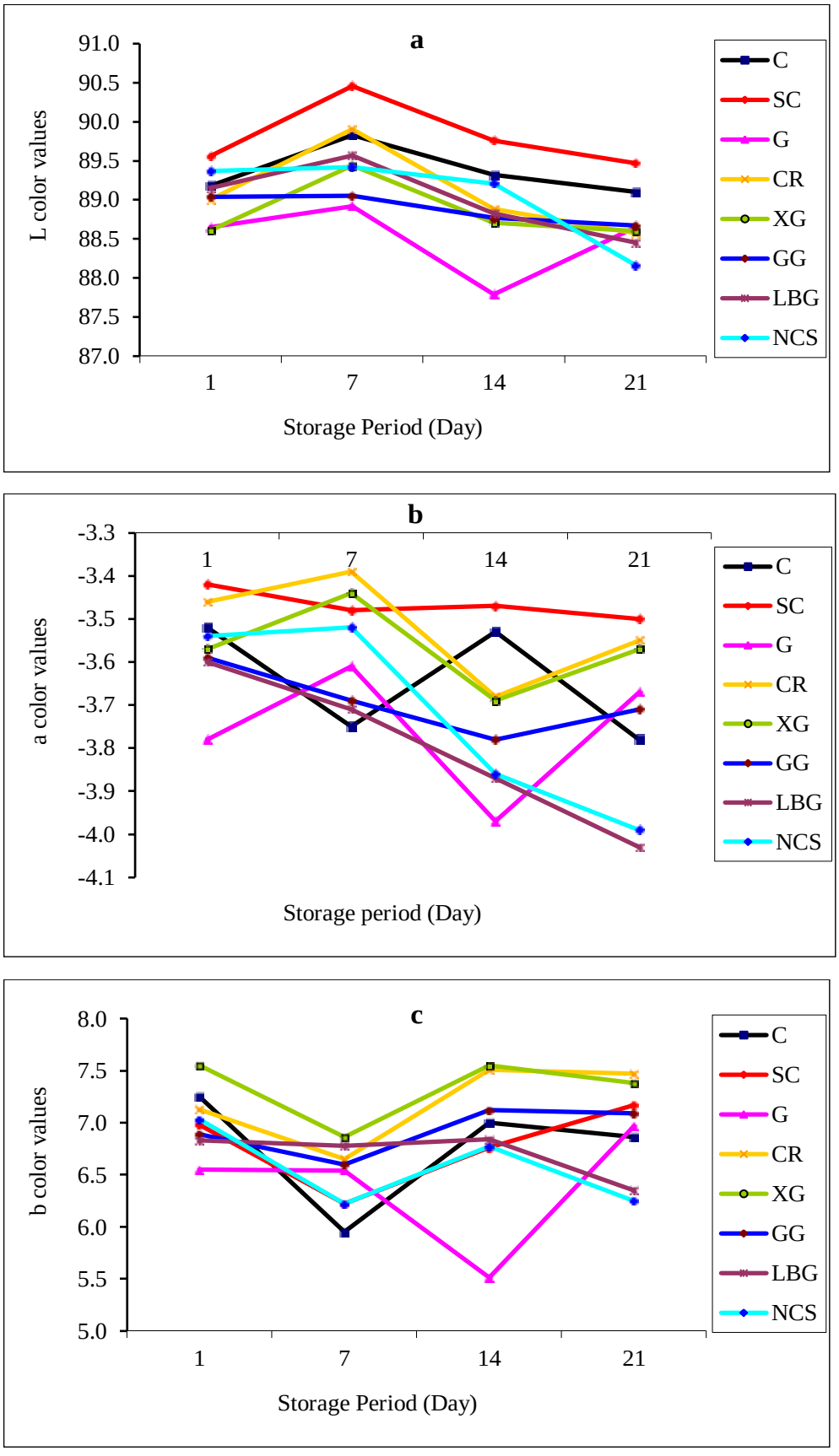

Figure 3. The change in L, a, b color values of yogurt samples during the storage period.

Table 2. The means and standard deviations of *S. thermophilus* and *L. debrueckii* subsp. *bulgaricus* counts of yogurt samples.

Sample code	*S. thermophilus* (log kob/g)	*Lactobacillus debrueckii* subsp. *bulgaricus* (log kob/g)
C	7.77 ± 0.41^{a}	7.01 ± 0.44^{c}
SC	7.38 ± 0.28^{c}	6.98 ± 0.61^{c}
G	7.52 ± 0.21^{bc}	7.14 ± 0.38^{bc}
CR	7.66 ± 0.39^{ab}	7.28 ± 0.33^{ab}
XG	7.11 ± 0.38^{d}	7.41 ± 0.40^{a}
GG	7.47 ± 0.29^{c}	7.27 ± 0.37^{ab}
LBG	7.72 ± 0.21^{a}	7.12 ± 0.32^{bc}
NCS	6.07 ± 0.19^{e}	7.39 ± 0.53^{a}
P	114.72^{**}	8.33^{**}
Storage period (days)		
1	6.99 ± 0.54^{c}	6.63 ± 0.34^{c}
7	7.45 ± 0.55^{ab}	7.33 ± 0.28^{b}
14	7.52 ± 0.58^{a}	7.48 ± 0.17^{a}
21	7.38 ± 0.62^{b}	7.35 ± 0.33^{b}
P	40.52^{**}	90.49^{**}

Letters a, b, c and d indicates means that are significantly different at $p < 0.01$ level.

(Table 1). Thus, it enabled yogurt to seem whiter by preventing the release of the serum phase from casein network. L values increased on the 7th day, and then a decrease was observed in these values. a Values decreased in parallel with the decrease in L color values. This situation resulted from a spontaneous syneresis in yogurt after the seventh day. The highest b value was determined in XG sample and the lowest value in sample G. Fat levels affected b color value.

Yogurt samples showed significant differences in terms of color values. The syneresis ratios of yogurt samples, the color and usage ratio of stabilizer had a significant effect on these differences.

Microbiological analysis

Stabilizer and storage period significantly affected *S. thermophilus* ($p<0.01$) and *L. delbrueckii* subsp. *bulgaricus* numbers ($p<0.05$) (Table 2). Stabilizers, except LBG, adversely affected the development of *S. thermophilus* but did not show the same effect on *L. delbrueckii* subsp. *bulgaricus*. This effect could be explained thus: reducing water activity of stabilizers affected negatively, the development of *S. thermophilus. S. thermophilus* needs higher water activity than *L. delbrueckii* subsp. *bulgaricus*.

It was considered that the lowest *S. thermophilus* count in the M sample may be due to the fact that water activity of the medium was lower when compared with other samples regarding the high corn starch concentration used in the M sample. *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* counts increased until the 14th day and decreased after 14th day. This decrease might have been due to the decrease in pH values as a result of acidity development.

Sensory analysis

Storage time had no effect on the sensory properties of yogurt samples. Stabilizers were effective at $p<0.01$ level on appearance, consistency and general acceptability, at $p<0.05$ on taste. C, SC and NCS samples for appearance and consistency, C sample for taste, and C and SC samples for general acceptability were the most preferred. C sample was the most popular example in sensory evaluations (Table 3).

Microstructure

The microstructure analysis of sample C showed that it had a homogeneous network formed by casein micelle (Figure 4a). The microstructure of sample C is in accordance with the study of Hess et al. (1997), Fiszman et al. (1999), Sanchez et al. (2000), Oh et al. (2007), Jaya (2009) and Rascón-Díaz et al. (2010). Sample SC had a denser network and fewer voids than the control group (Figure 4b). These results are in parallel with some physical and chemical analysis results found in yogurt samples with the addition of Na-caseinate. The microstructure of sample SC was similar to that of Modler et al. (1983).

Table 3. The means and standard deviations of sensory analysis results of yogurt samples.

Sample code	Odor	Appearance	Consistency	Taste	General acceptability
C	7.34±0.62	7.50±0.57^{a}	7.43±0.52^{a}	7.15±0.48^{a}	7.31±0.55^{a}
SC	7.35±0.67	7.40±0.58^{a}	7.21±0.54^{a}	6.91±0.51^{ab}	7.19±0.54^{a}
G	6.68±0.48	6.46±0.48^{bc}	5.96±0.87^{bc}	6.34±0.59^{abc}	6.22±0.49^{bc}
CR	6.73±0.27	6.49±0.44^{bc}	6.42±0.41^{bc}	6.25±0.91^{bc}	6.57±0.59^{abc}
XG	6.85±0.38	5.77±0.87^{c}	5.75±0.56^{c}	5.87±0.42^{c}	5.89±0.38^{c}
GG	7.11±0.46	6.92±0.45^{ab}	6.54±0.70^{b}	6.81±0.73^{ab}	6.84±0.67^{ab}
LBG	7.16±0.62	7.16±0.57^{ab}	6.49±0.59^{b}	6.78±0.50^{ab}	6.75±0.49^{ab}
NCS	7.21±0.71	7.35±1.02^{a}	7.29±0.74^{a}	6.53±0.99^{abc}	6.94±0.87^{ab}
P	1.18	5.61**	7.51**	2.45*	4.26**
Storage period (days)					
1	7.13±0.59	6.87±0.74	6.62±0.64	6.66±0.48	6.90±0.61
7	6.99±0.47	6.76±0.76	6.50±1.07	6.62±0.68	6.66±0.72
14	7.04±0.61	6.83±1.06	6.56±0.97	6.60±0.92	6.59±0.79
21	7.05±0.65	7.07±0.79	6.86±0.59	6.44±0.87	6.70±0.76
P	0.12	0.56	0.96	0.26	0.67

Letters a, b and c indicates the means that are significantly different at $P < 0.01$ or $P < 0.05$ levels. $^{**}P < 0.01$, $^{*}P < 0.05$.

Sample G had a low porosity (Figure 4c). A number of researchers reported that gelatin could interact with casein and form a denser porosity in yogurt microstructure (Fiszman et al., 1999; Gonçalvez et al., 2005; Ares et al., 2007). This information was supported with a decrease in the syneresis values of the sample G. However, it is in contradiction with viscosity decrease. Modler and Kalab (1983) pointed out that no gelatin was visible in the microscopic appearance of yogurt with 0.5% gelatin and the gel network was similar to plain yogurt. The microstructural analysis of the sample CR showed that the carrageenan had a larger structure as compared to the milk components and the microstructure of yogurt had fewer voids (Figure 4d). Rascón-Díaz et al. (2012) reported that yogurt samples produced by adding carrageenan had a tense structure.

In sample XG (Figure 4e), the xanthan gum particles were bigger than the casein fractions. Consequently, they broke into the network of the casein micelles and created a more heterogeneous structure than the control sample. So, the use of xanthan gum in yogurt increased serum separation values and adversely affected the microstructural properties. The microstructure of sample XG was similar to that of Sanches et al. (2000) but was different from that of El-Sayed et al. (2002). Harwalkar and Kalab (1986) reported that large pores in the protein matrix would promote serum separation.

In the sample GG (Figure 4f), large guar gum particles caused buildup of a heterogeneous structure. The pores were small but their number was high. The guar gum was a neutral stabilizer and there was no electrical interaction with casein micelles (Everett and Leod, 2005). Rascón-Díaz et al. (2010) reported that the guar gum samples showed low porosity and low homogeneity and such a structure had low water holding capacity.

LBG molecules increased porosity and made yogurt structure more heterogeneous (Figure 4g). However, the serum separation values of LBG-added yogurt samples were close to that of the control group. This effect resulted from the fact that LBG had a more thickening function rather than being a gelling agent. Thaiudom and Goff (2003) determined that LBG particles were excluded from the structure formed by casein micelles.

In the samples NCS, casein micelles were closer to each other than those of the control sample and the pores of the network were smaller (Figure 4h). This effect was due to the fact that the starch granules absorbed some of the water in the medium during the heat treatment and the water amount in the pores of the network decreased. Oh et al. (2007) concluded that swollen starch granules appeared as dark globules embedded in the protein network and this structure increased in parallel with the increase in starch concentration.

Conclusion

A variety of stabilizers can be used to improve the textural properties of yogurt. This study was carried out to reveal the effects of various stabilizers on the quality characteristics of set type yogurts. Physical, chemical, color sensory and microstructural properties of yogurt were investigated and the counts of yogurt bacteria were performed during storage period of of 21 days. In conclusion, the yogurts produced without the stabilizer

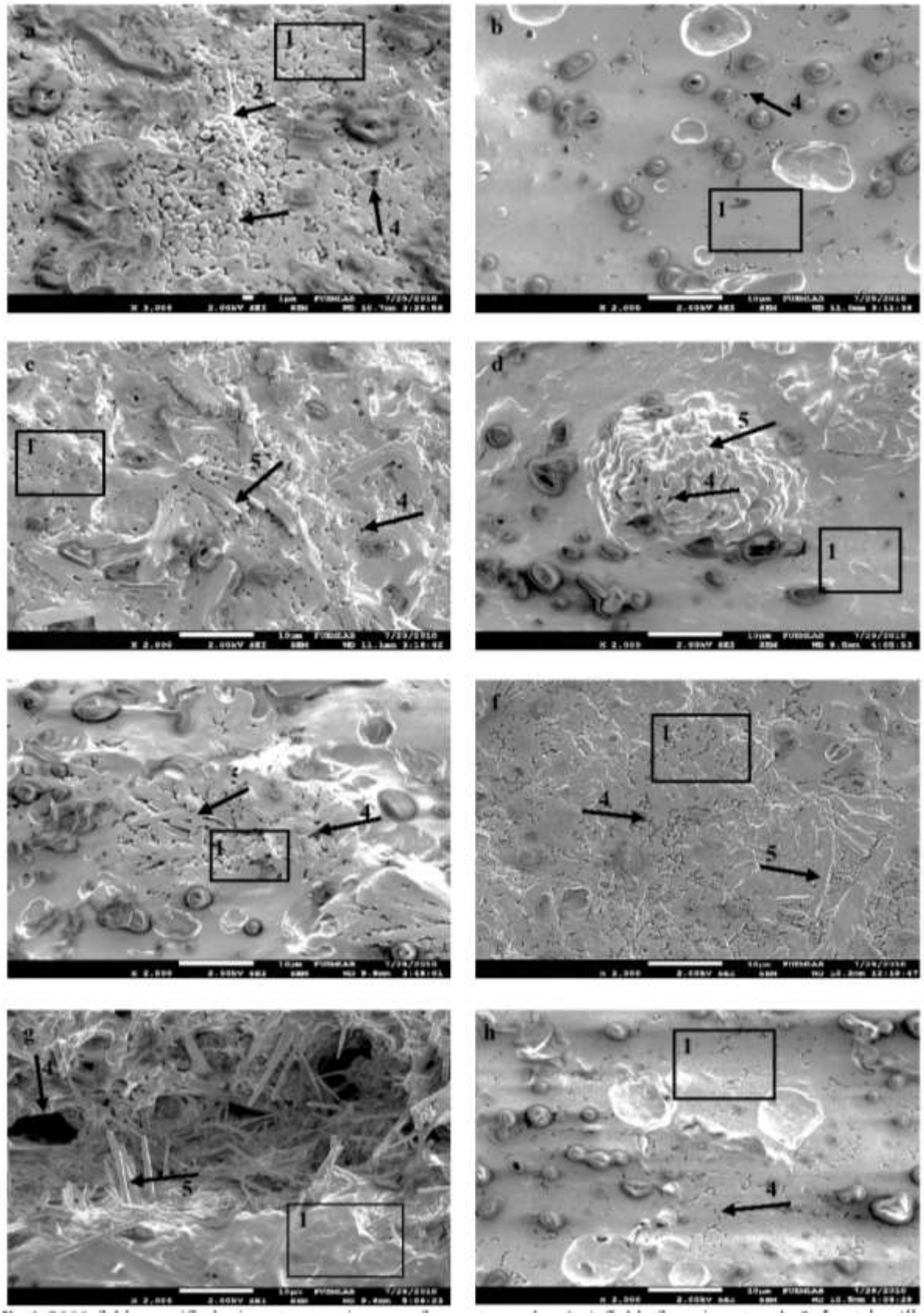

Figure 4. 2000-fold magnified microstructure images of yogurt samples. 1, A field of casein network; 2, *L. debrueckii* subsp. *bulgaricus*; 3, *S. thermophilus*; 4, A void; 5, stabilizer.

were more favorable in terms of sensory properties. However, Na-caseinate could increase viscosity and water holding capacity and gelatin was capable of being used to reduce serum separation in the event that stabilizer is required for use. Carrageenan, xanthan gum, guar gum, LBG additives alone and in the concentrations used, had no positive effect on the these properties of the yogurt.

