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Effects of autoclaving on the proximate composition of stored castor (*Ricinus communis*) seeds

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Abstract. *Negedu A, Ameh JB, Umoh VJ, Atawodi SE, Rai MK. 2013. Effects of autoclaving on the proximate composition of stored castor (*Ricinus communis*) seeds. Nusantara Bioscience 5: 51-56.* The effect of autoclaving on the proximate composition, free fatty acids and peroxide value of castor (*Ricinus communis* L.) seeds in storage were studied. Seeds of castor were surface sterilized, dried and divided into two equal sets of 300g each. One set was autoclaved at 15 1b pressure for 30 minutes at 121^{o}C and the other set served as control. Each set was prepared in triplicates and both sets were stored under same room temperature conditions for a period of 180 days and agitated intermittently. Analysis of the proximate composition showed that autoclaving treatment caused an increased total fat content, reduced moisture, protein, nitrogen-free extract (soluble sugar) and ash contents of the seeds in storage, as well as a non-significant increase in crude fiber (non-soluble sugar) content. It increased the free fatty acid content and decreased the peroxide value of seed oil.

Keywords: autoclaving, castor seeds, free fatty acids, peroxide value, proximate composition

Abstrak. *Negedu A, Ameh JB, Umoh VJ, Atawodi SE, Rai MK. 2013. Pengaruh perlakuan autoklaf terhadap komposisi proksimat dari benih jarak kepyar yang disimpan (*Ricinus communis*). Nusantara Bioscience 5: 51-56.* Pengaruh perlakuan autoklaf terhadap komposisi proksimat, asam lemak bebas dan nilai peroksida benih jarak kepyar (*Ricinus communis* L.) dalam penyimpanan dipelajari. Biji jarak kepyar disterilkan permukaannya, dikeringkan dan dibagi menjadi dua kelompok yang sama, masing-masing sebanyak 300g. Salah satu kelompok itu diautoklaf pada tekanan 15 lb selama 30 menit pada suhu 121^{o}C, sedangkan kelompok lainnya digunakan sebagai kontrol. Setiap kelompok diperlakukan dalam tiga ulangan dan kedua kelompok disimpan dalam kondisi suhu ruangan yang sama dalam jangka waktu 180 hari serta beberapa kali dibalik-balik. Analisis komposisi proksimat menunjukkan bahwa perlakuan autoklaf menyebabkan peningkatan kadar lemak total, mengurangi kadar air, protein, ekstrak nitrogen bebas (gula larut) dan kandungan abu dari biji dalam penyimpanan, serta peningkatan secara tidak signifikan kandungan serat kasar (non larut gula). Perlakuan ini meningkatkan kadar asam lemak bebas dan menurunkan nilai peroksida minyak biji.

Kata kunci: perlakuan autoklaf, biji jarak kepyar, asam lemak bebas, angka peroksida, komposisi proksimat

INTRODUCTION

Vegetation belt influences dietary pattern in West Africa. For instance, in Southern Nigeria, legumes, nuts, seeds, starchy roots or tubers dominate, while cereals dominate the northern part (Ajayi et al. 2005). In southeastern Nigeria, popular among the oilseeds used in soups for emulsification and stabilization is *Irvingia gabonensis* (Ataga and Ota-Ibe 2006); *Brachystegia eurycoma* and *Detarium microcarpum* (Ohegbu et al. 2002). But, of particular interest in this study is the castor *(Ricinus communis)* because of versatile industrial applications.

Castor (*Ricinus communis* L.) (Figure 1) bean plant is a dicotyledonous and monoecious herb of the family Euphorbiaceae and it is considered by most authorities to be native to tropical Africa and may have originated in Abyssinia/Ethiopia (CSIR 1976). The castor is cultivated for its seeds which yield versatile oil known as castor oil. The seed contains 45-50.6% oil, 12-16% protein, 23-27% fibre, 3-7% NFE, 5% moisture and 2% ash (CSIR 1976). The annual worldwide production stands at 1,311, 669 metric tonnes. The demand for castor oil is about 453,590 metric tonnes and valued at more than US $500 million (FAO 2005). The oil has about one thousand patented industrial applications and has been used in the production of over four hundred industrial products such as paints, dyes, soaps, cosmetics, polishes, lubricants, plastics, paper, hydraulic fluids, inks, lacquers, machining oils, pigments, sealants, electrical liquids etc. (Roetheli et al. 1991; Gobin et al. 2001). Castor oil enjoys tremendous world demand in the pharmaceutical, cosmetic, textile, paint, leather, lubricant, chemical, plastic, synthetic fiber, automobile and engineering industries (Roetheli et al. 1991; Anjani et al. 2004).

