Comparative study of physicochemical analysis of *prosopis africana* seeds fermented with different starter cultures

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original scientific paper
DOI: 10.17508/CJFST.2017.9.1.04

Summary

Prosopis africana (African mesquite) is one of the lesser known legume seed crops in Nigeria, which in a fermented state, gives a food condiment. Because of its rich protein content (about 34%), efforts are made to utilize some lesser known legumes to improve the nutritional status of the people. This study was carried out by fermenting *Prosopis africana* seeds with mono and mixed cultures of bacterial isolates to produce a local condiment called *Okpehe*. Standard AOAC methods were used to determine the pH, total sugar and crude protein content of the fermented seeds. During the production of *Okpehe* with mono cultures of bacteria, the pH ranged between 6.80 and 8.92; total sugar between 10.2 and 7.5 mg/g, and crude protein between 34.62 and 41.25%. In the mixed culture inoculated samples, the pH increased from 7.00 to 8.92; total sugar decreased from 9.4 to 7.4 mg/g, while the crude protein increased (35.02 - 44.61%) significantly ($p < 0.05$) as the fermentation progressed. The highest crude protein content of 44.61% was obtained with the combination of *Bacillus subtilis* and *Bacillus licheniformis*, while the lowest protein content referred to the combination of *Bacillus megaterium* and *Bacillus pumilus*. The result of this study showed that *Prosopis africana* seeds could be utilized for the production of *Okpehe* using mixed cultures of *B. subtilis* and *B. licheniformis*, so as to increase the protein intake of the populace.

Keywords: Prosopis africana, fermentation, starter culture, protein content

Introduction

Prosopis (mesquite) consists of about 45 species of leguminous spiny pod-bearing trees and shrubs found in subtropical and tropical regions (Balogun and Oyeyiola, 2011). *Prosopis africana* (Guill and Perr) Taub (syn *P. oblonga* Benth) belongs to the family Fabaceae and subfamily Mimosoideae (USDA, 2011). It grows wildly in the Middle Belt and Northern parts of Nigeria (Barminas et al., 1998; Aremu et al., 2006). Its seeds are usually inedible in their raw unfermented or uncooked state. The seeds can be consumed when processed and fermented using the metabolic apparatus of microorganisms (Balogun et al., 2015). Protein rich seeds are often fermented to make food condiments so as to enhance the flavor of foods. The use of hydrolyzed vegetable proteins in seasoning has long been recognized (Odunfa and Oyewole, 1998). Most of the reported fermented vegetable proteins are from leguminous seeds (Ogunshe et al., 2007), and are used as food condiments.

The fermentation of condiments is still being carried out by the traditional (spontaneous) village-art method. It has been suggested that fermentation processes in developing countries could be improved by using starter cultures, and back slopping, which entails application of brine from prior fermentation cycles (Holzapfel, 2002; Naiba, 2003). A starter culture is essential for the controlled fermentation processes. Most starter cultures not only help to reduce fermentation time, but alsoguarantee consistency, product safety and quality (Achi, 2005a; Oguntoyinbo et al., 2010). *Bacillus*, *Staphylococcus* and *Streptococcus* species have been used as pure starter cultures in a controlled experimental fermentation (Suberu and Akinyanju, 1996; Omafuvbe et al., 2003). Controlled fermentation of soybeans was achieved by using pure single cultures of *Bacillus subtilis, B. licheniformis,* or in combinations (Suberu and Akinyanju, 1996, Omafuvbe et al., 2003) to obtain *soyiru*. Report by Gberikon et al. (2010) also showed that fermenting seeds of *Parkia biglobosa*, *Glycin max*, and *Prosopis africana* inoculated with a consortium of *B. subtilis* and *B. pumilus* exhibitedhigher values of protein and lipids than when the seeds were fermented naturally. The fermentation time of 3-4 days was shortened to 2 days in the production of *ugba* using a mixed starter culture of *B. subtilis* and *B. megaterium* (Mbata and Orji, 2008). Parkouda et al. (2009) reported the development of the following starter cultures for the production of the following condiments; *B. subtilis* var natto (*natto*), *B. subtilis* B7, and B15 for *soumbala; B. subtilis* MM-4:B12 for *ugba*, and *B. subtilis* $24BP_2$ and *B. subtilis* $FpdP_2$ for *soydawadawa*. The use of a single strain

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may seem too restrictive for the production of a food with a generous range of organoleptic characteristics. The use of a mixture of microorganisms with complementary physiological and metabolic properties might be the best approach for obtaining a product with the desired nutritional and sensory properties (Achi, 2005b).