Sodium caseinate and gelatin were the most suitable stabilizers that could be used in the production of set yogurt.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effect of adenine sulphate, casein hydrolysate and spermidine on *in vitro* shoot multiplication of two banana varieties (FHIA-21 and PITA-3)

Oumar Silué[1,2*], Kan Modeste Kouassi[2], Kouablan Edmond Koffi[1], Konan Eugène Pacome Kouakou[3] and Séverin Aké[2]

[1]Laboratoire Central de Biotechnologies (LCB), Centre National de Recherche Agronomique (CNRA), KM 17 Route de Dabou, 01 BP 1740 Abidjan 01, Côte d'Ivoire.
[2]Laboratoire de Physiologie Végétale, UFR Biosciences, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire.
[3]Programme Palmier à huile, Centre National de Recherche Agronomique (CNRA), Station de Recherche de La Mé, 13 BP 989 Abidjan 13, Côte d'Ivoire.

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A highly reproducible and efficient *in vitro* shoot regeneration system was developed for two banana varieties (FHIA-21 and PITA-3) using shoot tip as explant. Shoot tip was inoculated onto Murashige and Skoog (MS, 1962) medium supplemented with cytokinins [benzylaminopurine (BAP), kinetin (Kin) and 2-isopentenyl (2-iP)] and additives [Adenine sulphate (Ads), spermidine (Spd) and casein hydrolysate (CH)] for shoot multiplication. In all varieties, the maximum number of shoots and shoot length was obtained with 3 and 4 mg/L BAP, respectively. This rate was further enhanced by adding Ads (25 mg/L), CH (25 or 50 mg/L) and Spd (100 or 200 mg/L). *In vitro* raised shoots were successfully rooted on 1 mg/L 2-iP in combination with 0.5 mg/L naphthaleneacetic acid (NAA). Rooting was significantly enhanced by adding casein hydrolysate (25 or 50 mg/L). The well rooted plantlets were successfully acclimatized on different substrates (compost, forest soil, sand, forest soil + sawdust from dead tree and sand + sawdust from dead tree). The compost substrate was found to be preferable. Finally, after one month in the greenhouse, the hardened plants were transfered to the field environment for utmost survivability.

Key words: Additives, banana, cytokinins, *in vitro* shoot multiplication, rooting, shoot tip.

INTRODUCTION

Bananas and plantains (*Musa* species) are among the most important fruit crops in the world and are staple food for millions across the globe (FAO, 2010). World total banana and plantain production ranks at the 5th place after cereals and there is still much scope for yield improvement (Jain and Swennen, 2004). They are at the

*Corresponding author. E-mail: silueoumarnatogoma@gmail.com. Tel: (+225) 01672718/(+225) 07761372.

second place after cassava (Manihot esculenta Crantz) in sub-Saharan Africa where they provide diet to millions of people (FAOSTAT, 2005). Plantains account for about 32% of total *Musa* production, from mostly Central and West Africa (Lescot, 2008). It represents an essential source of nutrients for millions of people, particularly in tropical and subtropical regions, as well as a cash crop in many developing countries.

Bananas are multipurpose plants with parts that can be used in various ways, depending on the species. Nutritionally, banana's fruit is rich in carbohydrate, vitamins (A, B, and C), and potassium (Aurore et al., 2009).

Despite the importance of bananas and plantains, the crop is threatened by many pests and diseases (Viljoen, 2010). Based on the high contribution of bananas and plantains to food security and human's health, there is great need for optimization of their production.

Basically, bananas are cultivated by conventional methods and *in vitro* cultivation. Banana propagation through the conventional method of using young shoots (suckers) or part of the tuber is not an ideal method. This method takes a long growth period and prone to various diseases (Matsumoto et al., 2003). Moreover, 5 to 10 suckers can be obtained per plant over a year. The traditional clonal propagation method appears to be unable to supply the increasing demand for disease free and healthy planting materials of banana. In order to increase conventional propagation and to avoid pathogens related constraints, *in vitro* approach has been considered (Tripathi, 2003). Methods developed using this approach offers more effective and better controlled condition for banana cultivation.

Positive role of different growth additives such as adenine sulphate, casein hydrolysate and spermidine has been reported extensively in the *in vitro* propagation of several species (Walia et al., 2007; Sanghamitra and Satyabrata 2011; Siwach et al., 2012). Earlier, it was reported that spermidine is essential for shoot multiplication in cucumber (Vasudevan et al., 2008), sugarcane (Shankar et al., 2011) and *Wrightia tomentosa* (Joshi et al., 2014).

Additives have been used in banana micropropagation media. However, there is no study on their specific effect. Hence, the present study was conducted to understand the influence of these growth additives such as adenine sulphate, spermidine and casein hydrolysate in the *in vitro* shoot multiplication from shoot-tip explants of two banana varieties.

MATERIALS AND METHODS

Explant source

Suckers (Figure 1a) as explants source were collected from the banana collection of Azaguie Research Station of National Agronomic Research Center (CNRA) of Côte d'Ivoire. All the experiments were conducted at the Central Laboratory of Biotechnology of CNRA at Adiopodoume.

Selection of explant and surface sterilization

Healthy meristematic shoot tip from sword suckers were initially washed thoroughly with soap water and then were surface presterilized with 15% (v/v) calcium hypochloride containing few drops of 0.01% tween-20 for 10 to 15 min. In the laminar air flow cabinet, shoot tip were sterilized with ethanol (98% v/v) for 5 min followed by three time rinsing in sterile distilled water. Then, explants were again sterilized with commercial bleach (3.8% of active chloride) containing few drops of 0.01% tween-20 for 20 min. At the end, shoot tips were rinsed thoroughly with sterile distilled water to remove any traces of commercial bleach.

Influence of different cytokinins on shoot multiplication

For shoot induction, explants of 2 cm long (Figure 1b) were placed in 200 mL glass bottles (Figure 1c) containing 30 mL of induction medium consisted of Murashige and Skoog (MS, 1962) (Sigma-Aldrich Chemie Gmbh Munich, Germany) medium supplemented with BAP (5 mg /L) (Sigma-Aldrich Chemie Gmbh Munich, Germany) and sucrose (30 g/L). The pH of the media was adjusted to 5.7 prior to adding agar (7 g/L). One month later, microshoots were isolated and inoculated in media supplemented with different cytokinins BAP, Kin (Sigma-aldrich Chemie Gmbh Munich, Germany) and 2-iP (Sigma-aldrich Chemie Gmbh Munich, Germany) at various concentrations (1.0, 2.0, 3.0, 4.0 and 5.0 mg/L) for shoot proliferation. After 4 weeks, mean number of shoots per explant and shoot length of shoot per explant were recorded. All the cultures were kept on dark at 25 ± 2°C.

Shoots multiplication in MS media supplemented with different additives

In order to increase shoot multiplication rate, adenine sulphate (Sigma-aldrich Chemie Gmbh Munich, Germany), casein hydrolysate (Sigma-aldrich Chemie Gmbh Munich, Germany) and spermidine (Sigma-aldrich Chemie Gmbh Munich, Germany) were added to the multiplication media. Microshoots were isolated and cultured in 500 mL glass bottles containing 150 mL of shoot multiplication media. This multiplication medium consisted of MS medium supplemented with adenine sulphate (Ads), casein hydrolysate (CH) at various concentrations ranging from 0 to 50 mg/L and spermidine (Spd) (50 to 200 mg/L). These components were individually or in combination added to the medium, with the best concentration of cytokinins in order to determine their individual and combined effects on shoot induction and multiplication. After 4 weeks, the mean number and the mean length of shoots were recorded.

In vitro rooting of microshoots

For root induction, microshoots were transferred into MS medium containing indole butyric acid (IBA; Sigma-Aldrich Chemie Gmbh Munich, Germany) or naphthaleneacetic acid (NAA ; Sigma-Aldrich Chemie Gmbh Munich, Germany) (0.5 mg/L) in combination with 1 mg/L of BAP or Kin or 2-iP. Two concentrations (25 and 50 mg/L) of casein hydrolysate were individually added to the most suitable cytokinin-auxin combination to enhance rooting. After three weeks of culture, rooting frequency, mean number of roots, and mean lenght were recorded.

Acclimatization of regenerated plants

In vitro plants with well-developed roots and three new leaves were carefully removed from culture vessels and washed thoroughly with

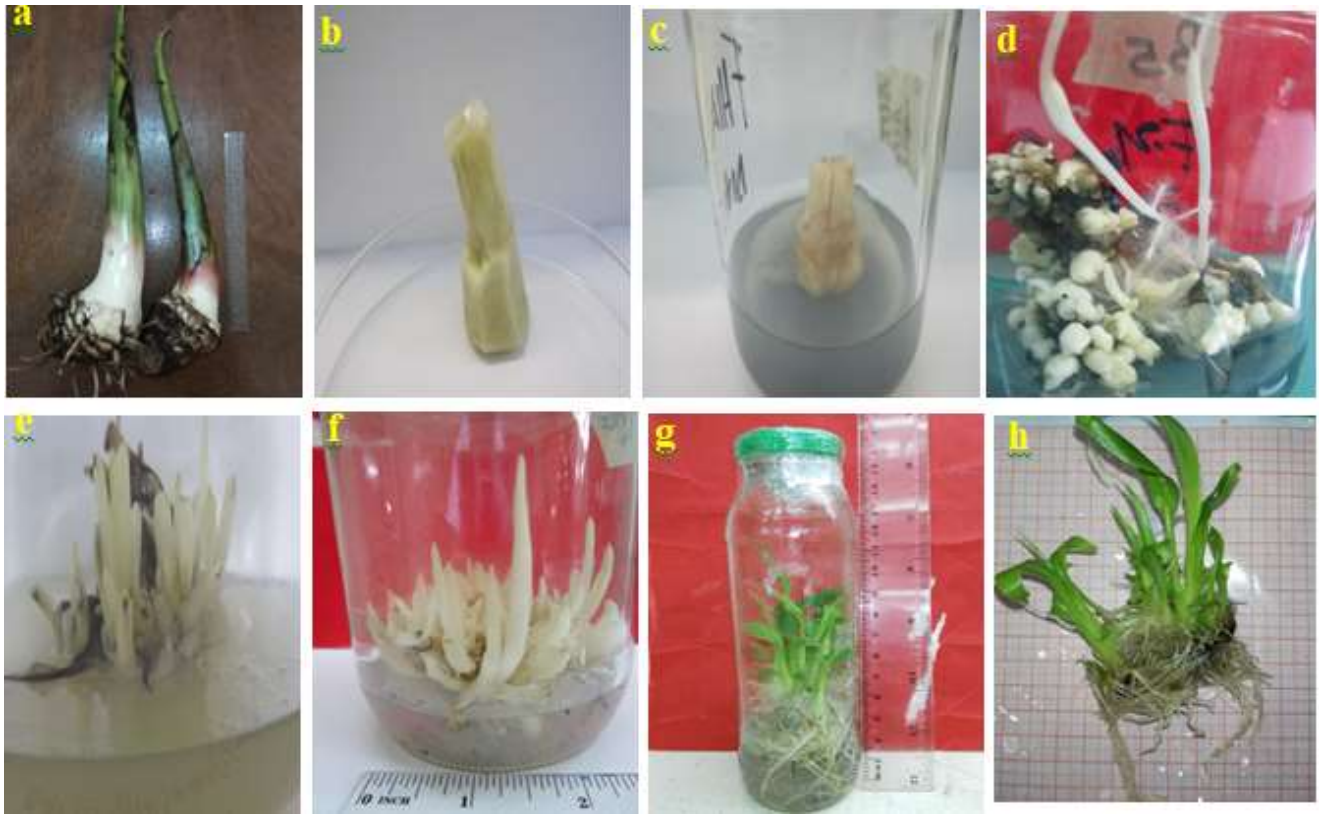

Figure 1. *In vitro* shoot multiplication and rooting of two banana varieties. (a) Sword shaped suckers of banana. (b) Healthy meristematic shoot tip. (c) Initiation of healthy meristematic shoot tip on MS + 5 mg/L BAP medium. (d-e) Shoot multiplication on MS + 3 mg/L BAP medium after 4 weeks. (f) Shoot proliferation on MS + 3 mg/L BAP + Ads + CH + Spd medium after 4 weeks. (g-h) In vitro rooting of regenerated shoots on MS + 1 mg/L 2-iP + 0.5 mg/L NAA + CH medium after 3 weeks.

distilled water to remove the remaining gelled media. Plants were then transfered to plastic bag containing five different substrates: S1 (compost), S2 (forest soil), S3 (sand), S4 (forest soil and sawdust from dead tree in 1:1 ratio), and S5 (sand and sawdust from dead tree in 1:1 ratio). Cultures were transferred to greenhouse. Three weeks later, rate of surviving plants was calculated. Pseudostem growth (length and circumference) and the appearance of new leaves (number, length and width) were also observed. After one month in greenhouse, cultures were transfered to field and survival rate was assessed two months after.

Culture conditions

All explants were cultured on different types of media and transferred to growth room in dark for shoots multiplication or under 16 : 8 h (light : dark) photoperiod at 25 ± 2°C and with light intensity of 20 µmol m^{-2} s^{-1} for rooting.

Statistical analysis

Experiments were carried out using completely randomized design. Each treatment was repeated three times with ten replicates per repetition. Each replicate consists of one explant per glass bottles. Data were registered four weeks after inoculation and were analyzed using the analysis of variance (ANOVA). Differences among treatment means were compared by using Least Significance Difference (LSD) test at 5% probability level. Data statistical analysis was carried out using STATISTICA 7.1 version.

RESULTS

Influence of different cytokinins on shoot mutiplication

Microshoots cultured onto MS medium supplemented with various cytokinins were able to regenerate multiple shoots of the two banana varieties. The mean number of shoots per explants and the mean length of shoot in each tested medium are shown Table 1. Amongst various concentration, BAP (3 mg/L) gave the highest mean number of shoots per explants both for FHIA-21 (6.00 shoots) and PITA-3 (5.80 shoots) (Figure 1d and e). The longest induced shoots from shoot-tip explants were obtained in the presence of 4 mg/L BAP. The mean shoot length was 3.40 cm (FHIA-21) and 3.57 cm (PITA-3).

Shoots multiplication in MS media supplemented with different additives

Significant differences were observed when Ads, CH and

Table 1. Effect of cytokinins on *in vitro* multiple shoots regeneration of two banana varieties cultured on MS medium after 30 days of culture.