Figure 1. *Ricinus communis*. A. Habit, B. Development of flowers into fruit.

After the extraction of castor oil, the high nitrogen-containing pomace (meal) is suitable for fertilizer, and when detoxified, it can be employed in livestock feed formulation (Uzogora et al. 1990; Joshua 2005).

Castor oil is used as an ingredient in the folk medicine for arthritis, cancer, cholera, convulsion, dog bite, guinea worm, osteomyelitis, rheumatism, venereal diseases, tuberculosis and considered an antidote, bactericide, emetic, emollient, insecticide, larvicidal, laxative, purgative etc (Roetheli et al. 1991). It has been reported that the proximate composition of the seeds of some used as soup thickeners, such as *Mucuna flagellipes* (Udensi et al. 2010); *Mucuna utilis* (Ukachukwu and Obioha 2000; Udensi et al. 2008) have been improved by autoclaving. However, there appears to be dearth of information on the effect that autoclaving would have on the proximate composition of castor seeds.

This study was, therefore, undertaken to evaluate the effect that autoclaving treatment will have on the proximate composition of castor seeds to recommend it as a pre-storage treatment for the preservation of the nutritional values of seeds.

MATERIALS AND METHODS

Collection of seed samples

Shortly after harvesting and sun-drying of castor seeds by farmers, seed samples were purchased from local farmers at Ankpa, Kogi State, Nigeria. Visibly moldy as well as necrotic lesioned seeds were handpicked and whole seeds that failed to pass through ¾ x ¾ inch mesh were used for treatments.

Sample preparation

The approximately uniform and clean seeds were surface sterilized using 1% sodium hypochlorite solution (NaOCl) and rinsed consecutively in sterile de-mineralized water. The surface sterilized seeds were divided into two sets of 300g each and placed in 1-liter autoclavable plastic jars. Each set was prepared in triplicates and one set was autoclaved at 15 1b pressure for 30 minutes at 121^{o}C and cooled, while the second set served as control (raw seed). Both sets were stored under same normal room temperature of 27±1^{o}C for a period of 180 days (6 months). At intervals of sixty days (2 months), samples were taken from each set (autoclaved and control) and analyzed for proximate composition using standard methods of AOAC (1995). The biochemical changes that occurred in both sets of seed samples were compared.

Analysis of proximate composition

Moisture content of the samples was determined by drying to a constant weight of 105^{o}C in a forced draught oven. Crude protein content was determined using the micro Kjeldahl digestion method described by AOAC (1995). The total ash content was determined using the method of Kirk and Sawyer (1991). The total ash present in 5g of the sample was determined by incinerating the sample in a muffle furnace at 550^{o}C for 3 hours. The

method described by Kirk and Sawyer (1991) was used to determine the crude fiber content of the samples. The protocol for the crude fiber content is briefly given. Two grams of defatted sample was boiled in 200cm^3 of 0.1275 M sulphuric acid solution for 30 minutes with constant agitation. The boiling mixture was poured into a Buckner funnel and washed with boiling water twice. Then the residue was boiled in a 0.313 M sodium hydroxide solution for 30 minutes with constant stirring. The residue was then washed twice with boiling water followed by 1% HCl, then washed with boiling water until free from acid. It was then dried in an oven to a constant weight. Carbohydrate was determined by difference (100-(protein + fat + moisture + ash).

The nitrogen value, which is the precursor for protein of a substance, was determined by micro-Kjeldahl method (Guelbel et al. 1991). The nitrogen value was converted to protein by multiplying with a factor of 6.25. The crude lipid content of the sample was determined using Soxhlet type of the direct solvent extraction method. The solvent used was petroleum ether (boiling range 40-60 ^{o}C). All proximate values were reported in percentage (AOCS 2000; Okwu and Morah 2004).

