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Physicochemical characteristics of fermenting castor seeds containing lime and NaCI as additives

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The effect of addition of lime and NaCl during the natural fermentation of castor bean seeds was studied in this research. Fermentation was carried out for four days at room temperature (29° C). The p H and temperature of the fermenting castor bean seeds were monitored and found to increase steadily. The sample with 3% NaCl attained the highest temperature of (31.50° C) on the fourth day of fermentation followed by the sample without any lime (31.00° C). There was an increase in the crude protein (13.88 to 17.90%) and crude fat (48.76 to 57.00%) contents of the fermenting castor bean seeds. The crude protein content was highest in the sample containing no lime (17.90%), followed by the samples with 1% lime (17.85%) and 3% NaCl (17.85%) on the fourth day of fermentation. The fermentation process however reduced the soluble carbohydrate content of the castor bean seeds. The most preferred level of lime inclusion was found to be in the range of 1 to 3%. At this level the quality was close to the sample with 3% NaCl addition.

Key words: Fermentation, Ogiri, lime, NaCl, castor bean.

INTRODUCTION

Fermented foods are essential components of the diet in many countries and are consumed either as main dishes or as condiments (Steinkraus, 1996). Indigenous fermented foods were developed through traditional technologies which were preserved over the years in order to maintain their uniqueness and identity (Valyasevi and Rolle, 2002). They are prepared from both plant and materials, using processes in animal which microorganisms play active roles in the physical, nutritional and organoleptic modification of the starting materials (Aidoo, 1994).

Legume seeds have continued to make significant contributions to human nutrition (Nwosu and Ojimelukwe, 1993). Seeds of legumes may account for 80% of dietary protein and may be the only source of protein for some groups. Their cooked forms are eaten as meals and are commonly used in fermented form as condiments to enhance the flavor of foods (Oniofiok et al., 1996).

The castor oil bean plant probably indigenous to the South-eastern Mediterranean region and parts of East Africa is today widespread throughout the tropical regions of the world. The castor oil bean is inedible because the seed contains a toxic protein, ricin and other toxic constituents, ricinic and ricinoleic acids but this can be completely eliminated reduced or be through fermentation (Odunfa and Oyeyiola, 1985). Castor oil bean seeds are fermented to give condiments (Omafuvbe et al., 2004). The fermented seeds can be ground to make an oily paste 'Ogiri' which has a characteristic ammoniacal odour. This characteristic flavor enhances the taste of traditional soups and sauces (Omafuvbe, 2006). It also contributes protein and essential fatty acid intake in West Africa (Oke and Umoh, 1998).

Lime (*Citrus aurantifolia*) is known worldwide for its tart, tangy flavor. The values reported for acid content (as citric acid) for lime juices have ranged from 6.10 to 8.32%; about 47 g/L in the juices (Penniston et al., 2008). Citric acid which is a weak organic acid found in citrus is a good natural preservative. It selectively suppresses the growth of microorganisms by inactivating enzyme

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systems vital to the cell, slowing or stopping growth.

Lime juice also contains various limonoid compounds, particularly D-limonene, reduces tumorgenesis under experimental conditionsand carotenoids, such as lycopene, which are associated with decreased risks of heart attacks (IFT, 1994).

Lime and (sodium chloride) are additives used in some cultural settings to control the natural fermentation of legume condiments and are known to impact a preservative effect on the end product. Antimicrobials are most often used with other preservation techniques such as refrigeration in order to inhibit the growth of spoilage and pathogenic micro organisms (Ademola et al., 2011).

Previous studies have shown that inclusion of salt in fermented food condiments improves the organoleptic properties and also exerts a selective inhibitory action on certain contaminating micro organisms (Ikenebomeh, 1989; Amin et al., 2004; Omafuvbe, 2006). Putrefactive or proteolytic micro organisms and also spore forming organisms are particularly susceptible to even relatively low concentration of salt (up to 6%). Pathogenic micro organisms including Clostridium botulinum are inhibited by salt concentrations up to 10 to 12%. However many organisms particularly Leuconostoc micro and Lactobacillus species are capable of rapid growth in brine with the development of acid to inhibit undeseriable organisms (Ihekoronye and Ngoddy, 1985). Addition of 1% salt in the traditional production of soy-daddawa has been reported to improve the organoleptic quality of the product (Omafuvbe, 1994). Effect of salt on the fermentation of soybean into daddawa using Bacillus subtilis as starter culture has also been reported by Omafuvbe (2006). Also Ademola et al. (2011) reported that inclusion of salt during the fermentation of locust bean improved the physical and proximate characteristics as well as had a preservative effect. Amin et al. (2004) carried out a study on the effect of salt on tempoyak fermentation and sensory evaluation using a range of 0 to 3% sodium chloride (common salt). This present study seeks to comparatively evaluate the effects of addition of 0 to 5% lime in fermenting castor been seeds with the effect of addition of 3% salt on the quality characteristics of fermenting castor bean seeds. The results will help to improve the traditional condiment from castor bean seeds.