Cytokinins (mg/L)			FHIA-21		PITA-3	
BAP	Kin	2-iP	Mean number of shoots	Mean length of shoots (cm)	Mean number of shoots	Mean length of shoots (cm)
1	-	-	3.70 ± 0.44^{bcd}	2.61 ± 0.78^{a}	3.00 ± 0.29^{de}	1.91 ± 0.31^{cde}
2	-	-	4.10 ± 0.72^{bc}	2.57 ± 0.57^{a}	5.10 ± 0.48^{ab}	2.13 ± 0.53^{cd}
3		-	6.00 ± 0.71^{a}	3.19 ± 0.70^{a}	5.80 ± 0.38^{a}	2.22 ± 0.30^{bc}
4	-	-	5.00 ± 0.53^{ab}	3.40 ± 0.48^{a}	4.80 ± 0.57^{ab}	3.57 ± 0.70^{a}
5	-	-	3.50 ± 0.54^{bcd}	1.01 ± 0.18^{b}	3.20 ± 0.59^{cde}	3.10 ± 0.37^{ab}
-	1	-	5.10 ± 0.90^{ab}	1.48 ± 0.17^{b}	4.60 ± 0.61^{abc}	1.65 ± 0.45^{cdef}
-	2	-	4.30 ± 0.65^{abc}	1.39 ± 0.22^{b}	4.80 ± 0.53^{ab}	1.52 ± 0.23^{cdef}
-	3	-	3.80 ± 0.48^{bcd}	2.65 ± 0.42^{a}	5.00 ± 0.33^{ab}	1.36 ± 0.14^{cdef}
-	4	-	3.40 ± 0.42^{bcd}	1.21 ± 0.26^{b}	3.20 ± 0.78^{cde}	1.30 ± 0.20^{def}
-	5	-	3.50 ± 0.45^{bcd}	1.09 ± 0.17^{b}	3.10 ± 0.99^{de}	1.15 ± 0.07^{ef}
-	-	1	3.20 ± 0.55^{cd}	0.83 ± 0.07^{b}	3.10 ± 0.40^{de}	0.79 ± 0.07^{f}
-	-	2	4.10 ± 0.64^{bc}	0.95 ± 0.09^{b}	4.20 ± 0.57^{bcd}	0.89 ± 0.05^{f}
-	-	3	2.90 ± 0.62^{cd}	1.43 ± 0.14^{b}	2.80 ± 0.32^{de}	1.02 ± 0.09^{f}
-	-	4	2.90 ± 0.84^{cd}	1.04 ± 0.07^{b}	2.50 ± 0.34^{e}	1.07 ± 0.12^{ef}
-	-	5	2.30 ± 0.47^{d}	0.82 ± 0.12^{b}	2.10 ± 0.23^{e}	1.14 ± 0.08^{ef}

Spd were used alone or in combination (Table 2). Results showed that all additives supplemented individually or in combination, influenced shoot multiplication significantly. Supplied individually, Ads (25 mg/L), CH (50 mg/L) and Spd (100 mg/L) improved shoot multiplication in variety FHIA-21, while Ads (25 mg/L), CH (25 mg/L) and Spd (200 mg/L) were found to improve shoot multiplication in PITA-3. Additives combination was found to be better than their individual effects (Figure 1f). Dealing with FHIA-21 variety, the highest shoots mean (12.50 cm) per explants were observed on MS medium supplemented with Ads (25 mg/L) in combination with CH (25 mg/L) and Spd (100 mg/L). In respect with PITA-3 variety, Ads (25 mg/L) in combination with CH (50 mg/L) and Spd (200 mg/L) gave the highest shoots average (11.12 cm). Additives in combination had significant positive effect on shoot elongation (Figure 1f). The higher concentration of Spd (200 mg/L) was found more appropriate for shoots elongation. The highest mean shoot lengths were 3.35 cm (FHIA-21) and 2.97 cm (PITA-3) (Table 2).

In vitro rooting of microshoots

Except control, all media induced roots and the best results were observed on medium supplemented with NAA (0.5 mg/L) in terms of number of root per shoot with 11.20 in FHIA-21 and 13.80 in PITA-3. While, in terms of root length, IBA (0.5 mg/L) was found to be the best with 4.26 cm (FHIA-21) and 4.65 cm (PITA-3). Addition of CH was found to be effective in enhancing the shoot number and length per explant (Table 3 and Figure 1g to h). In FHIA-21, the number of roots per explants was high in the medium supplemented with NAA (0.5 mg/l) + CH (50 mg/L); an average number of 20.80 roots and average root length of 6.67 cm were obtained. Whereas, in PITA-3, medium supplemented with NAA (0.5 mg/L) + CH (25 mg/L) was found to be the most suitable with 22.40 number of roots and 6.39 cm root length average (Table 3).

Acclimatization of regenerated plants

One month acclimatization in greenhouse showed that survival rate that ranged from 96 to 100% in both varieties and with all substrates.

Addition of sawdust from dead tree to forest soil

Table 2. Different adenine sulphate, casein hydrolysate and spermidine concentrations effects on shoot multiplication of two banana varieties after 4 weeks of culture.

Ads (mg/L)	CH (mg/L)	Spd (mg/L)	FHIA-21		PITA-3	
			Mean number of shoots	Mean length of shoots (cm)	Mean number of shoots	Mean length of shoots (cm))
Control	-	-	2.12 ± 0.22^{f}	1.42 ± 0.10^{ef}	2.62 ± 0.37^{j}	1.23 ± 0.10^{f}
5	-	-	4.50 ± 0.88^{de}	1.56 ± 0.24^{def}	3.75 ± 0.61^{ij}	1.33 ± 0.08^{f}
10	-	-	4.75 ± 0.52^{de}	2.15 ± 0.23^{c}	4.12 ± 0.51^{hij}	1.92 ± 0.09^{cd}
25	-	-	8.50 ± 0.50^{b}	2.66 ± 0.28^{b}	7.00 ± 0.75^{cdef}	2.25 ± 0.23^{bc}
50	-	-	5.25 ± 0.95^{de}	1.37 ± 0.13^{f}	3.62 ± 0.80^{ij}	1.44 ± 0.10^{ef}
-	5	-	5.00 ± 0.84^{de}	1.59 ± 0.11^{def}	6.00 ± 0.73^{fg}	1.59 ± 0.10^{def}
-	10	-	5.87 ± 0.74^{de}	1.63 ± 0.10^{def}	6.50 ± 0.26^{def}	2.11 ± 0.29^{c}
-	25	-	6.25 ± 0.31^{cd}	1.89 ± 0.07^{cde}	8.12 ± 0.78^{bcd}	1.96 ± 0.08^{cd}
-	50	-	8.87 ± 0.93^{b}	2.04 ± 0.21^{cd}	5.62 ± 0.59^{fgh}	1.59 ± 0.19^{def}
-	-	50	4.00 ± 0.32^{ef}	1.43 ± 0.12^{ef}	4.50 ± 0.46^{ghi}	1.89 ± 0.21^{cde}
-	-	100	9.12 ± 0.69^{b}	1.90 ± 0.28^{ce}	6.25 ± 0.49^{efg}	1.85 ± 0.10^{cde}
-	-	200	5.00 ± 0.65^{de}	1.48 ± 0.07^{ef}	8.87 ± 0.74^{b}	1.94 ± 0.20^{cd}
25	25	100	12.50 ± 0.42^{a}	3.07 ± 0.20^{ab}	7.87 ± 0.93^{bcde}	2.82 ± 0.13^{a}
25	50	100	8.00 ± 0.46^{bc}	2.92 ± 0.20^{ab}	7.00 ± 0.62^{cef}	2.63 ± 0.19^{ab}
25	25	200	9.25 ± 0.88^{b}	2.90 ± 0.16^{ab}	8.50 ± 0.42^{bc}	2.97 ± 0.16^{a}
25	50	200	9.87 ± 0.97^{b}	3.35 ± 0.28^{a}	11.12 ± 0.85^{a}	2.73 ± 0.14^{a}

Mean values within a column followed by the same letters are not significantly different at $p < 0.05$ according to least significance difference (LSD).

Table 3. Effect of different concentrations of casein hydrolysate (CH) in combination with NAA and IBA number and mean length of roots after three weeks culture.

NAA (mg/L)	IBA (mg/L)	CH (mg/L)	FHIA-21			PITA-3		
			Rooting (%)	Mean number of roots	Mean length of roots (cm)	Rooting (%)	Mean number of roots	Mean length of roots (cm)
Control	-	-	0.00 ± 0.00^{c}	0.00 ± 00^{f}	0.00 ± 0,00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{f}	0.00 ± 0.00^{d}
0.5	-	-	80 ± 0.13^{ab}	11.20 ± 0.95^{d}	4.26 ± 0.41^{b}	100 ± 0.00^{a}	13.80 ± 0.78^{de}	3.91 ± 0.31^{c}
-	0.5	-	70 ± 0.15^{b}	9.00 ± 0.44^{e}	3.90 ± 0.36^{b}	90 ± 0.00^{a}	12.70 ± 0.70^{e}	4.65 ± 0.40^{bc}
0.5	-	25	100 ± 0.00^{a}	16.10 ± 0.76^{b}	5.85 ± 0.59^{a}	100 ± 0.00^{a}	22.40 ± 0.49^{a}	6.39 ± 0.72^{a}
0.5	-	50	100 ± 0.00^{a}	20.80 ± 0.61^{a}	6.67 ± 0.57^{a}	100 ± 0.00^{a}	19.60 ± 0.79^{b}	6.05 ± 0.66^{a}
-	0.5	25	90 ± 0.10^{ab}	13.20 ± 0.75^{c}	5.35 ± 0.73^{ab}	80 ± 0.13^{a}	15.40 ± 0.74^{cd}	5.30 ± 0.45^{ab}
-	0.5	50	100 ± 0.00^{a}	14.50 ± 0.81^{bc}	5.22 ± 0.57^{ab}	80 ± 0.13^{a}	16.10 ± 0.90^{c}	5.40 ± 0.29^{ab}

Mean values within a column followed by the same letters are not significantly different at $p < 0.05$ according to least significance difference (LSD).

or sand improved plantlets growth though different response on compost substrate was significantly higher. Pseudostem growth (length and

Table 4. Effect of different substrates on plant growth after one month of acclimatization of the two banana varieties

Substrates	FHIA-21					PITA-3				
	Length of pseudostem (cm)	Circumference of pseudostem (cm)	Number of leaves	Leaf length (cm)	Leaf width (cm)	Length of pseudostem (cm)	Circumference of pseudostem (cm)	Number of leaves	Leaf length (cm)	Leaf width (cm)
Compost	8.80 ± 0.46^{a}	3.74 ± 0.25^{a}	7.60 ± 0.30^{a}	11.36 ± 0.64^{a}	3.43 ± 0.36^{a}	10.64 ± 0.52^{a}	3.63 ± 0.15^{a}	6.60 ± 0.16^{a}	11.55 ± 0.36^{a}	3.57 ± 0.29^{a}
Forest soil	7.50 ± 0.31^{bc}	2.50 ± 0.18^{b}	4.60 ± 0.22^{c}	10.71 ± 0.66^{a}	2.92 ± 0.18^{ab}	7.87 ± 0.36^{bc}	2.55 ± 0.17^{bc}	4.90 ± 0.52^{bc}	9.92 ± 0.56^{a}	2.98 ± 0.39^{ab}
Sand	6.57 ± 0.08^{c}	1.92 ± 0.34^{b}	4 .80 ± 0.32^{bc}	8.10 ± 0.60^{b}	2.89 ± 0.32^{ab}	7.20 ± 0.30^{c}	1.90 ± 0.13^{d}	4.60 ± 0.22^{c}	9.76 ± 0.82^{a}	2.74 ± 0.29^{b}
Forest soil + Sawdust from dead tree	7.84 ± 0.69^{ab}	2.14 ± 0.17^{b}	5.40 ± 0.54^{bc}	10.18 ± 0.70^{a}	2.16 ± 0.14^{b}	8.70 ± 0.41^{b}	2.43 ± 0.31^{cd}	5.80 ± 0.38^{ab}	11.29 ± 0.81^{a}	2.87 ± 0.13^{ab}
Sand + Sawdust from dead tree	7.60 ± 0.26^{bc}	3.20 ± 0.19^{a}	5.70 ± 0.33^{b}	10.54 ± 0.63^{a}	3.16 ± 0.26^{a}	8.42 ± 0.47^{b}	3.05 ± 0.13^{b}	5.30 ± 0.42^{bc}	10.35 ± 0.54^{a}	3.28 ± 0.26^{ab}

Mean values within a column followed by the same letters are not significantly different at $p < 0.05$ according to least significance difference (LSD).

Figure 2. Hardening of in vitro rooted plants in greenhouse and transplantation in the field. (a) Plantlets on different substrates (S1) compost, (S2) forest soil, (S3) sand, (S4) Forest soil + sawdust from dead tree, (S5) sand + sawdust from dead tree. (b) Acclimatized plants in the field after two months of transplantation.

circumference) was less important on sand substrate for the two varieties compared to the other substrates (Table 4 and Figure 2a). The mean (number, length and width) of leaves produced on compost substrate was also significantly higher than on others substrates. Six weeks later in the greenhouse, plantlets were finally transferred to the field (Figure 2b) and 2 months later the survival rate of the plants was

100%.

DISCUSSION

Various types and concentrations of cytokinins were used to study their effect on shoot multiplication using shoot tip explants of two banana varieties (FHIA-21 and PITA-3). Amongst the cytokinins, BAP is the most widely used, most effective and affordable for the proliferation of multiple shoots (Johnson and Manickam, 2003). BAP performance over other cytokinins has also been reported for some banana varieties. In our study, BAP (3 mg/L) was found to be suitable cytokinin for shoot proliferation. This result is in accordance with those obtained in banana varieties Dward and Poyo (Asmare et al., 2012; Kalimuthu et al., 2007). The same result was reported by Bikram and Bikram (2016) who stated that 3 mg/L BAP gave a maximum number of shoot buds. Similar results have been reported in banana with 4 mg/L BAP (Muhammad et al., 2007; Shiv et al., 2014). In addition, Shirani et al. (2011) mentioned that BAP (5 mg/L) was optimal for shoot proliferation as well as for shoot elongation from excised scalps of banana cultivars. Inclusion of Ads and CH in culture medium improved the frequency of multiple shoot production. The results of this study were similar with earlier observations made in *Citrus reticulata* Blanco (Siwach et al., 2012). In the study, 25 mg/L of Ads produced the highest average number of shoot 8.50 (FHIA-21) and 7.00 (PITA-3) shoots per explant. Similar results were reported by Sanghamitra and Maiti (2011) who stated that inclusion of Ads (25 mg/L) in the culture medium improved the frequency of multiple shoot production in *Chlorophytum arundinaceum* (Liliales : Liliaceae). Polyamines have been reported to play a vital role in cell division and differentiation (Yamada et al., 1986; Basu et al., 1989) and to help in the regulation of plant growth and development (Tisi et al., 2011). In the current study, 100 mg/L of spermidine in the medium increased the shoot multiplication in FHIA-21 (9.12 shoots per explant), while 200 mg/L of spermidine gave maximum number of shoot in PITA-3 (8.87 shoots per explant). Spermidine is essential for shoot multiplication, as reported in cucumber (Vasudevan et al., 2008), sugarcane (Shankar et al., 2011) and *W. tomentosa* (Joshi et al., 2014). Supplementation of CH to culture medium was effective in shoot multiplication. The number of shoots was found to be enhanced with 50 mg/L in FHIA-21 and 25 mg/L in PITA-3. Similar results have also been found with CH in *Anogeissus pendula*, *Anogeissus latifolia* (Saxena and Dhawan, 2001) and in *Crataeva nurvala* (Walia et al., 2007). Media components such as amino acids have demonstrated a profound effect on tissue culturing systems of several species (Benson, 2000; El-sharabasy et al., 2016). The mixture of amino acids like CH is frequently used as sources of organic nitrogen in culture media. On the other hand, it has been found that the combined effect of Ads, Spd and CH was more suitable for shoot multiplication when used in culture medium than their individual effects.