Data analysis

Data were expressed as mean±standard error of M (SEM). The data were subjected to one-way analysis of variance (ANOVA). SPSS software was used to analyze the data and $P< 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Effects of autoclaving on moisture content of castor seeds after 180 days of storage

In the control (un-autoclaved) and autoclaved, there was a similar trend in moisture content levels (Figure 2.). From an initial level of 7.9±0.21%, the moisture declined through 60 days to lower values (5.57±0.16% and 6.45±0.16%) in the control and autoclaved respectively. Following this point, the seed moisture content increased to 8.71±0.19% in the un-autoclaved. At the end of storage period of 180 days, the level of moisture in the control (un-autoclaved) was higher than in the autoclaved seeds. Statistical analysis shows that the difference in the values of moisture between the control and autoclaved was significant ($P\geq0.05$).

The significant decrease in moisture content of the autoclaved seeds (Figure 2.) agreed with Ward and Diener (1961) on steamed peanuts and Ohegbu et al. (2009) on *Brachystegia eurycoma*. The decrease could have been as a result of physical damage to the structural integrity of the seeds and denaturation of protein structure leading to reduced water holding capacity of the seeds during storage.

Effect of autoclaving on crude protein (cp) content of castor seeds after 180 days of storage

Figure 3 presents the trend in the level of crude protein content of the castor seeds after 180 days of storage. Within 0-60 days, in the control (un-autoclaved), there was a similar trend in crude protein values. A rise in the initial value (21.28±0.03%) to a higher value (27.07±1.76%) was observed. A slight decline from the initial value (21.28±0.03%) to a lower level (20.80±1.76%) was observed in the autoclaved seeds. Between 120-180 days, a decline in the crude protein value was observed in the un-autoclaved and autoclaved (from 26.36±0.40% to 24.47±0.55% and from 20.26±0.40% to 17.78±0.55% respectively). At the end of storage period, the level of crude protein in the control (un-autoclaved) was higher than in the autoclaved seeds). Statistical analysis shows that the difference in the crude protein values between the un-autoclaved and the control was significantly different ($P \leq 0.05$).

The decline in the level of crude protein in the autoclaved seeds (Figure 3.) agreed with Ward and Diener (1961) who reported similar result on steamed peanuts and Udensi et al. (2004) who reported similar observation on autoclaved seeds of *Mucuna utilis*. The increase in protein content could have been due to reduction/destruction of certain protease inhibitors and anti-nutrients like phytic acid and tannins which form complexes with protein and make it unavailable during hydrolysis.

Effect of autoclaving on total fat content of castor seeds after 180 days of storage

Figure 4 presents the mean values of the total fat content with respect to autoclaving treatment. In the un-autoclaved seeds, it was observed that the total fat declined from the initial value (47.55±0.42%) to a lower value (24.93±2.23%) at 60 days, 21.77±0.15% at 120 days and 13.19±1.44% at 180 days. But in the autoclaved, after the decline from the initial value (47.55±0.42%) to a lower value (32.84±2.23%), at 60 days, the value remained almost unchanged till end of storage (180 days). However, at the end of storage, the level of fat content in the autoclaved was higher than in the control (un-autoclaved) and the difference in the level, between the control and the autoclaved, was statistically significant ($P \leq 0.05$).

The higher total fat content of the autoclaved seeds (Figure 4.) agreed with the findings of Ezeokonkwo (2005) who observed increased total fat content of steamed seeds of an oilseed crop (African almond-*Terminalia catappa*). The significantly higher total fat value in the autoclaved seeds as compared to the control could be as a result of the preservation of the total fat by the autoclaving treatment which might have inactivated the endogenous enzymes (lipoxygenases) of the seeds. This reasoning is supported by the findings of Majunder (2007) and Sreerama et al. (2008) who reported that post-harvest practices accelerate moisture migration, together with thermogenesis leading to enhanced deterioration of seed constituents such as fat, but, because endogenous enzymes were inactivated in the autoclaved seeds, there was lesser deterioration of the total fat compared to the raw seeds in which the endogenous enzymes might have been reduced the total fat content.