MATERIALS AND METHODS

Castor bean seeds were purchased from Umuahia central market in Abia State, Nigeria. The analyses were carried out at the Central Laboratory Service Unit of the National Root Crops Research Institute (NRCRI) Umudike, Abia State, Nigeria. Chemicals used were of analytical grade.

Preparation of Ogiri samples

Approximately 1.3 kg of castor bean seeds were dehulled wrapped in clean heat treated plantain leaves and boiled for about 6 h. The

seeds were ground in a clean mortar into paste. The paste was divided into seven portions and different concentrations of lime (0 to 5%) and 3% NaCl were added. The seven portions were then wrapped in clean plantain leaves and allowed to ferment for four days at room temperature. The following analyses were carried out on daily basis:

Determination of pH

The pH of the samples was determined by weighing 1g of the fermenting mash, mashing and suspending in 9 ml of distilled water. The pH was measured after shaking with a pH meter (R1 - 02895 HANNA / Italy).

Determination of temperature

This was determined by putting a sterile thermometer in the fermenting castor bean seed mash and taking the reading on the thermometer after two minutes.

Determination of crude protein

Protein content of the samples was determined by the kjedahl method as reported by James (1995). The total N₂ was determined and multiplied by the factor 6.25 to obtain the protein content. A quantity of the sample was weighed into a kjedahl digestion flask. A tablet of selenium catalyst was added to it. 10 ml of concentrated H₂SO₄ was then added to the flask and digested by heating in a fume cupboard until a clear solution was obtained. It was then carefully transferred to a 100 ml volumetric flask and made up to mark. Ten ml of the digest was mixed with an equal volume of 45% NaOH solution in a Kjedahl distillation apparatus. The mixture was distilled and the distillate collected in 10 ml of 4% boric acid solution of the distillate was collected and titrated against 0.02 N H₂SO₄. The total N₂ content was calculated using the equation.

Determination of crude fat

The continuous solvent extraction method as described by James (1995) was used in the determination of ether extract. 2 g of the sample was wrapped in a porous paper (whatman no. 1 filter paper). The wrapped sample was placed in a soxhlet reflux flask. The flask was then placed in a weighed oil extraction flask containing 200 ml of petroleum ether and the upper end of the reflux was connected to a condenser. The petroleum ether was heated to boiling, with an electrothermal heater and the solvent vaporized and condensed into the reflux flask. The sample in the flask was immersed in the solvent and remained in contact until the flask refluxed carrying the oil extract down to the boiling flask. The cycle of vaporization, condensation, extraction and reflux was allowed to continue uninterrupted for 4 h. The defatted sample was removed and the solvent recovered leaving the oil extract (fat). The flask and its oil content was further dried in the oven at 60°C for 30 min, cooled in a dessicator and re-weighed. The weight of oil (fat) was determined by difference.

Determination of soluble carbohydrates

This was done by estimation as described by Balagopalam et al. (1988). Five grams of each sample was weighed into a flask and 10 ml of dilute HCl solution was added. The mixture was boiled until it tested negative to iodine solution. It was subsequently filtered and

Sampling period (days)					
Lime %	0	1	2	3	4
0	5.80ij±0.12	6.00gh±0.00	6.20f±0.06	6.80c±0.01	7.10b±0.09
1	5.70jk±0.04	5.80ij±0.12	6.00gh±0.07	6.60d±0.09	7.00b±0.01
2	5.70jk±0.04	5.80ij±0.12	5.90hi±0.12	6.40e±0.10	7.00b±0.00
3	5.30l±0.06	5.40l±0.12	5.70jk±0.04	6.20f±0.06	6.50de±0.00
4	4.90m±0.00	4.90m±0.00	5.40l±0.09	5.60k±0.00	6.00gh±0.00
5	4.90m±0.00	4.90m±0.00	4.90m±0.00	5.30l±0.06	6.00gh±0.00
3% Nacl	6.20f±0.06	6.40e±0.12	6.80c±0.06	7.00b±0.06	7.40a±0.00
LSD (0.05) =	0.18				

Table 1. Changes in the pH of castor bean seeds treated with lime and Nacl over a 4-day fermentation period.