Fermented seeds of *Prosopis africana* are produced and marketed on a small scale by the local producers. The fermented seeds can serve as a flavor intensifier in soups and stews, and also improve the protein quality in protein poor diets (Balogun et al., 2015). Traditionally, the production process of fermented *Prosopis africana* seeds (*Okpehe*) is tedious and it takes a long time to get the end product. The use of microorganisms as starters in the right proportion would guarantee consistency, shorten the production time, increase product safety, and make the production less tedious while achieving a better quality product. Therefore, the objectives of this study are to use a starter culture singly, and in consortium for the fermentation of *Prosopis africana* seeds, and to carry out physicochemical analysis of the fermented seeds.

Materials and methods

Sample collection and processing

Prosopis africana fruits (5 kg) were obtained from the campus of University of Ilorin, Ilorin, Nigeria and authenticated at the herbarium of the Plant Biology Department of the University with a voucher specimen number UIH/472. The fruits were beaten with a club on a concrete surface so as to remove its seeds. The seeds were processed in the laboratory using the hot plate method described by Balogun and Oyeyiola (2012) with some modifications. The seeds (2 kg) were boiled at 100 °C for 6 hours in a stainless steel pot on a hot plate, during which the seed coats became soft and the seeds swollen. The seeds were allowed to cool. The seed coats were removed by pressing between fingertips, and later decanted along with the washing water, leaving the clean cotyledons. The clean cotyledons were rinsed with sterile water before putting them in another clean pot with a small amount of water, and cooked on the hot plate set at 60 °C for 30minutes. The cotyledons were drained through a sterile sieve and allowed to cool prior to inoculation of starter organisms. The method of Balogun et al. (2014) was used for the inoculation with starter organisms. One hundred grams of processed, unfermented seeds were mixed with separate 1 ml broth cultures of the following organisms: *B. subtilis, B. licheniformis, B. megaterium,* and *B. pumilus.* After the single organism inoculation, a consortium of 2, 3 and 4 organisms was also done into separate one hundred grams of processed raw seeds in the ratio of 1:1, 1:1:1 and 1:1:1:1 respectively. Prior to inoculation, a suspension of each of the starter organisms was made in a nutrient broth and incubated at 37 °C for 24 hours. The resulting mash after inoculation was first wrapped with pawpaw leaves and then in two layers of the sterile aluminum foils (Balogun et al., 2015). Prior to their usage,used pawpaw leaves werecleaned, their surface was sterilized with 70% alcohol, and they were rinsed with sterile water. The wrapped cotyledons were left at 30 ± 2 ˚C for 72 hours in an incubating unit to ferment. Samples were taken at zero time (time of wrapping), and subsequently after every 24 hours for physicochemical analysis which lasted for 72 hours. The control was processed, packaged and stored like the other samples, except that it was not inoculated with any starter organisms.

Physicochemical analysis

Standard AOAC (2000) methods were used for the determination of pH, total sugars and crude protein content of samples. pH meter (model Denwer 20) was used to determine the pH of the samples, total sugars were determined using the spectrophotometric method (phenol-sulphuric acid method), while the Micro-Kjeldhal method (N × 6.25) was used for the determination of crude protein content.

Statistical analysis

The obtained data underwent one way Analysis of Variance (ANOVA), and the means were separated usingthe Duncan Multiple Range test.

Results and discussion

There was a significant increase in pH of the fermented seeds inoculated with both mono and mixed starter cultures of bacteria (Fig. 1, 2 and 3) as fermentation period progressed. The range of pH for the mono culture inoculated samples was between 6.80 and 8.92, while that of the mixed culture inoculated samples was between 6.98 and 8.92. The increase in pH is generally due to the production of ammonia, which is characterized by the pungent smell of fermented condiments (Balogun and Oyeyiola, 2012). The pH of fermented seeds being slightly alkaline was in agreement with earlier reports of Achi (1992); Omafuvbre et al. (2003) and Ogunshe et al. (2007), all of which recorded a slightly alkaline pH in fermented food condiments from vegetable proteins.