Rooting is a crucial step. Success of the protocol also depends on frequency of root induction (e.g., number roots and root length). In the present study, all media used were able to induce roots and exhibited good response in terms of number and length of roots produced. The inclusion of casein hydrolysate improved the number and length of roots. These results corroborate with those of Shereen et al. (2016) who found that casein hydrolysate was more effective for rooting in terms of number and length of roots in *Phoenix dactylifera* L. In contrast to this finding, Marvin et al. (2012) showed that casein hydrolysate did not have any significant influence on root production in *Hyoscyamus niger* L.

Transfer of *in vitro* plantlets to the *ex vitro* environment is extremely important as it can result in significant loss of propagated material. Loss could be the fact that, plantlets were produced under high humidity and low light intensity conditions.

In this study, acclimatization was successfully carried out in the greenhouse. The survival rate varied from 96 to 100% for all the tested substrates. Similar type of response was observed in terms of survival rate with different substrates mixture by various authors. One hundred percent of survival rate was obtained with the mixture soil : sand : farm yard manure (FYM) (2:1:1) in banana cv. Grand naine (Shahnawaz et al., 2014) and with sand, soil and vermicompost in banana cv. Matti (Lohidas and Sujin, 2015). In *Eurycoma logifolia* plants grown in potting media, jiffy 7 showed 100% survival (Muhammad et al., 2015). In the current study, compost was found to be more suitable for acclimatization process followed by the mixture of sawdust from dead tree and forest soil. Although, compost is a very good substrate, it is expensive and could not be accessible to small farmers. Its substitution by local substrates as a mixture of sawdust from dead tree and forest soil or sawdust from dead tree and sand could be more profitable to small farmers. In this study, all acclimatized plants were finally transferred to field conditions and grew normally in the natural environment. No phenotypic variability was observed in plants in this experiment.

Conclusion

The study showed advantages of the inclusion of Ads, CH and Spd in *in vitro* micropropagation of two banana varieties using shoot tip explants. A highly efficient micropropagation system, capable of sustainable multiplication of shoots, was obtained for two banana varieties. Inclusion of Ads, CH and Spd is most effective for shoot multiplication. MS medium supplemented with

1 mg/L 2-iP, 0.5 mg/L NAA, and 25 or 50 mg/L CH was suitable for PITA-3 and FHIA-21 root induction. Compost was suitable for acclimatization of *in vitro* derived plantlets. In absence of compost, a mixture of sawdust from dead tree and forest soil or sawdust from dead tree and sand could be used as a good substrate.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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Production and characterization of proteases from edible mushrooms cultivated on amazonic tubers

Ana Rita Gaia Machado[1*], Salomão Rocha Martim[1], Mircella Marialva Alecrim[2] and Maria Francisca Simas Teixeira[3]

[1]Universidade Federal do Amazonas, Rede de Biodiversidade e Biotecnologia da Amazônia Legal –Bionorte, Av. General Rodrigo Octavio Jordão Ramos, 1200 - Coroado I, Manaus - AM, 69067-005, Brazil.
[2]Departamento de Engenharia Agrícola e de Solos–UFAM. Faculdade de Ciências Agrárias, Universidade Federal do Amazonas, Av. General Rodrigo Octávio, 6200, Coroado I, Cep: 69077-000, Manaus, Amazonas, Brazil.
[3]Departamento de Parasitologia, Universidade Federal do Amazonas, Instituto de Ciências Biológicas, Coleção de Culturas DPUA –UFAM. Av. General Rodrigo Octávio, 6200, Coroado I, Cep: 69077-000, Manaus, Amazonas, Brazil.

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Proteases are important commercial enzymes, and among their numerous sources are the Basidiomycetes. The use of proteases in many industrial areas promotes the search for enzymes with new properties. The aim of this study is to produce and characterize peptidases of a biocomposite from mycelial biomass grown in Amazonic tubers. *Lentinus citrinus* DPUA 1535 and *Pleurotus ostreatoroseus* DPUA 1720 were cultivated on *Dioscorea trifida*, *Manihot esculenta* and *Dioscorea alata* supplemented with rice bran or manioc flour residue in different proportions. The highest proteolytic activity was determined in the crude extract from *P. ostreatoroseus* grown in *D. alata* (DA) without supplementation (142.22 U/mL). The enzymes showed optimum activities at 40°C and pH 7.0; and stability at 50°C and pH 8.0. The proteases were classified as cysteine proteases based on the effect of inhibitors used.

Key words: *Pleurotus, Lentinus, Dioscorea*, protease.

INTRODUCTION

Proteases are enzymes with important biotechnological use. They have applications in chemical and biochemical reaction on food, beverages, pharmaceutical products and cosmetics. Proteases represent one of the biggest groups of world industrial enzymes with perspective increasing around 7% until 2020 (Singh et al., 2016; Geng et al., 2016; Chandrasekaran et al., 2015).

Peptidases can be obtained from microorganism (bacteria and fungi), plants or animals. However, the microbial sources of these enzymes have preference to be used as protease producers due to their physiological and biochemical properties, suitability to genetic manipulation and short time of fermentation process (Sharma et al., 2017; Souza et al., 2016).

The edible mushrooms, *Lentinus crinitus*, *Lentinus citrinus*, *Pleurotus ostreatoroseus*, *Pleurotus florida* and

*Corresponding author. E-mail: ritamachado.nutri@hotmail.com.

Table 1. Substrates mixtures in solid state fermentation of *L. citrinus* and *P. ostreatoroseus.*

Substrate	Proportion (tuber : supplement)
ME	100:0
ME+RB10	90:10
ME+RB20	80:20
ME+MR10	90:10
ME+MR20	80:20
DT	100:0
DT+RB10	90:10
DT+RB20	80:20
DT+MR10	90:10
DT+MR20	80:20
DA	100:0
DA+RB10	90:10
DA+RB20	80:20
DA+MR10	90:10
DA+MR20	80:20

Substrates: ME= *Manihot esculenta*, DT= *Dioscorea trifida*, DA= *Dioscorea alata*, RB= rice bran, MR= manioc flour residue.

Pleurotus albidus have been reported as enzymes sources, including proteases (Fonseca et al., 2014; Kirsch et al., 2011; Martim et al., 2017; Souza et al., 2016). Edible mushrooms are consumed by many civilizations for centuries due to their nutritional and dietetic properties. They present high quantities of protein, fiber and low levels of fats and produce enzymes, vitamins, antimicrobial compounds, antioxidants and immune stimulants (Reid et al., 2017).

Edible mushrooms promote important benefit to health due to their nutritional composition. Their protein content is similar to the ones from animal and plant sources and higher than most of other food. Edible mushrooms contain all essential amino acids that are required in human diet (Nwoko et al., 2017).

The cultivation of mushrooms to produce biocompounds from the combination of mycelium and substrates of different compositions allows one to obtain many compounds of biological activity (Haneef et al., 2017). Pulp of tubers of *Dioscorea trifida* (cará-roxo), *Dioscorea alata* (inhame roxo) and *Manihot esculenta* (macaxeira) can serve as substrates with nutritional properties that can be used to cultivate edible mushrooms and also for the production of biocatalysts as peptidases. Considering the availability of edible tubers in Amazon, this research aims to produce and characterize peptidases of a biocomposite from mycelia biomass grown in Amazonic tubers.

MATERIALS AND METHODS

Edible mushrooms

L. citrinus DPUA 1535 and *P. ostreatoroseus* DPUA 1720 were the selected edible mushrooms species for this study (Micoteca DPUA, Federal University of Amazonas- UFAM). The mushrooms were cultivated on potato dextrose agar (PDA) with 0.5% (w/v) yeast extract to obtain the matrix culture.

Substrates

The tubers *D. trifida* (cará-roxo), *M. esculenta* (macaxeira) and *D. alata* (inhame-roxo) were obtained from a local market of Manaus (Amazonas/Brazil). They were washed and sanitized in sodium hypochlorite (50 ppm) for 10 min. The peel was removed and the tubers were cut in cubes of 1 cm, distributed in polyethylene bags and sterilized at 121°C for 10 min (Brasil, 2007).

Inoculum selection

The inoculum was chosen from selection of culture media. The mushrooms were cultivated on potato agar dextrose with 0.5% (w/v) yeast extract (PDA) and oat bran agar with 0.1% yeast extract (OMYA) distributed in Petri dishes. The cultures were maintained at 25°C for 8 days in the absence of light. The selected culture medium was the one that promoted significant radial growth. The radial growth was evaluated by measuring the colony diameter every 24 h until there was complete colonization of medium surface in the dish.

The mycelial vigor was classified by a subjective method of grades: grade 1 weakly dense, grade 2 moderately dense and grade 3 strongly dense (Fonseca et al., 2014). The medium that promoted significant radial growth was used for mushroom cultivation. From this culture, 10 mycelial discs (Ø = 10 mm) were inoculated in 50 mL of glucose, peptone and yeast extract (GYP). The fermentation was carried out at 25°C and 150 rpm. After five days, the biomass was separated from crude extract by filtration using an aluminum sieve (Ø = 75 mm).

Solid state fermentation

The recovered biomass of submerged fermentation was inoculated in the substrates supplemented with rice bran or manioc flour residue (*crueira*) in different proportions (Table 1). The patterns were the substrates without supplementation. The fermentation was carried out at 25°C, in the absence of light, 60% humidity, until there was complete colonization of the mycelium in the substrates. All the experiments were made in triplicate

After myceliation was completed in the tubers, they were dehydrated at 40°C in forced air oven for 24 h. Then, they were crushed and the granules were standardized with sieve of 10 mesh diameter.

Enzymes extraction and determination of proteolytic activity

The enzymes were extracted in distilled water using the proportion 1:5 (myceliated tuber : water). The mixture was maintained at 25°C, and 150 rpm for 1 h. The crude extract was recovered by vacuum filtration using Whatman no. 1 filter paper.

Proteolytic activity was determined according to the methodology described by Leighton et al. (1973). A mixture containing 0.15 mL of crude extract and 0.25 mL substrate [1% (w/w) azocasein in 0.2 M Tris-HCl buffer, pH = 7.2] was incubated for 60 min in the absence of light. The reaction was interrupted by addition of 10% (w/w) trichloroacetic acid and centrifuged (8000 rpm) for 15 min at 4°C. The supernatant (0.8 mL) was added to 1.4 mL of 1 M NaOH. One unit of proteolytic activity was defined as the amount of enzyme that promotes a 0.01 increase of absorbance in one hour at 440 nm.

Table 2. Morphological characteristics and mycelial growth (mm) of *L. citrinus* and *P. ostreatoroseus* cultivated in PDA + YE and OMYA + YE (after 6 days).

Mushroom	Media	Mycelium color	Mycelial vigor	Mycelial growth (mm)
L. citrinus DPUA 1535	PDA + YE 0.5% OMYA + YE 0.1%	White	2	48.8±0.2[b]
		White	2	65.5±0.3[a]
P. ostreatoroseus DPUA 1720	PDA + YE 0.5% OMYA + YE 0.1%	White	2	64.0±0.1[b]
		Pinksh	3	67.0±0.2[a]

Means with same letters in a row are not different according to Tukey's test ($p<0.05$). PDA = Potato dextrose agar; YE = yeast extract, OMYA = oat bran agar.

Effect of pH and temperature on enzyme activity and stability

To assay optimum pH, proteolytic activity was determined at 25°C, with azocasein in different pH ranges using the following 0.1 M buffer solutions: citrate (5.0 and 6.0), phosphate (7.0 and 8.0) and carbonate-bicarbonate (9.0 and 10.0). Optimum temperature was determined by incubating the enzyme extract with azocasein at temperatures ranging from 25 to 80°C and assaying the activity at the pH determined as optimum.

For the pH stability, the crude extract was dispersed (1:1), for one hour, in the following 0.1 M buffer solutions: citrate (5.0 and 6.0), phosphate (7.0 and 8.0) and carbonate-bicarbonate (9.0 and 10.0); it was incubated in azocasein and maintained at optimum temperature for 1 h. For thermal stability study, the enzyme extracts were incubated in azocasein at different temperatures ranging from 25 to 80°C for 1 h. All samples were prepared in triplicate.

Effect of protease inhibitors and metal ions on enzyme activity

The effect of inhibitors and metal ions on enzyme activity was investigated by using 10 mM of calcium chloride ($CaCl_2$), potassium chloride (KCl), sodium chloride (NaCl), copper sulphate ($CuSO_4$), ferrous sulphate ($FeSO_4$), zinc sulphate ($ZnSO_4$) and protease inhibitor compounds such as phenyl-methylsulfonyl fluoride (PMSF), ethylene-diaminetetraacetic acid (EDTA), iodoacetic acid and pepstatin A (10 mM). The crude extracts were incubated with the solutions of ions and inhibitors at 50°C for 1 h. After this time, they were incubated in 1% (w/v) azocasein at 40°C for 60 min, in the absence of light. Residual enzyme activities were determined and compared with the control which was incubated without the inhibitors (0% inhibition) and metal ions and corresponds to 100% of enzyme activity. All samples were prepared in triplicate (Alecrim et al., 2015).

RESULTS AND DISCUSSION

Inoculum selection

Table 2 shows the results of radial growth of *L. citrinus* and *P. ostreatoroseus* in solid medium, for six days. The significant value of growth and higher mycelial density in both cultures was observed in oat bran agar and yeast extract (OMYA+YE) medium (67.0 and 65.5 mm, respectively).

L. citrinus presented moderately dense white mycelium in OMYA+YE and in potato dextrose agar and yeast extract (PDA+YE), while *P. ostreatoroseus* presented moderately dense white mycelium in PDA+YE and strongly dense pinkish mycelium in OMYA+YE. According to these results, OMYA+YE medium was considered appropriate for the growth of both cultures in the analyzed conditions. The results are similar to other studies that presented mushroom growth and morphology in pure culture using different culture media. This proves that this condition influences fungi growth (Sastre-Ahuatzi et al., 2007; Wiriya et al., 2014; Masoumi et al., 2015).

The effect of different media cultures on the mycelial growth of basidiomycetes was reported in the study of Okwulehie and Okwujiako (2008). They observed that OMYA stimulated the growth of *P. ostreatus* var. *florida* Eger. *Lentinula edodes* presented a dense mycelium when cultivated on OMYA in the study of Escobar et al. (2007). The oat is considered a food with high nutritional value containing carbohydrates, amino acids, minerals and vitamins (Rasane et al., 2015). The *in vitro* cultivation aims to clarify the optimum conditions of fungi species growth related to availability of nutrients in the medium culture, temperature and time of incubation. This knowledge is an important prerequisite to possible cultivation in large scale (Andrade et al., 2010).

Proteolytic activity

The proteolytic activity of the extracts obtained from *L. citrinus* and *P. ostreatoroseus* solid fermentation in different mixtures of tubers and supplements is shown in Table 3. In all studied conditions of solid fermentation, proteases were produced, but the protease activity was different according to the type of supplementation and fungi species. The highest proteolytic activity was determined from *P. ostreatoroseus* grown in *D. alata* (DA) without supplementation (142.22 U/mL), while the lowest proteolytic activity (24.88 U/mL) was determined in *M. esculenta* supplemented with 10% of manioc residue (ME+MR 10). *L. citrinus* presented high proteolytic activity in DF+RB 20 (52.40 U/mL) and low in DA+RB 10 (10.73 U/mL). The results of this study with *P. ostreatoroseus* were higher than the ones reported by Fonseca et al. (2014), who observed significant proteolytic activity (7.89 U/mL) from *P. ostreatoroseus* using *cupuaçu* exocarp supplemented with 20% rice bran as

Table 3. Proteolytic activity of *L. citrinus* and *P. ostreatoroseus* cultivated in tropical tubers supplemented with rice bran or manioc flour residue.