*Values with the same letters are not significantly (P<0.05) different from one another.

the filtrate collected. 1 ml of the filtrate was measured and 6 ml of Anthrone reagent was added to it. Each sample mixture was put in a test tube and the tubes were corked and boiled in a beaker of water for 10 min. It was allowed to cool and filtered. Absorbance of the filtrate was read in a spectrophotometer and the values recorded.

Statistical analysis

The data obtained in this study were subjected to analysis of variance (lwe, 2002). Statistical significance was accepted at P value was equal to or less than 0.05. The SPSS package (version, 2000) was used for statistical analysis.

RESULTS AND DISCUSSION

Effect of fermentation on pH

Table 1 shows the pH changes in fermenting castor bean, 'Ogiri' with different levels of fresh lime inclusion (0 to 5%) during a four day fermentation period. There was simultaneous increase in the pH of the fermenting castor seed as fermentation period progressed. Similar trends have been observed during the fermentation of some other protein rich plant materials (Steinkraus et al., 1983; Odunfa, 1985; Achinewhu, 1986; Ikenebomeh et al., 1986; Barber et al., 1988; Njoku and Okemadu, 1989; Omafuvbe, 2004, 2006; Yabaya, 2006; Popoola et al., 2007). The pH of fermenting castor seeds ranged from 4.90 to 7.40. Increases in pH during fermentation of protein rich oil seeds have been attributed largely on the production of proteases by fermenting microorganisms which breakdown the proteins of the seeds to amino acids, peptides and free ammonia. It is the free ammonia that causes the pH increase in the substrates. The rise in pH could also depict the period when the desirable flavor components of the condiments are developed (Achi, 1992).

From Table 1, the sample with the highest pH value was that containing 3% NaCl followed by those of 0 to 3% lime. The least values were those of samples 4 and

5% lime inclusion. A rise in pH could be as a result of proteolysis and the release of ammonia following the utilization amino the of acids by fermenting predominantly microorganism, Bacillus species (Omafuvbe, 2006). The release of ammonia or other basic end products have been reported to be common features in the fermentation of vegetable proteins (Hesseltine, 1965; Sarkar et al., 1993). There has been a report on decrease in pH during fermentation of tempoyak an Asian food condiment, from day 0 to 2, followed by gradual increase afterwards. This was attributed to the production of metabolites other than lactic acid such as acetic acid and carbon dioxide produced from heterofermenters LAB (Amin et al., 2004; Leisner et al., 2001). It was also observed that there was significant difference (P<0.05) in the pH values of the Ogiri samples as the concentration of lime increased, the pH values decreased. The reduction was particularly observed at 4 to 5% lime inclusion levels. This could be attributed to the level of citric acid in the lime juice used. The preservative effects of citric acid in food is pH dependent and food products with high acidity always have a long shelf life and can keep without refrigeration even when exposed to microbial contamination (Hersom and Hulland, 1980).

Effect of fermentation on temperature

From Table 2, it was observed that the temperature of the fermenting castor bean seeds increased as the fermentation process progressed. The values ranged from 29.39 to 15.10%. The sample containing 3% NaCl had the highest value of 31.50℃ followed by that with 0% lime inclusion level, with 31.00℃. The results from Table 2 show that addition of lime suppressed rise in temperature at the initial stage which is an indication of suppressed microbial activity. The increase in temperature being attributed to heat or the liberation of carbon dioxide by the fermenting organisms. This might have been as a result of exerted pressure on the substrate.