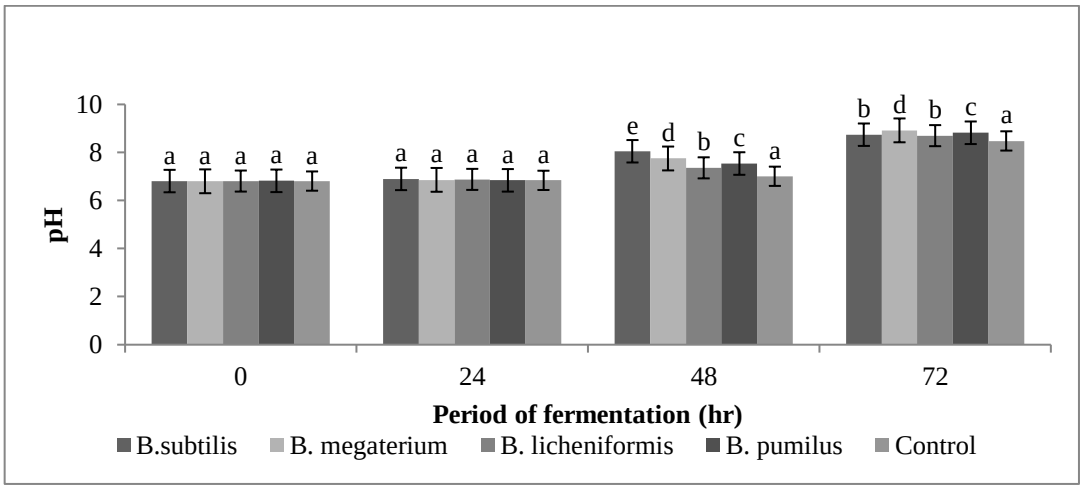

Fig. 1. Changes in pH values during the fermentation of *Prosopis africana* seeds inoculated with monocultures of bacteria

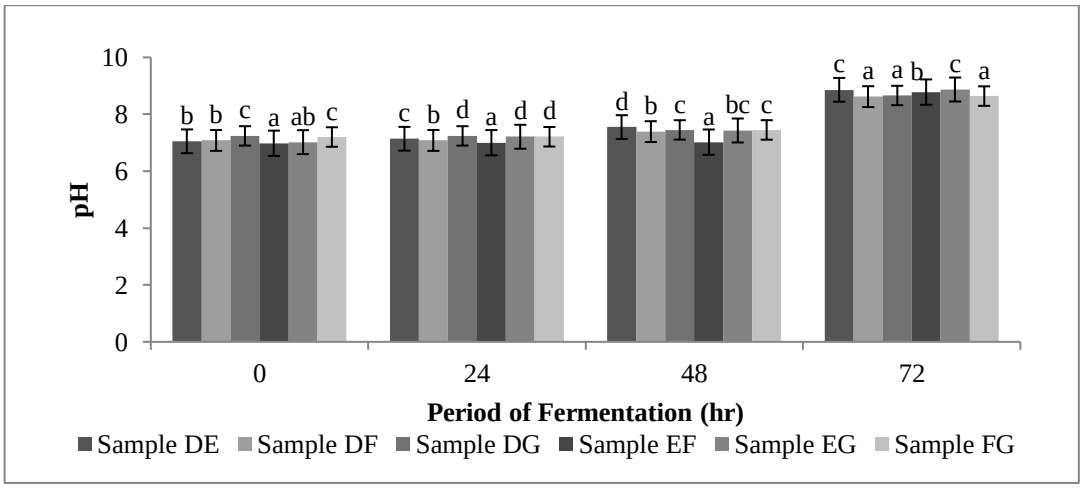

Fig. 2. Changes in pH values during the fermentation of *Prosopis africana* inoculated with mixed cultures of bacteria. D – *B. subtilis;* E – *B. megaterium;* F – *B. licheniformis;* G – *B. pumilus*