Treatment	*L. citrinus* (U/mL)	*P. ostreatoroseus* (U/mL)
ME	23.11±1.9^{l}	31.26±0.6^{ij}
ME+RB20	51.11±0.7^{cd}	43.11±3.1^{fg}
ME+RB10	41.33±2.3^{g}	22.46±0.4^{l}
ME+MR20	43.55±1.5^{fg}	37.33±2.6^{h}
ME+MR10	32.66±0.6^{i}	18.00±0.6^{m}
DA	29.97±0.21^{ij}	142.22±0.8^{a}
DA+RB20	28.37±0.7^{jk}	48.89±2.3^{cd}
DA+RB10	10.73±0.2^{n}	24.88±0.3^{kl}
DA+MR20	30.11±0.3^{ij}	45.15±0.5^{ef}
DA+MR10	30.40±0.1^{ij}	44.31±0.1^{efg}
DT	42.47±0.1^{fg}	51.04±0.2^{cd}
DT+RB20	52.40±0.11^{bc}	54.82±0.7^{b}
DT+RB10	23.97±0.23^{l}	49.04±0.1^{cd}
DT+MR20	47.80±0.1^{de}	32.26±0.3^{i}
DT+MR10	44.37±0.2^{efg}	44.66±0.5^{efg}

Substrates: ME = (1) *Manihot esculenta*, DT = *Dioscorea trifida*, DA= *Dioscorea alata*, RB = rice bran, MR= manioc flour residue. Means with same letters in the line are not different according to Tukey's test ($p<0.05$).

substrate. Similar results were reported by Machado et al. (2016) and Souza et al. (2016) that cultivated *L. citrinus* on *cupuaçu* exocarp supplemented with litter and pineapple bark without supplementation, respectively. The results of this investigation revealed that the use of the tubers, especially *D. alata*, as substrates promoted the production of proteases by *P. ostreatoroseus* and *L. citrinus*.

Effect of pH and temperature on enzyme activity and stability

The proteolytic enzymes presented activity in all pH ranges analyzed. However, the optimum activity was observed at pH 7.0, maintaining around 88% of activity at pH 6.0. These results are in agreement with the ones reported by Fonseca et al. (2014) who cultivated *P. ostreatoroseus* on amazonic substrates (sawdust, açai seeds, *cupuaçu* exocarp, pineapple peel and pineapple pulp) and observed optimum activity of the enzymes at pH 6.0 to 7.0.

The production of extracellular proteases from *P. ostreatorouseus* cultivated on DA is probably associated with the mushroom that needs to be hydrolyzed in different types of substrates as nutritional source of protein. Nirma et al. (2011) reported that fungi can produce acid, neutral and alkaline proteases. One single species is capable to produce more than one kind of these enzymes with optimum activity in a wide range of pH (4.0 to 11.0).

P. ostreatoroseus also showed activity in all temperatures tested. But, the optimum activity was observed at 40°C. From this temperature, there was an increased activity (Figure 1B). At high temperatures, the enzymes suffer protein denaturation because the intramolecular bonds are affected (Ahmed et al., 2011). In the studies of Fonseca et al. (2014), Guan et al. (2011) and Machado et al. (2016), the optimum temperature activity of the mushrooms *P. ostreatoroseus*, *Pholiota nameko* and *L. citrinus* was also determined at 40 and 50°C, respectively.

The stability of pH and temperature is an important parameter in the enzymes application due to their determination of economic availability in the industrial processes (Moretti et al., 2012). The proteases of *P. ostreatoroseus* maintained stability at pH 7.0 and 8.0 with relative activity of 95 and 100%, respectively, for 60 min. The reduction of pH stability was observed at pH 9.0 (Figure 2A). At 40 and 50°C, the stability was maintained in 94 and 100% during 60 min. The inactivation of the enzymes was determined at 70°C (Figure 2B). According to Cheng et al. (2012), the thermostability of mushrooms proteolytic enzymes can be variable. Proteases from *L. citrinus* cultivated on *cupuaçu* exocarp and litter were active in all the temperatures tested. However, at 30°C they exhibited high activity for 60 min (Machado et al., 2016).

Effect of protease inhibitors and metal ions on enzyme activity

The enzymes of *P. ostreatoroseus* were inhibited at 95,

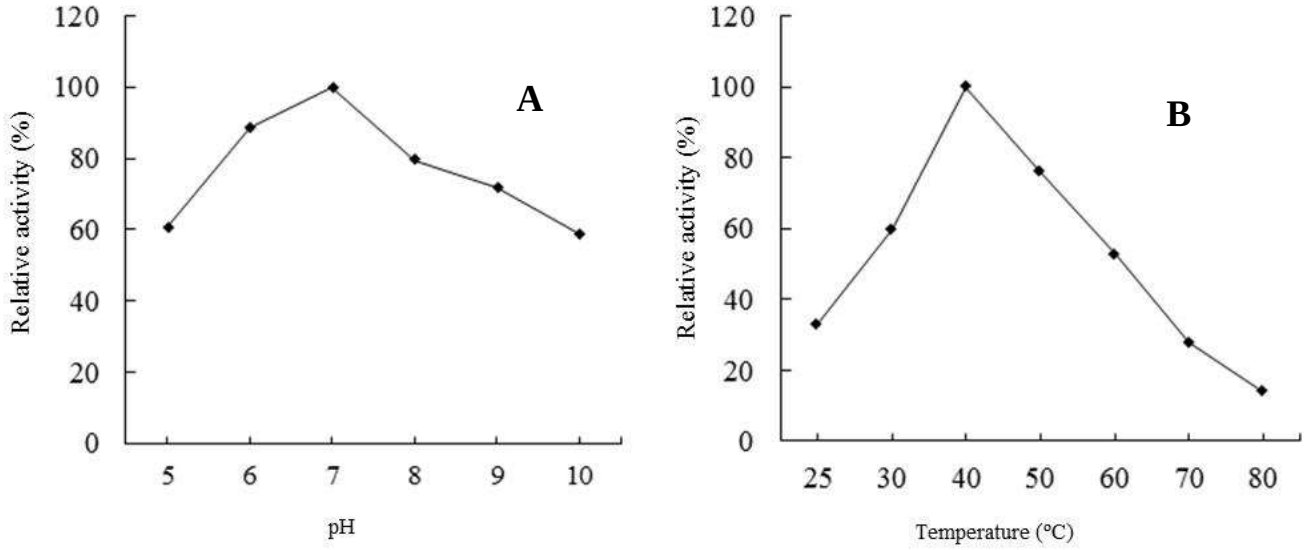

Figure 1. Effect of pH (A) and temperature (B) on proteolytic activity of *P. ostreatoroseus* cultivated on *Dioscorea alata*.

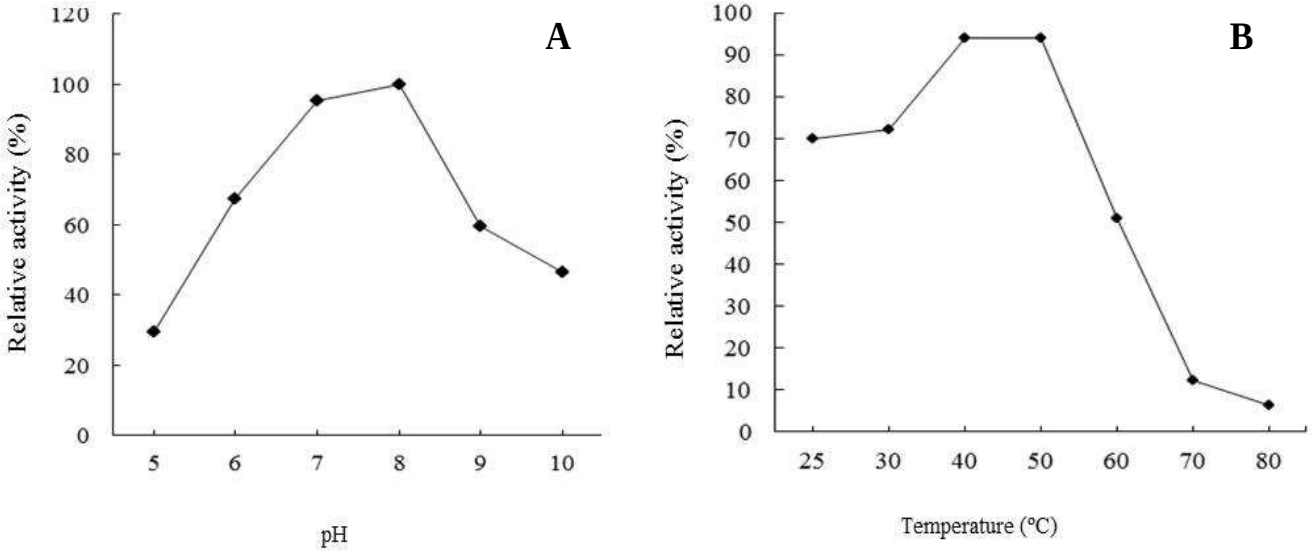

Figure 2. Effect of pH (A) and temperature (B) on stability of proteolytic activity of *P. ostreatoroseus* cultivated on *D. alata*.

94 and 87% by iodine acetic acid, PMSF and EDTA, respectively. These results suggest that the proteases be classified as cysteine, metalo and serine proteases. Some studies report the production of different types of proteases by mushrooms. Lebedeva and Proskuryakov (2009) and Zhang et al. (2010) observed inhibition in the proteases activity of *P. ostreatus* (Fr.) Kumm and *Hypsizigus marmoreus*, respectively using PMSF. This suggests the presence of serine proteases.

Based on the effect of metallic ions on the activity of *P. ostreatoroseus* enzymes, the ions Cu^{2+} and Zn^{2+} caused a reduction of 95% (Table 4). However, at similar conditions, the ions K^{+}, Mg^{2+} and Ca^{2+} did not have high influence on the proteases activity. Martim et al. (2017) showed that Zn^{2+} increased the activity of *P. albidus* enzymes at 78%. Ahmed and Helmy (2012) also observed the influence of Zn^{2+} at 67.7% with *Bacillus licheniformis* 5A5 enzymes. Couto and Sanromán (2006) showed that the interaction of metallic ions with fungi enzymes white rot is particularly important to the comprehension of biotechnology processes regulation of fungi degradation. The metallic ions can bond to amino acid residues and modify the protein structure that can have positive or negative proteolytic activity (Merheb-Dini

Table 4. Effect of metallic ions and inhibitors on the activity of proteases from *P. ostreatoroseus.*

Metallic ion and inhibitor	Inhibition (%)
Control	0
Cu^{2+}	95±0.7
Zn^{2+}	95±0.3
Fe^{2+}	34±0.6
Mg^{2+}	11±0.7
Mn^{2+}	52±0.3
Ca^{2+}	17±0.1
K^{+}	11±0.7
Na^{+}	21±0.6
Iodoacetic acid	95±0.2
EDTA	87±0.1
PMSF	94±0.8
Pepstatin A	36±0.1

et al., 2010).

Conclusion

The significant values of proteolytic activity were determined in the bioproduct from *P. ostreatoroseus myceliation* in D. alata tuber. In the experimental conditions, the data suggested the predominant presence of cysteine ande serine proteases. The protease expressed optimum activity at 40 °C and pH 7.0 with highest stability at 50 °C and pH 8.0.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Conversion of lignocellulose from palm (*Elaeis guineensis*) fruit fibre and physic (*Jatropha curcas*) nut shell into bio-oil

Onifade, T. B.[1]*, Wandiga, S. O.[2], Bello, I. A.[3], Jekayinfa, S. O.[1] and Harvey, P. J.[4]

[1]Department of Agricultural Engineering, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.
[2]Institute for Climate Change and Adaptation, University of Nairobi, Nairobi, Kenya.
[3]Department of Pure and Applied Chemistry, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.
[4]Faculty of Engr and Science, University of Greenwich Central Avenue, Kent, United Kingdom.

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Harmful gases are released into the atmosphere through burning of residues which is commonly practiced in Nigeria and can be attributed to climate change issues. Agricultural residues have the potentials to be used as energy and chemical source and meet its deficit in the country. This paper focuses on utilization of lignocellulosic materials obtained from two agricultural residues through renewable technology to produce bio-energy and chemical feedstock. The lignocellulosic materials were extracted from palm fruit (*Elaeis guineensis*) fibre and physic nut (*Jatropha curcas*) shell, and pyrolyzed under low temperature and pressure at various particle sizes. The main properties of solid (lignocellulosic) materials were tested and the bio-oil produced was analyzed using GC-MS. Results show proximate analyses (volatile, ash and fixed carbon contents) and ultimate analysis (carbon, oxygen, nitrogen, magnesium, phosphorus and zinc). The pH value of the bio-oil from both residues increased with increase in temperatures. The density, viscosity and calorific value of the palm and physic residue oil are 831.99 and 947.5 kg/m^3, 0.695 and 1.58 cPa at room temperature, 22.33 and 14.169 kJ/g, respectively. Aromatics and other compounds are major dominant compounds in the palm fruit fibre oil which is characterized for bio-fuel production. Physic nut shell oil contains aromatic ethers, cyclic ethers, secondary amides and organic halogen compound which are important chemical feedstock. Conversion of these residues to useful products will alleviate the energy supply deficit, improve social and economic development, promote clean and healthy atmosphere of the nation and significantly contribute to global climate change mitigation.

Key words: Palm fruit fibre, physic nut shell, lignocellulose, pyrolysis, bio-energy, climate change.

INTRODUCTION

Climate change can be described as a change in the statistical properties of the climate system when considered over long periods of time, regardless of cause (GCC, 2001). Climate change could be caused by human activity, as opposed to changes in climate that may have resulted as part of Earth's natural processes (NASA, 2011) and fluctuations over periods shorter than a few decades. In the context of environmental policy, the term *climate change* has become synonymous with anthropogenic global warming. Global warming is stated

as gradual increase in the average temperature of earth's surface and its ocean (Idowu et al., 2011) while climate change includes global warming and everything else that will be affected by increasing greenhouse gas levels (UNFCCC, 1994). Climate change also refers to a change of climate which is directly or indirectly attributed to human activity that affects the composition of the global atmosphere and which is in addition to natural climate variability observed over comparable time periods. Improper disposal and burning of agricultural residue are commonly practiced in rural areas of Southwest Nigeria and these are examples of the human activities that can contribute to climate change; extreme temperature and heat waves from burning activities release greenhouse gas emission into the atmosphere which can attribute to the global warming resulting to climate change. Therefore, it is profitable if these residues are converted to bioenergy and biochemical from affordable and surplus biomaterials to alleviate the climatic problem and hence the positive outcome can be resulted to policy making on environmental protection.

Biomass in general, can displace most of other fuel or source of energy, from the fact that biomass fuels have a very significant part to play in meeting our energy needs. It is a unique part with the realization of the finite limitation of the fossil resources, a sense of urgency has developed in the exploration of alternative energy and feedstock sources. Bioenergy technology could be defined as the conversion of biomass energy into other useful forms of energy for industrial and domestic purposes (FAO, 2015; Elum et al., 2016). Other forms of renewable energy that are predominantly used are from water and coal for generation of power, heating, cooling and transport fuels due to advanced technologies. Ackom and Ertel (2005) reported that about 60% of total energy consumed in Africa comes from biomass and by its projection in 2030; about 823 million people in Africa will depend on biomass. It is observed that more than 70% of the people living in the rural areas in Nigeria are using fuel wood, and the country uses more than 50 million tonnes of fuel wood annually (Oyedepo, 2014). This is due to insufficient and high prices of conventional fuel and this situation has led to both deforestation and desertification that are going on at the rate of 350,000 ha/annum while reforestation is only about 10% (Amigun et al., 2008) due to negligence attitude of people and weak policy in the country (Elum et al., 2016).