Sampling period (days)					
Lime %	0	1	2	3	4
0	28.00h±0.00	28.00h±0.00	28.50g±0.00	29.50c±0.00	31.00b±0.00
1	28.00h±0.00	28.00h±0.00	28.50g±0.00	29.50c±0.00	30.00d±0.00
2	28.00h±0.00	28.00h±0.00	28.00h±0.00	29.00f±0.00	30.00d±0.00
3	28.00h±0.00	28.00h±0.00	28.00h±0.00	29.50c±0.00	30.00d±0.00
4	28.00h±0.00	28.00h±0.00	28.00h±0.00	29.00f±0.00	30.50c±0.00
5	28.00h±0.00	28.00h±0.00	28.00h±0.00	29.00f±0.00	30.00d±0.00
3% Nacl	28.00h±0.00	28.00h±0.00	28.00g±0.00	29.00f±0.00	31.50a±0.00
LSD (0.05) =	0.00				

Table 2. Changes in the temperatures of castor bean seeds treated with lime and Nacl over a 4-day fermentation period (°C).

*Values with the same letters are not significantly (P<0.05) different from one another.

Table 3. Changes in the crude protein content of castor bean seeds treated with lime and Nacl over a 4-day period.

Sampling period (days)				
Lime %	1	2	3	4
0	14.58mn±0.04	15.82gh±0.06	17.25b±0.04	17.90a±0.06
1	14.50mn±0.06	15.02ij±0.12	17.00cd±0.12	17.85a±0.06
2	14.50mn±0.06	15.50jk±0.06	16.75c±0.06	17.10bc±0.06
3	14.35n±0.06	15.00l±0.00	15.85gh±0.04	16.34f±0.05
4	14.00o±0.12	14.55mn±0.04	15.35k±0.06	16.00g±0.06
5	13.880±0.05	14.00o±0.12	15.10l±0.06	15.71i±0.34
3% Nacl	14.67m±0.04	15.80gh±0.12	16.32dc±0.05	17.85a±0.00
LSD (0.05) =	0.20			

*Values with the same letters are not significantly (P<0.05) different from one another.

Temperature increases have also been reported by Odunfa (1981). Similar trends have also been observed in other plant materials (Achi, 1992; Ogueke and Nwagwu, 2007). The rise in temperature indicates that the fermentation reaction is exothermic with the changes being due to metabolic activities of the microorganisms (Achi, 1992). The heat generated in the fermenting mash possibly provided the conditions for the optimal activity of the proteolytic enzymes (Odunfa, 1985). An initial increase in temperature followed by a gradual decrease thereafter has been reported during 'ugba' fermentation (Odunfa and Oyeyiola, 1985; Njoku and Okemadu, 1989). During 'Iru' production, Odunfa (1981) reported a continuous increase in temperature during fermentation process.

Effect of fermentation on crude protein

The results from Table 3 show the changes in crude protein of the fermenting castor seeds. There was increase in values of the crude protein content. The highest value 17.90% was recorded on day four in the sample containing 0% lime. This value was not

significantly (P<0.05) different from the values obtained for the samples with 1% lime and 3% NaCl on the fourth day of fermentation. The least value 13.95% was observed on day one for the sample containing 5% lime. This value was significantly (P<0.05) lower that the other values obtained during the fermentation period.

From Table 3, it can be deduced that the fermentation process increased the protein content of the fermented castor bean seed and this will enhance its nutritional quality and increase utilization. Fagbemi et al. (2005) also reported protein increase in fermented fluted pumpkin (*Telfairia occidentalis*) seeds for production of 'Ogiri ugu'. Achinewhu (1982) reported high content of essential amino acids in African oil bean seeds thereby making it a potential source of protein when used as fermented food condiment. Similar trends have been observed with Enujiugha (2003), Omafuvbe et al. (2004) and Pelig-Ba (2009).

Achi (2005) also observed that four day fermentation period for castor oil seed caused the highest increase in protein and tannin as well as decreases in ash, lipid and also non-protein nitrogen. NaCl has been reported to influence the hydrolysis of protein in 'sufu' (fermented soybean curd) to a large extent resulting in increased

Sampling period (days)				
Lime %	0	1	2	4
0	52.67f±0.06	54.31de±0.06	55.80c±0.06	57.00a±0.60
1	52.00g±0.60	54.00c±0.12	54.76d±0.05	56.53ab±0.04
2	51.16h±0.60	52.83f±0.05	54.61d±0.06	56.00bc±0.60
3	50.00j±1.15	51.89g±0.04	52.70f±0.06	54.64d±0.05
4	50.00j±1.15	50.75hi±0.04	51.92g±0.04	53.00f±0.50
5	48.76k±0.03	50.25ij±0.04	50.99h±0.06	51.84g±0.05
3% Nacl	52.00g±0.00	54.00c±0.06	54.89d±0.05	56.71a±0.12
LSD (0.05) =	0.60			

Table 4. Changes in the ether content of castor bean seeds treated with lime and Nacl over a 4-day fermentation period.