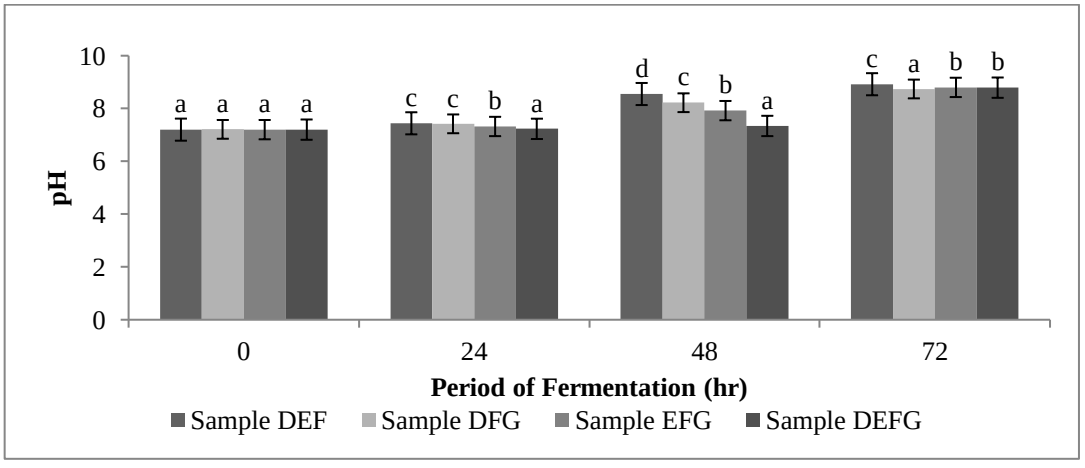

Fig. 3. Changes in pH values during the fermentation of *Prosopis africana* inoculated with mixed cultures of bacteria. D – *B. subtilis;* E – *B. megaterium;* F – *B. licheniformis;* G – *B. pumilus*

The changes in total sugar content of *Prosopis africana* seeds inoculated with mono and mixed cultures of bacterial isolates are presented in Fig. 4, 5 and 6. There was a decrease in total sugars (10.2-7.5 mg/g) for the mono inoculated samples as the period of fermentation progressed (Fig 4). The highest total sugars (9.4 mg/g) at zero hour was recorded in Sample EGH (*B. subtilis, B. licheniformis* and *B. pumilus*), while sample EG (*B. subtilis* and *B. licheniformis*) containedthe least (7.4 mg/g) total sugars after 72 hours of fermentation for the mixed culture inoculated samples (Fig 5 and 6). The fermentation of all samples resulted in a decrease in their total sugars. This decrease may be attributed to the utilization of the sugar by the fermenting organisms. The pattern of a change in soluble sugar levels has been reported in similar fermented condiments (Omafuvbre and Oyedapo, 2000; Omafuvbre et al., 2000). Oligosaccharides are present in the unfermented vegetable glycoproteins, but the quantity decreases during fermentation (Oyewole and Odunfa, 1998).

The result of the crude protein analysis of *P. africana* seeds inoculated with mono- and mixed cultures of bacterial isolates are shown in Tables 1 and 2. As the fermentation period increased from 0-72 hours, the crude protein increased significantly ($p < 0.05$) for both the mono and mixed culture inoculated samples. *B. subtilis* had the highest (41.25%,), while *B. pumilus* had the lowest crude protein of 38.45% for the mono inoculated seeds (Table 1). The highest crude protein content of 44.61% was obtainedwhen*B. subtilis* and *B. licheniformis* (EG) were combined, while the lowestone (42.24%) was obtainedin FH, which was a combination of *B. megaterium* and *B. pumilus* (Table 2).

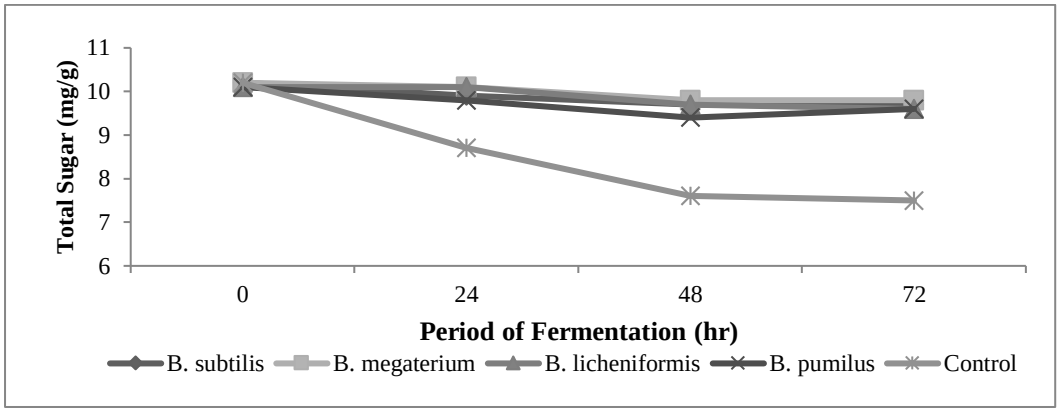

Fig. 4. Changes in total sugars of *Prosopis africana* seeds inoculated with monocultures of bacteria