Agricultural residues can be transformed into various forms of biofuels and biogas. Bio-energy are produced from biomass and could be in the form of liquid, gaseous or solid state which can be used for cooking, heating, electricity generation, as fuels for transport and to replenish farm soil (FAO, 2015). Generally, biofuels and biogas are produced from biomass through biochemical (fermentation and anaerobic digestion) or thermochemical processes such as gasification, pyrolysis and liquefaction (Amigun et al., 2008). Biofuels can be made from the use of energy crops that have been cultivated exclusively for their production or from crop and forestry residues. These biofuels are wood-based methanol and biodiesel from soy, rapeseed oil and switch grass, also from corn and sugarcane-based ethanol known as bioethanol derived from fermentation of organic, sugar-rich substrates into alcohol through microbial degradation (Adebayo et al., 2013; Nehrenheim, 2014) for use in vehicles, machinery and generators (Oniya and Bamigboye, 2012). Biodiesel is basically a type of biofuels having about 38% higher energy than ethanol that could be made from oil crops such as canola, palm, rapeseed, sunflower, coconut and soybean (Fekete, 2013) and non-food energy crop such as physic nut (*Jatropha curcas* and *Eucalyptus* spp.) was discussed by Jatropha World Team (JWT, 2010). Biodiesel is meant for diesel engines and the demand for biodiesel in Nigeria has been projected to increase to 900 million litres in 2020 from the 480 million litres demanded as of 2007 (Samuel and Adekomaya, 2012). Biogas is formed when organic materials are degraded in the absence of oxygen (Itodo et al., 2007). It is a mixture of methane (60 to 70%), carbon dioxide (30 to 40%) and traces of hydrogen sulphide, ammonia, and carbon monoxide (Ngumah et al., 2013; Usman and Ekwenchi, 2013). Biogas technology is anaerobic digestion of organic materials which has been in existence since 1850s, and the main products are biogas (energy) and char (biofertilizer) which is the residual effluent (Ngumah et al., 2013; Usman and Ekwenchi, 2013). The black solid product, obtained after pyrolysis process known as char, has potential soil-quality benefits which include an increase in organic matter and soil carbon (Bello et al., 2009).

Pyrolysis is a process of the thermo-chemical conversion of biomass to char, bio-oil and gas, in the absence of oxygen and other reactants (Balat et al., 2009; Goyal et al., 2006). It is a non-equilibrium process where the biomass undergoes multistage decomposition resulting in large changes in specific volume. In this conversion process, combustion and gasification occur where complete or partial oxidation is allowed to proceed. The reaction rate, order and product yields depend on parameters such as temperature, heating rate, pre-treatment, catalytic effects, particle size etc (Bridgewater and Peacookie, 2000) and these parameters influence the optimum value in a fast pyrolysis process (Faisal et al., 2011). Moreso, Tsai et al. (2006) reported that fast pyrolysis is the most suitable process route to maximize

*Corresponding author: E-mail: tbonifade@lautech.edu.ng.

the yield of liquid product. It is on this note that the paper emphasizes the importance of pyrolysis on the extract lignocellulosic content of two agricultural residues to simultaneously respond to bioenergy and chemical production.

Lignocellulosics are three-dimensional, naturally occurring, polymeric composites primarily made up of cellulose, hemicelluloses, lignin, and small amounts of extractives and ash (Rowell, 1992). Cellulose is the focus of much research because it is the dominant lignocellulosic compound and “next generation fuels” mostly based on cellulose (Kullander, 2010; Valentine et al., 2012). Some sources of lignocellulosics include agricultural residues, water plants, grasses, wood, and other plant substances (Limayem and Ricke, 2012; Sanchez and Cardona, 2008). In addition, Bello et al. (2009) stated that cellulosic material is recognized as a possible supplement for long term requirement as abundant renewable resources in organic materials obtained from plants. Lignocellulosic materials have also been called photomass because they are a result of photosynthesis. Wood and other lignocellulosics have been used as “engineering materials” because they are economical, low in processing energy, renewable and strong (Rowell, 1992). Lignocellulosic can decompose because it is organic in nature and burn under high temperature. However, a key factor affecting the efficiency of bioethanol production is the complex composition of lignocellulosic materials during the conversion processes (Jordan et al., 2012; Himmel et al., 2007; Dixon, 2013). The plant cell walls consist of cellulose, hemicellulose, and lignin as the major components that comprise around 90% of its dry biomass (Gibson, 2012; Harris and Stone, 2008; Pauly and Keegstra, 2008). Lignocellulosics can also be combined in an inorganic matrix to produce composites and also be combined with plastic in several ways. One example of this technology is reinforced thermoplastic composites, which are lighter in weight, have improved acoustical, impact and heat reformability properties, and cost less than comparable products made from plastic alone. The concept of combining lignocellulosics with other materials provides a strategy for producing advanced composites to achieve enhanced properties of all types of materials. These advantages make possible the exploration of new processing techniques, new applications, and new markets (Rowell, 1992).

This work focus on alleviating and coping with the negative effects that emanates from fossil fuel-based energy sources, renewable energy utilization plays a vital role in meeting current and future energy demands. At different processing sites visited in the study community, it is observed that the selected agricultural residues (palm fruit fibre and physic nut shell) used in this work are improperly disposed and leads to land and water pollution. Sometimes, the residues are burnt which causes air pollution by releasing greenhouse gas into the atmosphere which can attribute to the problem of climate change and global warming. This study employed renewable technology for purpose of converting waste to energy and greatly contribute to global climate change mitigation. The detrimental effects of climate change require that alternative forms of energy such as biogas be utilized to avoid environmental catastrophes. The renewable technology activities can improve social development and economy identity and strength of the villages and cities and it also has beneficial and better environmental implications on the nation.

Figure 1. Fresh physic nuts (*Jatropha curcas*).

MATERIALS AND METHODS

Material and sample preparation

Physic nut shell is an agricultural residue obtained, after dehusking process of physic nut fruit (*Jatropha curcas*), which is left in the farm unused but decomposed over a long period. Palm fruit fibre is a waste material of African palm kernel fruits (*Elaeis guineensis*) after oil extraction process is done. Heap of this waste is formed at the processing site but this residue is not easily decomposed because of oil reminant in it and this has disrupted the landscapes, soil percolation and water infiltration are difficult resulted to low cultivation of other arable crops in the surrounding. The two residues (palm fruit fibre and physic nut shell) used for this research were collected from small scale processing centres in nearby villages via Ogbomoso town (8°07’ N, 4°16’E), Nigeria. Figures 1 to 4 show the fresh fruits and the residues.

The samples used were sun dried and ground into fine particle size. The samples were then screened to give various fractions using different wire mesh sizes. The diameter of the particle size of the residues used ranged from 0.250 to 0.550 mm in form of 0.250, 300, 0.425 and 0.550 mm. Each sample was mixed with sodium anhydrous in order to make it dried and remove totally the moisture in it. The extraction, pyrolysis and proximate analyses of the samples were carried out at the laboratory of the Department of Chemistry, University of Nairobi, Kenya while the samples were taken to Kenya Agricultural and Livestock Research Organization Laboratory for ultimate analyses. Proximate composition was investigated to determine the fuel property of the solid sample residue and this was carried out in the Department of Physics,

Figure 2. Physic nut shell (residues).

Figure 3. Fresh palm kernel fruits (*Elaeis guineensis*).

Figure 4. Palm fruit fibre (residue).

University of Nairobi, Kenya. The samples were analyzed for the volatile matter, ash and fixed carbon contents to determine the composition of residues (Faisal et al., 2011).

Experimental procedure

Extraction of the lignocellulosic material from the samples

Lignocellulose was prepared from physic nut shell and palm fruit fibre according to the methods reported by Bello et al. (2009). Fifty (50) grammes of the raw ground sample residues were weighed using an electronic weighing balance Mettla Toledo, with an accuracy of 0.01 g, the sample was put in a 500 ml round bottom flask. Then, 200 ml of ethanol and distilled water was measured (1:1 v/v) using a 100 ml measuring cylinder, and poured into the sample. The flask containing the sample was put on an electric heating mantle set at 70°C and allowed to boil for 30 min. Each purification process was maintained for all particle sizes. After the final decantation of the final supernatant, the resultant material (purified sample) was drained and dried in an oven at 105 for 48 h and cooled in an air tight dessicator with dessicant. Extraction or purification process of the sample was achieved to obtain lignocellulosic contents from the samples.

Pyrolysis process

The lignocellulosic (purified) samples were pyrolysed in batch-type reactors in which the reactors are the ampoule and tubular systems. One end-opened narrow cylindrical pyrex tubes of about 3 cm diameter and 15 cm long was used with a tight lid. The pyrex ampoules was made by joining bulbs of 5 cm diameter with the cylindrical tubes. 2 g of lignocellulose was measured in each case and introduced into the reactor by means of narrow plastic funnel. In order to ensure quantitative transfer of the charge, a small Teflon rubber tubing about 2 cm long was used in the joining of the funnel and reactor outlet, and by gently tapping and varying the amount of the charging materials until the exact weight of 2 g as required was introduced into the reactor. Then, 0.2 g of glass wool was placed at the constriction level of the reactor. The wool was held fixed at the constriction point above the reactor so as to prevent discharge of gas during the evacuation period. High vacuum pump was used for the evacuation and the glass was sealed with a hand torch burner when the pressure reaches 0.1 mmHg as was measured with a pirant vacuum gauge. The duration of the evacuation was 5 to 30 min for the tubular and ampoule reactors. A thermostat oven furnace was required as shown in Figure 5; the isothermal temperature was allowed to run for ten minutes before introducing reactor and was maintained throughout the run. Each pyrolysis reaction lasted for 120 min at each temperature selected ranging from 200 to 400°C. To terminate a run, the reactor was removed from the furnace and placed in a dessicator and allowed to cool to ambient temperature level. The gas produced was collected through the tight fitted tap joined to the upper part of the reactor, with a small teflon rubber tubing inserted for easier discharge into an air tight gas bag. The weight of the gas produced was obtained by subtracting the final weight of the glass reactor after gas discharge from the initial weight before heating in a furnace. The pyrolysed sample (solid) was collected from the reactor for further process so that liquid extract can be produced using soxhlet extraction as shown in Figure 6; the extraction was carried out for 72 h.

2 g of the pyrolysed sample were measured and put into dried thimble container and introduced into soxhlet extractor. Then 100 ml of methanol and dichloromethane solvent, (1:1 v/v) each was poured into a round bottom (250 ml) flask and placed on the electric heating mantle set at 20°C for 24 h. Then, the extracted

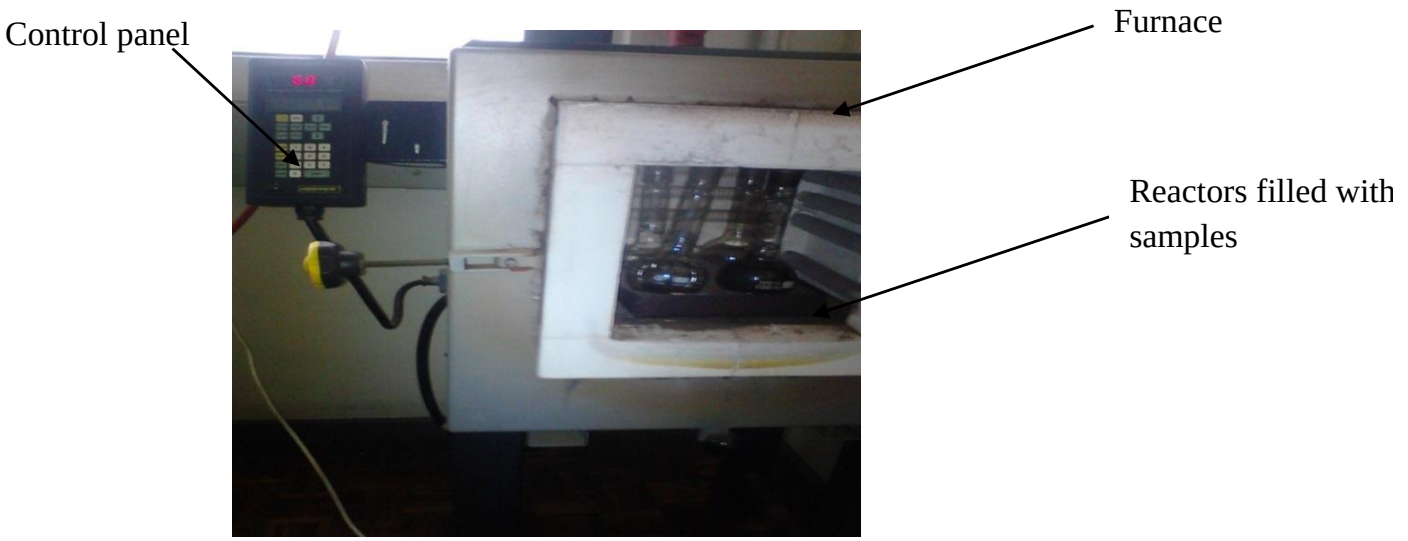

Figure 5. Pyrolysed samples inside the furnace**.**

Figure 6. Soxhlet extraction experiment for the residues.

sample was taken out of the thimble and put into crucible and dried in an oven for 48 h at a temperature of 105°C. The extracted solvent in the round bottom flask was then concentrated using the vacuum pump set. The concentrated extract was cleaned using a glass column (10 cm) filled with sodium sulphate to remove water or impurities. The clean sample filled into 0.2 ml chromatograph vials and then introduced to GC/MS for analysis.

Experimental design

The response surface methodology (RSM) was employed in this study and this is necessary to evaluate the performance of variables in pyrolysis system in other to optimize the bio-oil and biogas in the samples (physic nut shell and palm fruit fibre).The effects of tested parameters such as temperatures and particle sizes on the pyrolysis efficiency were determined to identify optimal bio-oil and gas conditions. RSM helps proper design of experimental work and optimization can either be minimum or maximum variables of design parameters. Design expert 6.0.8 software was used to analyze the tested parameters and response. This was obtained by the response surface regression procedure using the following second-order polynomial equation (Yunardi et al., 2011; Gratuito et al., 2008; Faisal et al., 2011).

The temperatures chosen were 200, 250, 300, 350 and 400°C while the particle sizes were 0.25, 0.300, 0.425 and 0.55 mm and each experiment was replicated three times. The reaction time and product yield are the responses. The model used for predicting is generally a quadratic equation or second order model. The model equation can be expressed as follows:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum \sum_{i<j=1}^{k-1} \beta_{ij} x_i x_j \quad (1)$$

Where, y is the predicted response (Y_{char} , Y_{gas} and Y_{oil}); x_i and x_j are the coded independent variables corresponding to temperature and particle size and β_0, β_i, β_{ii}, and β_{ij} are intercept, linear, quadratic and interaction constant coefficients respectively. RSM package was also used for regression analysis and analysis of variance (ANOVA). Response surfaces, normal probability and plots were developed using the fitted quadratic polynomial equation obtained from regression, holding one of the independent variables at a constant value corresponding to the stationary point and changing the other variable. The independent variables being studied were temperature and particle size. The dependent variables were the retention time, yields of oil, gas and char. The design of three levels low, medium and high are coded as -1, 0 and +1 was applied to this study. Another step is checking the adequacy of the model. To achieve this aim is testing of the lack-of-fit which is defined as a measure of a model failure in representing data in the experimental domain (Faisal et al., 2011).