*Values with the same letters are not significantly (P< 0.05) different from one another.

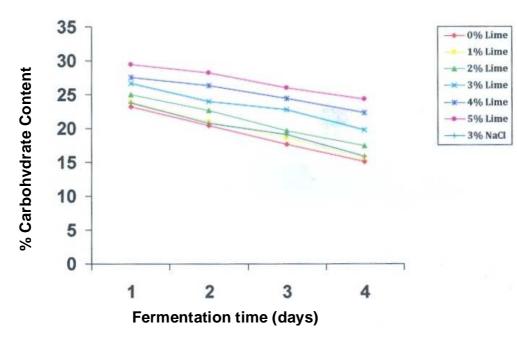


Figure 1. Changes in soluble carbohydrate content of fermenting castor bean.

level of free amino acids (Han et al., 2004).

Effect of fermentation on crude fat

Table 4 shows the changes in the ether extract of the fermenting castor seeds. In all the samples, the ether extract increased as the fermentation period progressed. The values ranged from 48.76 to 57.00. Samples with the highest ether extract values were those of 0% lime and 3% NaCl; followed by those of 1-3% lime inclusion. The increase in the ether extract of the fermenting castor seed as the fermentation process increased is in agreement with the findings of Eka (1980) and Omafuvbe

et al. (2004). Also Achinewhu (1986) reported that 'Ogiri' has a high percentage of fat. Ogbonnaya, et al. (2010) and Odebunmi et al. (2010) also reported increase in lipid enrichment in fermented locust bean.

Effect of fermentation on soluble carbohydrate

The results in Figure 1 shows the changes in soluble carbohydrate of the fermenting castor bean samples. The results show that there was a decrease in the soluble carbohydrate content as the fermentation period increased. The values ranged from 15.10 to 29.39%. It was also observed that the inclusion of lime has an effect

on the soluble carbohydrate content of the samples. As the concentration of lime increased, the soluble carbohydrate content decreased. This indicates that the inclusion of lime reduced the utilization of soluble carbohydrate by the microorganisms in the substrate especially at 4 and 5% lime inclusion throughout the four day fermentation period.

Production of fermented castor bean resulted in a decrease in carbohydrate content. Decrease in carbohydrates has been reported in previous studies on similar products like maari, bikalga and daddawa (Parkouda et al., 2011, 2008; Ibrahim and Antai, 1986). The decrease is likely to be explained by cooking and loss of carbohydrate in the cooking water and by the fermentation where carbohydrates are metabolized by the micro organisms responsible for fermentation (Parkouda et al., 2008). The formation of some volatile aroma compounds belonging to ketones and aldehydes groups which are generally formed via maillard reactions between saccharides and peptides may also play a role (Ledauphin et al., 2003).

Nwosu and Ojimelukwe (1993) observed a similar trend in fermentation of melon and groundnut into 'ogiri' as the total carbohydrate content was appreciably reduced by the fermentation process. Achinewhu (1986) also reported the presence of a reasonable amount of maltose, fructose, glucose and other identified sugars in 'Ogiri'. Ogbonnaya et al. (2010) also reported a reduction in total carbohydrate in fermented locust bean. Ademola et al. (2011) also reported a significant decrease in carbohydrate content in salted and unsalted fermented locust beans. They also observed that the inclusion of salt was able to preserve the physical characteristics of the fermented locust bean seeds.

Conclusion

The results show that the increase in the protein and lipid contents add to the quality of the fermenting castor seeds. Addition of 1 to 3% lime to castor bean prior to fermentation has shown an improvement in the nutritional and physiochemical properties of the fermented castor bean product and lime also acts as natural preservative. The addition of 3% salt also proved efficient in preserving the physiochemical characteristics of fermented castor seed. It is therefore recommended that a range of 1 to 3% of lime inclusion is suitable in 'Ogiri' fermentation having improved on the quality parameters.

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