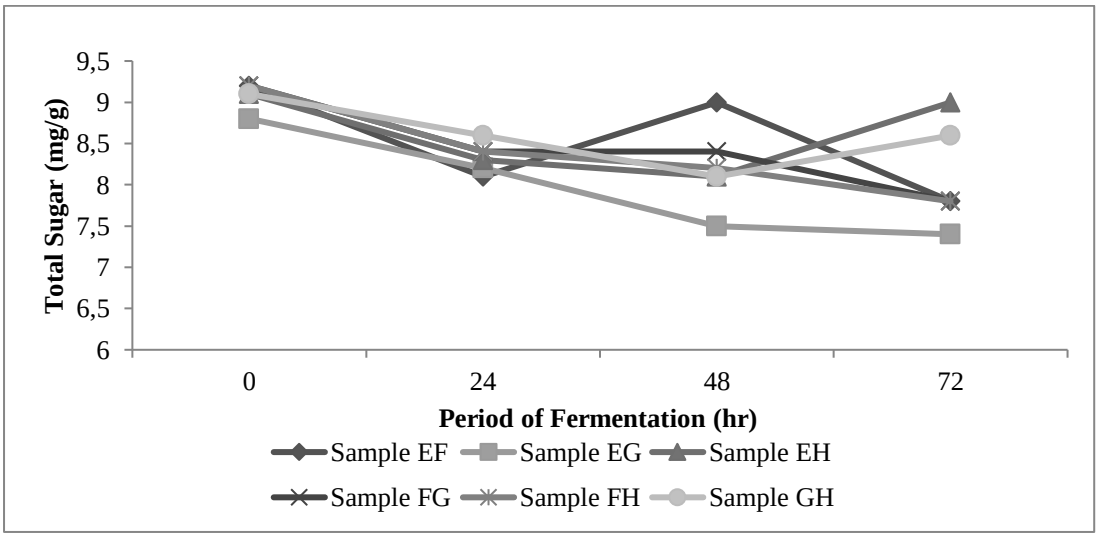

Fig. 5. Changes in total sugars of *Prosopis africana* seeds fermented with mixed cultures of bacteria. E – *B. subtilis;* F – *B. megaterium;* G – *B. licheniformis;* H – *B. pumilus*

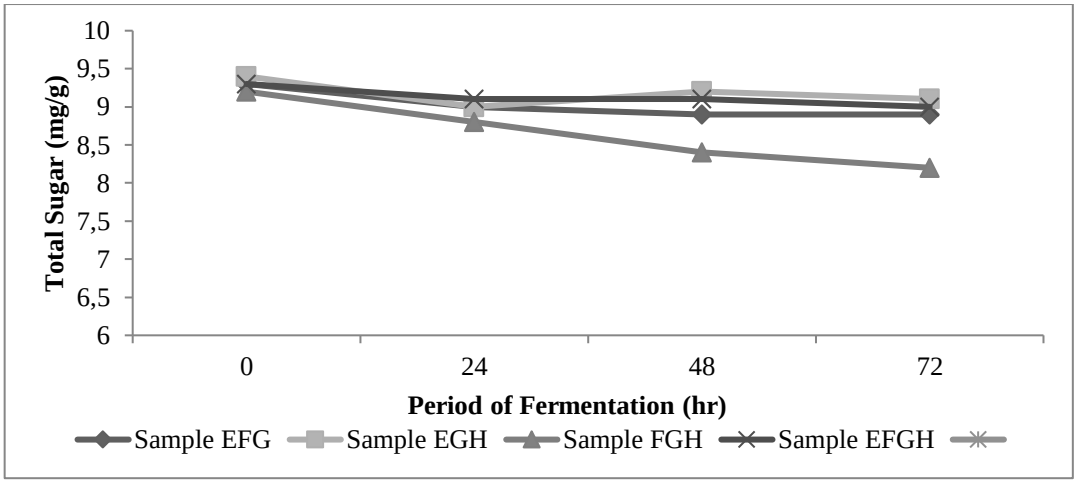

Fig. 6. Changes in total sugars of *Prosopis africana* seeds fermented with mixed cultures of bacteria. E – *B. subtilis;* F – *B. megaterium;* G – *B. licheniformis;* H – *B. pumilus*

The fermented seeds obtained from the mixed culture had a higher crude protein than boththe monoculture inoculated and control samples. The higher crude protein obtained is in line with the work of Gberikon et al. (2010), where the protein values were higher when a mixed culture of *Bacillus pumilus* and *B. subtilis* were used as a starter in the fermentation of *Glycin max*, *Parkia biglobosa*, and *P. africana* seeds as compared to when these seeds were fermented naturally. A better quality *ugba* was also produced with a mixed starter culture of *B. megaterium* and *Corynebacterium* sp, and *B. megaterium* and *Alkaligenes viscolatis*, which gave a 98% and 97% overall quality respectively in the controlled fermentation of *ugba* as compared to using single organism (*B. megaterium*) that gave a 87% quality (Nwagu et al., 2011). This is due to the fact that when organisms responsible for legume seeds fermentation are used as inoculum or starters in the right percentage, it guarantees consistency, product safety and quality (Holpzapfel, 2002; Achi, 2005a; Oguntoyinbo et al., 2010).