Characterization of bio-oil

The bio oil obtained, from lignocellulose of physic nut shell and palm fibre, was used for characterization. Litmus papers blue and red were used to check its acidity and alkalinity. A microprocessor pH meter (HANNA pH 211) was used to measure the pH of the oil. Analysis was done at room temperature; the meter was calibrated by measuring pH of buffer solution to be 7.03 at 23.6°C. The density of oil was conducted at room temperature of 23.6°C. The density is defined as sample mass divided by a fixed empty volume

Table 1. Main properties of the physic nut shell and palm fruit fibre.

Property	Values		Unit
Lignocellulosic analysis	**Pns**	**Pff**	
Cellulose	74.89±0.0128	28.99 ±0.029	%
Hemi cellulose	78.66±0.0148	38.96±0.0897	%
Lignin	50.92±0.005	60.36±0.41	%
Proximate analysis			
Moisture	4.61	5.93	%
Dry matter	95.24	94.09	%
Fat	84.37±10.12	20.13±0.12	%
Ash	13.60± 0.83	61.30±1.15	%
Volatile	56.01±11.15	49.89±3.12	%
Fixed carbon	25.79±0.83	38.70 ±1.15	%
Ultimate analysis			
Nitrogen	1.40	1.05	%
Phosphorus	0.43	0.24	%
Potassium	6.57	0.29	%
Calcium	6.48	3.22	%
Magnesium	0.59	0.08	%
Iron	96.02	20.17	wt%
Copper	0.834	2.17	wt%
Manganese	7.67	4.33	wt%
Zinc	15.67	1.83	wt%

pns, Physic nut shell: *pff*, palm fruit fibre.

of pycnometer. Then a 2 ml pycnometer was used to determine the density, the bio-oil was filled into the flask and weighed the mass. Viscosity of bio-oil was measured using Ostwald Viscometer (a U shape glass viscometer). All experiments were carried out three times and average readings were recorded.

Also, GC-MS was used to identify the chemical compositions of the bio-oil. The analysis was performed with Angilent HP 6890 (version 4.10) N gas chromatograph equipped with Agilent HP 5075 mass-selective detector (mass spectrometer), using a 30 m by 0.25 mm DB-5 ms capillary column (0.25 µm film thickness) with temperature capacity of -60 to 325°C. The GC oven was heated to 30°C for 3 min then to 290°C at a rate of 3.5°C /min while injection port and detector were set at 300°C. The carrier gas was helium with flow rate of 28 cm^3/min and the effluent was monitored using a flame ionization detector (FID). Nitrogen was the makeup gas while hydrogen and compressed air are the lightning flame for FID.

A separate constituent of the pyrolysis mixture together with helium carrier gas goes to the flame ionization detector. It was at this zone that the separated organic compounds ionized at a high temperature in contact with hydrogen or air flame. A polarized electric grid captures the resultant ions which generates a current that will be recorded as a chromatogram. Peak areas of individual gases were measured and the abundance was determined from the prepared calibration. The calibration which is an external standardization has a technique involving essentially, the injection of known amounts of pure compounds as reference substance at the same conditions with that of the sample (pyrolytic products). The sample peak was compared with that of the standard. Also, individual compound was identified by matching their elution times along the base line with that of the standard. So, different components in the liquid solvent and their retention time displayed on the screen of the GC-MS monitor.

RESULTS AND DISCUSSION

Main properties of palm fibre

The results of the main properties of the agricultural residues used (physic nut shell and palm fruit fibre) are presented in Table 1. The proximate analyses are the physical characteristics such as moisture, volatile, ash and fixed carbon contents. The ultimate analysis is the composition of the materials in weight percentage such as carbon, oxygen, nitrogen, magnesium, phosphorus and zinc while the lignocellulosic values are based on the hemicellulose, lignin and cellulose of the materials.

Table 1 presents the main composition of the sample which includes the estimated value for cellulose, lignin and hemicellulose contents. The results revealed that the cellulose, lignin and hemicellulose values of physic nut shell and palm fibre are 28.99, 60.36 and 38.96% and 74.89, 78.66 and 50.92%, respectively. The values of latter residue are higher than the former; this might be because lignocelluloses in palm fibre do not decompose easily and next generation fuels are based on cellulose (Kullander, 2010; Valentine et al., 2012). From previous findings, cellulose is the strongest component of lignocellulose because it is polymer in nature. It also has fiber which is the smallest unit that can be used to produce high-yielding lignocellulosic composites (Rowell,

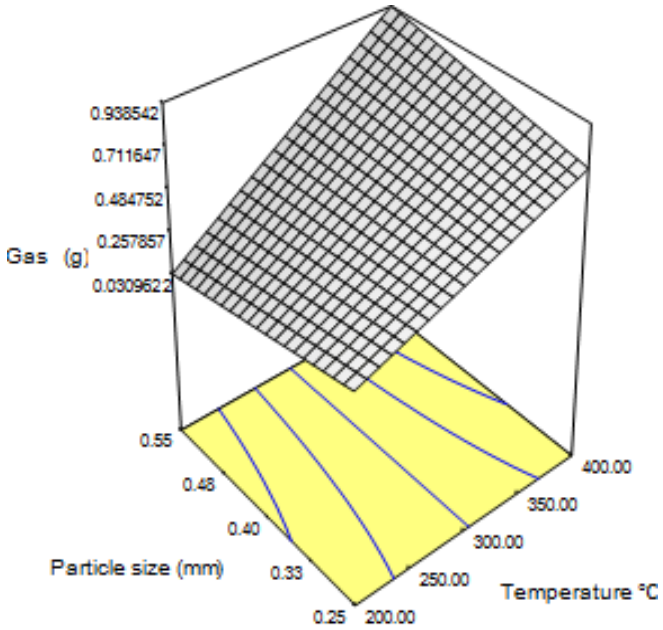

Figure 7. Effect of temperature and particle size on gas yield from physic nut shell.

1992). Similar findings are reported from other materials, such that mango pod had 22.96, 50.59 and 26.45% (Olaleye, 2013), wheat straw had 38, 15 and 29% (Hongzhang and Liying, 2007) while palm shell contained 27.7, 44 and 21.6%, respectively (Faisal et al., 2011).

From Table 1, the moisture content (dry base) and dry matter of physic nut shell and palm fruit fibre are 4.61 and 95.24%, and 5.93 and 94.09%, respectively. Agricultural residues have different values like that of mango pod is 7.31% (Hongzhang and Liying, 2007) and coconut shell has 6.0% (Ojha and Michael, 2006). Several works had been done on proximate analysis of agricultural materials; the value of ash content for mango pod is 2.1% (Hongzhang and Liying, 2007); coconut has 0.6% (Sundaram and Natarajan, 2009) rice straw contains 7.56% (Ahamed et al., 2013), palm shell has 2.1% (Faisal et al., 2011). In this study, the higher ash content value of *pff* (61.30%) than that obtained from pns (13.60 %) and other materials and it might be because palm fibre is a product of oil crop and high ash content indicates quality energy source and high fuel property.

Moreover, the results of volatile content of pns and pff gave 56.01and 49.90%, respectively while other reports gave 66.89% for volatile content of rice straw (Ahamed et al., 2013); while palm shell had 67.2% (Faisal et al., 2011). This indicates that the particles of others are less dense than the former which makes its component to escape into the air. The fixed carbon content of 25.79 and 38.70% are for *pns* and *pff*. The values reported by other researchers were lower; rice straw contained 14.56% by Ahamed et al. (2013) and palm shell had 19.7% (Faisal et al., 2011). There is higher value of fixed carbon content of physic nut shell, 86.40%. From previous research, it was illustrated that fresh physic nut could absorb more carbon content from the atmosphere that is more carbon can be emitted with fresh fruits (Onifade and Jekayinfa, 2015) but burning its residues can do more harm to human because of its high volatility and ability to release gases into the atmosphere. This is one of the most promising solutions for tackling the growing carbon emissions from atmosphere (JWT, 2010), hence reducing the problem of ozone layer. This implies that physic nut has greater potential to absorb carbon emission, thereby making the atmosphere carbon neutral. This factor can be a strategy for climate change adaptation or mitigation, if more cultivation practice of physic nut is established or encouraged in different countries of the world.

Effects of process parameters on product yield

Figures 7 to 13 present the effect of temperature and particle size on gas, char and liquid yields from physic nut shell and palm fibre. It is observed from Figures 7 and 11 that gas yield increased with increase in temperature and particle sizes. The increase in temperature leads to formation of more gaseous molecules released, the higher the temperature the higher the gases released to the atmosphere. This means if the residues are burnt in an open space, some is inhaled by human and the remaining affects the climate.

As reported from previous research, the burning of wood emits obnoxious GHGs that are harmful to the

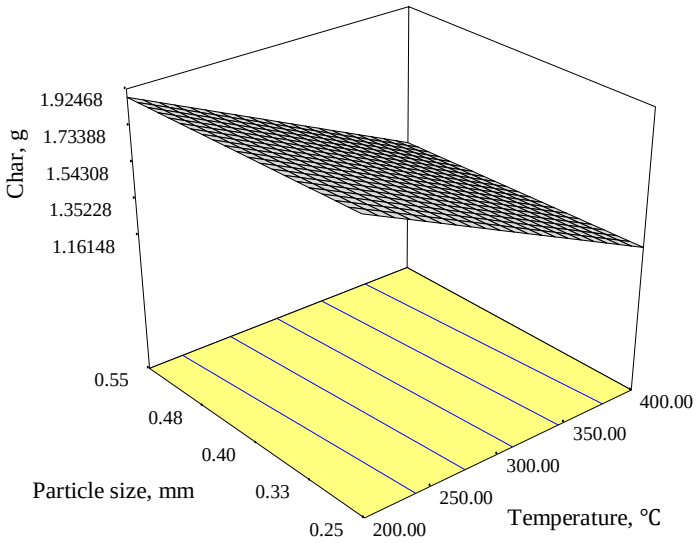

Figure 8. Effect of temperature and particle size on char yield from physic nut shell.

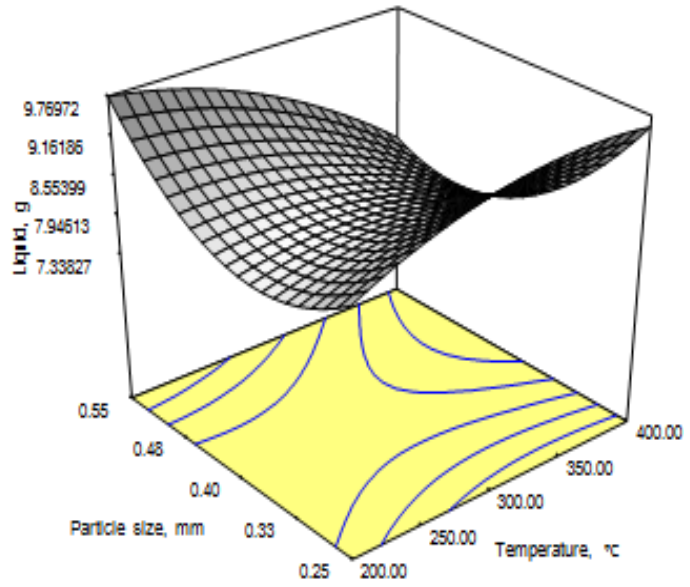

Figure 9. Effect of temperature and particle size on liquid yield physic nut shell.

environment (Pimentel et al., 1994; OECD/IEA, 2007; Davidsdottir, 2013).

Figures 8 and 12 depicted the effect of process parameters on the char yield. It was observed that increased in temperature and particle sizes lead to decrease in quantity of char produced. Figure 10 presented the char produced after pyrolysis. The char has potential soil-quality benefits for farming activities when it is incorporated into the soil. Application of residue char into the soil improves physical and chemical properties of farm land (UNL, 2016). From Table 1, the results of ultimate analysis indicate that the residues have traces of Nitrogen, Phosphorus and Potassium which are good compounds to enrich the soil if the char is impoverished into the farm soil.

From Figures 9 and 13, it was observed that as temperature and particle sizes increased, increase in liquid yield occurred but liquid yield decreased as temperature increased. The increase in temperature aids formation of newer pores and an opening of the existing

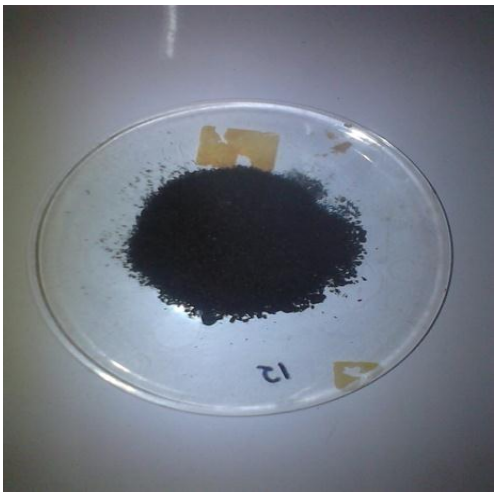

Figure 10. The char produced after pyrolysis.

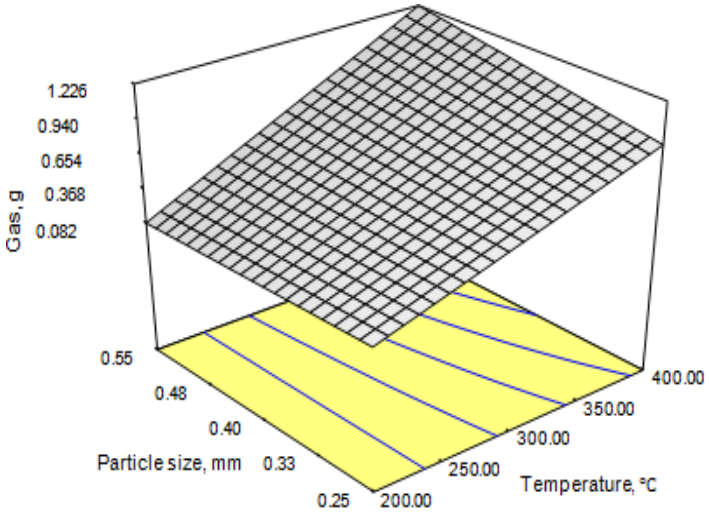

Figure 11. Effect of temperature and particle size on gas yield from palm fibre.

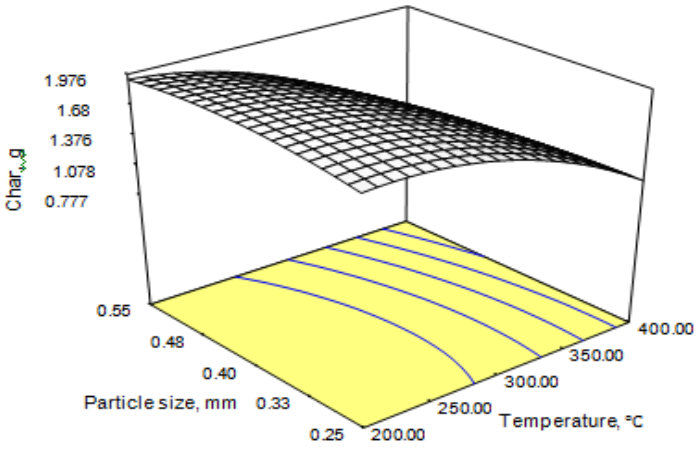

Figure 12. Effect of temperature and particle size on char yield from palm fibre.

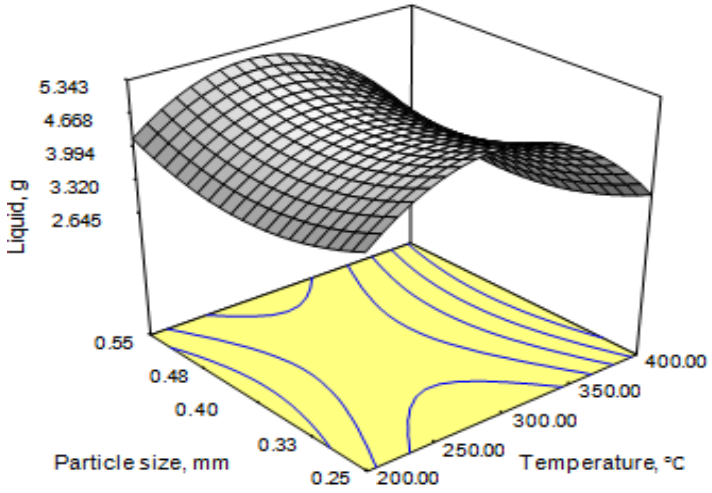

Figure 13. Effect of temperature and particle size on liquid yield from palm fibre.

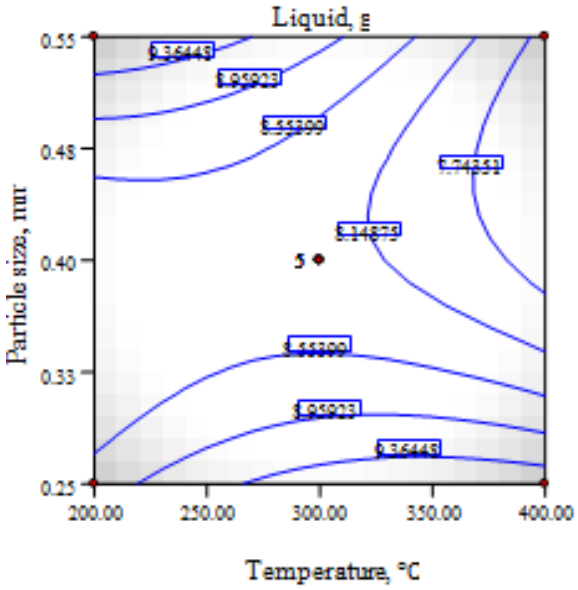

Figure 14. Effect of temperature and particle size on liquid yield from palm fibre.

pores for the liquid extract which leads to more liquid yield. If temperature is increased further, the pore wall of the materials could be collapsed; due to thinning porosity of the particle size as the surface area is reduced (Azargohar and Dalai, 2005) not only this but also increased ash content which offered a resistance to the diffusion of activating agent (Dutta et al., 2011).

Figure 14 shows the 3D surface plots obtained from numerical optimization of pyrolysis process where the optimum values of two process parameters for response product were found. It can be seen that the optimum values of process parameters varied slightly in each case. Therefore, application of numerical optimization was used to optimize the process where two process parameters were considered at a time. The desired objective of optimization was to maximize the temperature, particle size and product yield (liquid, gas and char). The optimum values of temperature and particle size predicted by the model were 300°C and 0.55 mm.

Physical characterization of bio-oil

The physical properties of bio-oil produced from the two

Table 2. Physical properties of bio-oil.

Properties	Pns	Pff	Unit
Viscosity	1.58	0.695	cP
pH	6.94-7.72	4.64 - 6.43	Acidic
Density at 23.6°C	947.5	831.99	kg/m^3
Calorific value	14.169	22.33	kJ/g
Water content	4.6	5.39	%
C	60.73	65.24	%
H	6.88	5.07	%
N	0.98	1.10	%
O	17.81	28.60	%

residues (physic nut shell and palm fibre) investigated are presented in Table 2. The liquid extracts of *pns* were neutral because it has little or no effect on litmus paper because the oil pH values ranged from 6.94 to 7.72 at 22.8°C. The liquid extracts of *pff* were acidic in nature because it turned blue litmus paper to red, the pH values ranged from 4.64 to 6.43. The pH of the bio-oil increased with increase in temperatures, this meant that oil obtained at high temperatures are slightly acidic while oil obtained at low pyrolitic temperatures indicate low pH readings which is more acidic.

The density of the oil from *pns* and *pff* is 831.99 and 947.5 kg/m^3, respectively at 23.6°C. It was observed that all density values at different temperatures and particle sizes gave similar readings. The density and viscosity are related to phenomenon of liquid floatation which can have significant effect on fluid atomizers. The respective viscous values of bio-oil produced in this study gave 1.58 and 0.695 cPa at room temperature; this value was less than that of water (0.9107) used as standard. This indicated that the viscous period of bio-oil is less than that of water. Hence, this could be due to low level of water content (5.93%) in the samples which caused it to be less viscous. The presence of water content in bio-oil shows the presence of lignin in the raw material of *pns* and *pff* which is 50.92 and 60.36%. It has been reported that lignins are not crystalline, but highly branched and their structure and chemical composition is a function of their source. Lignins play a role in the natural decay resistance of the lignocellulosic substance and are associated with the hemicelluose (Rowell, 1990).

The calorific values of the bio-oil of *pns* and *pff* are 14.169 and 22.33 kJ/g as shown in Table 2, this shows the amount of energy produced by the complete combustion of 0.291 g of the oil. The calorific value is an important factor to determine the energy content of the fuel. Compared to other common fuel, gasoline (47 kJ/g), diesel (45 kJ/g), ethanol (29.7 kJ/g), wood (15 kJ/g) coal (15 kJ/g) and natural gas (54 kJ/g) (NIST), this indicates that bio-oil from palm fruit fibre is a potential source of energy and can be upgrade first before using it as fuel.

Chemical characterization of bio-oil

GC-MS was used to analyze and identify the chemical components in the liquid (bio-oil). Table 3 presents the details results of GC-MS analysis of bio-oil of physic nut shell. The most abundant products and highest peak area achieved by hexadecanoic (82.2%). Other prominent products are pentadecanoic acid (12-48.9%), octadecanoic (4.16-35.5%), eicosanoic (1.55-3.53%), linoleic acid (3.72-18.4), octanamine (2.71-12.3), ethynefluoro (8.75) and 2-hexanamine (15.13). It was observed that different values were obtained at various temperature and particle sizes. This shows effect of experimental parameters (temperature and particles sizes) on the chemical compounds produced from physic nut shell. For instance, highest peak of hexadecanoic (82.2%) was obtained at 300°C, 0.25 mm, pentadecanoic acid was high (38.5%), at 200, 0.55 mm; eicosanoic had value of 3.53% at 200°C, 0.55 mm, heptadecanoic has highest peak of 26.5% at 400°C and 0.42 mm. Only 200°C, at 0.42 mm produced amine compounds. Table 4 presents the details results of GC-MS analysis of bio-oil of palm fibre, GC-MS was used to analyze and identify the chemical components in the liquid. The most abundant products and highest peak area was achieved by hexadecanoic (81.3%).

Other prominent products are pentadecanoic acid (1.47-14.5%), octadecanoic (2.6-70.1%), eicosanoic (3.5-11.3%), 2-2-hydroxyethoxy (2.71-12.3%), ascorbic 2, 6-dihexadecanoic (7.1-14.3) and isopropyl palmitate (7.31-41.0). It was observed that different values were obtained at various temperature and particle sizes. This shows effect of experimental parameters (temperature and particles sizes) on the chemical compounds produced from palm fruit fibre. For instance, highest peak of methyl-hexadecanoic (81.3%) was obtained at 158.8°C, 0.42 mm, methyl-pentadecanoic acid was high (14.5%) at 441.42°C, 0.42 mm, eicosanoic had value of 11.3% at 300°C, 0.25 mm. Hepta decanoic has highest peak at 200°C, 0.55 mm. Only 300°C, 0.25 mm and 400°C, 0.25 mm contained ascorbic 2,6-dihexadecanoic.

Summarily, there are a great number of other compounds but their peak areas are low, so this study did not examine them further. The dominant compounds presented in Tables 3 and 4 are classified as aromatic oxygenated and hydrocarbon compounds found from lignocellulose bio-oil of both in physic nut shell and palm fibre. Oxygenated content is favorable to be used for fuels. Other compounds found in palm fibre show the potentiality to be used as polymer. Amines and organic halogen compounds are highly present in the physic nut shell oil which can be useful as chemical feedstock for further productions. The results obtained in this study showed different level of concentration of each compound and the values are higher than those obtained from palm kernel shell by Faisal et al. (2011). Evidently, from previous research, it was stated that lignocellulosic

Table 3. Identification and analysis of chemical compounds in bio-oil of physic nut shell by GC-MS.

Chemical compounds	Molecular formula	Molecular weight (g/mol)	Peak probabilty (%)
9-octadecenoic	$C_{21}H_{40}O_2$	356	6.7- 35.3
Hexadecanoic acid	$C_{18}H_{34}O_2$	284	4.0 -82.2
n-propyl 11-octadecenoate	$C_{21}H_{40}O_2$	324	7.27
14-methylPentadecanoic acid	$C_{17}H_{34}O_2$	270	48.9
Linoleic acid	$C_{17}H_{34}O_2$	308	3.72-18.4
Ethyl Oleate	$C_{20}H_{38}O_2$	310	6.53
Eicosanoic acid	$C_{22}H_{44}O_2$	340	1.55-3.53
Butyl 9-octadecanoate	$C_{22}H_{42}O_2$	338	2.53-4.46
Trans -13-octadecanoic	$C_{19}H_{36}O_2$	296	4.16-5.99
Pentadecanoic acid	$C_{17}H_{34}O_2$	289	12-38.5
Methyl10-trans12-cis-octadecadienoate	$C_{19}H_{34}O_2$	294	2.57-8.65
Ethyl 9-cris, 11 trans-octadecadienoate	$C_{20}H_{36}O_2$	308	28.4-33.8
n-propyl 9, 12-octadecenoate	$C_{21}H_{38}O_2$	322	2.91-5.36
EthyneFluoro	C_2HF	44	8.74
2-Aminoheptane	$C_7H_{17}N$	115	6.69
2-hexanamine	$C_7H_{17}N$	115	15.13
Octanamine	$C_9H_{21}N$	143	2.71-12.3
Benzene thanamine	$C_9H_{11}F_2NO_3$	219	2.56-3.82
Dodecylmethylamine	$C_{13}H_{29}N$	199	2.77-3.0
Octodrine	$C_8H_{19}N$	129	2.36
Cyclopropanebutanoic	$C_{25}H_{42}O_2$	374	2.83
Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	4.72
14-methyl-hexadecanoate	$C_{19}H_{38}O_2$	298	3.43
3,9-Epoxypregnane	$C_{25}H_{41}NO_2$	435	1.89
Diethylene glycolmonolaurate	$C_{16}H_{32}O_4$	288	1.82
Tridecanoic	$C_{17}H_{34}O_2$	270	0.9-5.41
Docosanoic	$C_{24}H_{48}O_2$	368	0.37-3.7
Undecanoic	$C_{14}H_{28}O_2$	228	2.68
Oleic acid	$C_{18}H_{34}O_2$	282	2.29
Heptadecanoic	$C_{20}H_{40}O_2$	312	1.61-81.3
Bromodecanoic	$C_{12}H_{23}BrO_2$	278	0.73
Octadecadienoyl Chloride	$C_{18}H_{31}ClO$	298	2.80
Cyclopentanetridecanoic	$C_{19}H_{36}O_2$	296	1.07
Octadecanoic	$C_{20}H_{40}O_2$	312	35.3

Table 4. Identification and analysis of chemical compounds in bio-oil of palm fibre by GC-MS.

Chemical compounds	Molecular formula	Molecular weight (g/mol)	Peak probability (%)
3-methyl pentadecanoic acid	$C_{16}H_{32}O_2$	256	1.47-2.85
2,16-methyl hexadecanoic acid	$C_{18}H_{34}O_2$	284	1.30-10.15
Methyl hexadecanoic acid	$C_{17}H_{34}O_2$	270	81.3
14-Methyl pentadecanoic acid	$C_{17}H_{34}O_2$	270	9.92-28.7
Tridecanoic acid	$C_{17}H_{34}O_2$	270	2.58
Ethyl pentadecanoic acid	$C_{17}H_{34}O_2$	270	6.53
Ethyl eicosanoic acid	$C_{22}H_{44}O_2$	340	3.53-11.3
Ethyl hexadecanoic acid	$C_{19}H_{38}O_2$	298	2.84
Ethyl tridecanoate	$C_{15}H_{30}O_2$	242	1.46
Methyl octadecanoic	$C_{19}H_{38}O_2$	298	4.04-66.6
16-methyl heptadecanoate	$C_{19}H_{38}O_2$	298	10.5

Table 4. Contd.

Ethyl octadecanoic acid	$C_{20}H_{40}O_2$	312	60.7
Nonadecanoic acid	$C_{21}H_{42}O_2$	326	0.93
Cyclop1ropanepentanoic	$C_{20}H_{38}O_2$	310	3.73
Tetradecanoic	$C_{16}H_{32}O_2$	256	2.7- 4.63
Ethyl heptadecanoic	$C_{19}H_{38}O_2$	298	5.48
2-2-hydroxyethoxy	$C_{22}H_{44}O_4$	372	2.71-12.3
Hexadecanoic acid	$C_{18}H_{36}O_2$	284	49.4
Isopropyl palmitate	$C_{19}H_{38}O_2$	298	7.31-41.0
Ascorbic 2,6-dihexadecanoic	$C_{38}H_{68}O_8$	652	7.1-14.3
Ascorbic acid, 6-octadecanoate	$C_{24}H_{42}O_7$	442	2.8

fibers can be combined in an inorganic matrix to produce composites which are dimensionally and thermally stable, and majorly used as substitutes for asbestos composites (Rowell, 1992). It allows the scientist to design materials based on end-use requirements within the framework of cost, availability, renewability, recyclability, energy use, and environmental considerations.

Conclusion

This research was set up to explore and study the lignocellulosic characteristics of two agricultural residues as a provider of renewable energy and chemicals in Nigeria. The residues were pyrolyzed considering these experimental factors; temperature and particle size. The optimum process condition of bio-oil was produced at 300°C and 0.55 mm. It further examined the impact and advantage of biotechnology on farm residues studied to produce biogas, biochar and bio-oil and positive effects on climate change. This paper also revealed and identified the bioenergy and biochemical from lignocellulosic materials in physic nut shell and palm fibre which can be used for further processing in small and large scale industries. Aromatic oxygenated and hydrocarbon compounds present in lignocellulose bio-oil of physic nut shell and palm fibre are favorable to be used for fuels. Other compounds found in palm fibre show the potentiality to be used as polymer. Amines and organic halogen compounds are highly present in the physic nut shell oil which can be useful as chemical feedstock for further productions. The work analyses the use of lignocellulosic content of the residues studied as a great opportunity for bioenergy in its efficient forms and such engagement will enhance economic development and improve social wellbeing of the nation. It will promote innovation and imagination of the youth and means of empowering them. This showed possible ways that bioenergy would enhance Nigeria's economic diversification, reduce dependence on crude and export products. However, climate change has become one of the major challenges for mankind and the natural environment. It is reflected from these findings that both residues studied contained a lot of useful products that can be processed further, and if such biofuel and chemicals are burnt consistently will enhance gas emissions released into the atmosphere and rapidly growing volumes are recognized to be responsible for this climate change. It is profitable and highly beneficial that farm residues are rather used for energy and chemicals production than burning them that enhance less pollution and a cleaner environment. Thereby, this study is recommended for policy making on environmental protection in the nation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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