Table 1. Crude Protein Content of *Prosopis africana* Seeds Fermented with Monocultures of Bacterial Isolates

Bacterial Isolate	Crude Protein (% w/w)			
	0hr	24hr	48hr	72hr
B subtilis	$34.62^{a} \pm 0.04$	$36.32^{d} \pm 0.07$	$38.89^{d} \pm 0.03$	$41.25^{d} \pm 0.08$
B. megaterium	$34.62^{a} \pm 0.04$	$35.02^{a} \pm 0.06$	$35.61^{a} \pm 0.03$	$39.85^{b} \pm 0.06$
B. licheniformis	$34.62^{a} \pm 0.04$	$36.01^{c} \pm 0.07$	$38.28^{c} \pm 0.05$	$41.20^{c} \pm 0.06$
B. pumilus	$34.62^{a} \pm 0.04$	$35.42^{b} \pm 0.06$	$35.63^{b} \pm 0.05$	$38.45^{a} \pm 0.05$
Control	$34.62^{a} \pm 0.04$	$36.38^{e} \pm 0.06$	$38.92^{e} \pm 0.05$	$41.91^{e} \pm 0.04$

Values are means of triplicate determinations ± SD on dry weight basis; means within rows with different superscripts differ significantly ($p < 0.05$). SD – Standard Deviation

Table 2. Crude Protein Content of *Prosopis africana* Seeds Fermented with Mixed Cultures of Bacterial Isolates

Bacterial Isolate	Crude Protein (%)			
	0hr	24hrs	48hrs	72hrs
EF	$35.02^{a} \pm 0.06$	$36.82^{f} \pm 0.09$	$37.24^{d} \pm 0.11$	$43.50^{i} \pm 0.15$
EG	$35.02^{a} \pm 0.06$	$36.98^{g} \pm 0.12$	$39.58^{j} \pm 0.11$	$44.61^{j} \pm 0.07$
EH	$35.02^{a} \pm 0.06$	$36.65^{e} \pm 0.09$	$37.42^{f} \pm 0.09$	$43.42^{h} \pm 0.13$
FG	$35.02^{a} \pm 0.06$	$37.01^{g} \pm 0.12$	$37.84^{h} \pm 0.09$	$43.35^{g} \pm 0.13$
FH	$35.02^{a} \pm 0.06$	$36.26^{c} \pm 0.12$	$36.31^{a} \pm 0.12$	$42.24^{a} \pm 0.11$
GH	$35.02^{a} \pm 0.06$	$36.16^{a} \pm 0.09$	$36.92^{b} \pm 0.08$	$43.20^{f} \pm 0.10$
EFG	$35.02^{a} \pm 0.06$	$36.42^{d} \pm 0.11$	$38.05^{i} \pm 0.08$	$43.09^{e} \pm 0.11$
EGH	$35.02^{a} \pm 0.06$	$36.61^{e} \pm 0.08$	$37.51^{g} \pm 0.08$	$42.77^{d} \pm 0.10$
FGH	$35.02^{a} \pm 0.06$	$36.19^{ab} \pm 0.09$	$36.97^{c} \pm 0.10$	$42.33^{b} \pm 0.10$
EFGH	$35.02^{a} \pm 0.04$	$36.23^{bc} \pm 0.11$	$37.32^{e} \pm 0.10$	$42.60^{c} \pm 0.09$

Values are means of triplicate determinations ± SD on dry weight basis; means within columns with different superscripts differ significantly ($p < 0.05$), SD – Standard Deviation. E – *B. subtilis;* F – *B. megaterium;* G – *B. licheniformis;* H – *B. pumilus*

Conclusions

Prosopis africana seeds can be fermented to produce *Okpehe* using mixed cultures of *Bacillus subtilis* and *Bacillus licheniformis* in ratio 1:1. The use of these organisms on *Prosopis africana* seeds yielded a higher crude protein content of the condiment which can be used as a protein supplement in poor protein foods.

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Received: September 22, 2016
Accepted: December 19, 2016