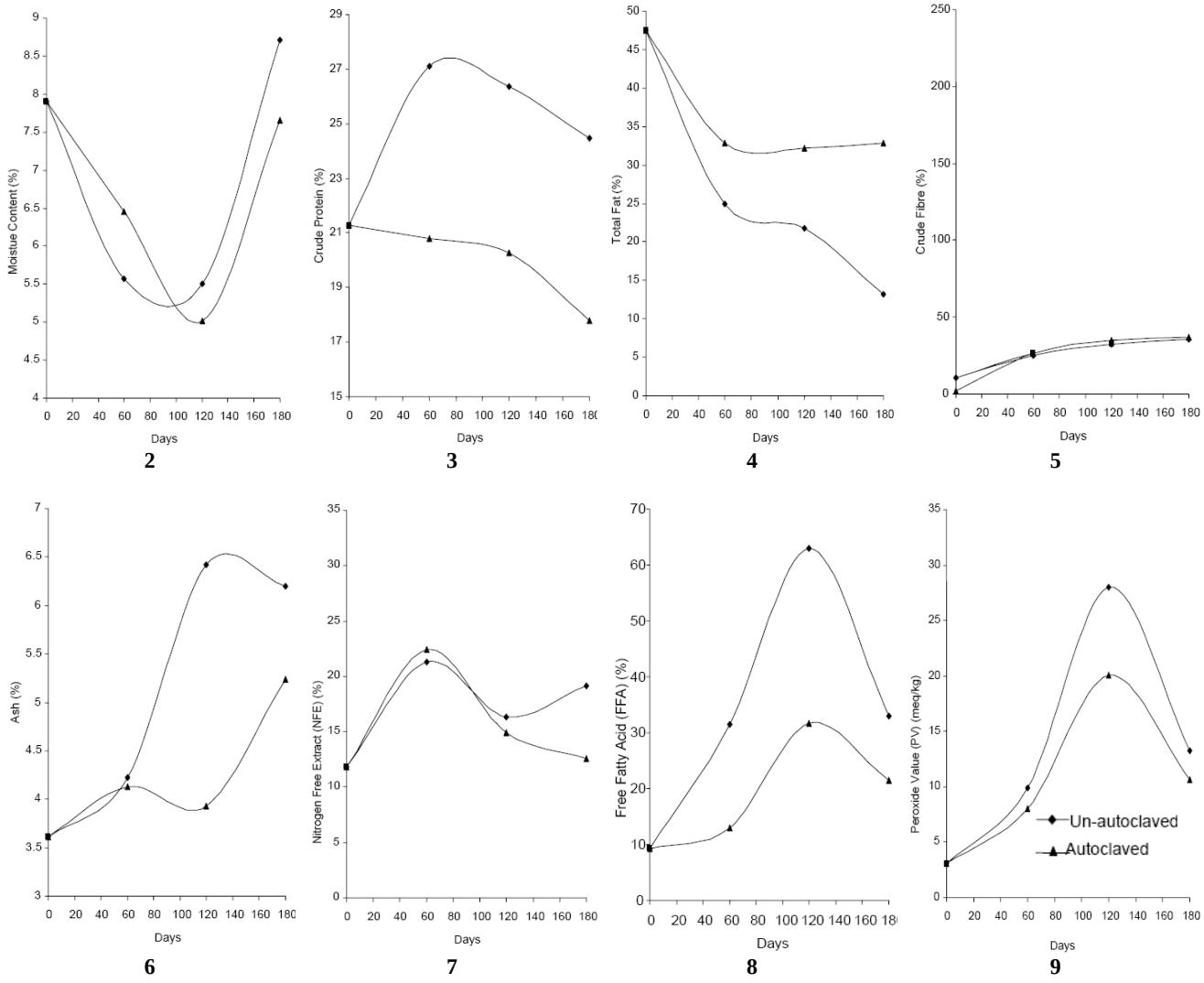

Figure 2. Effects autoclaving on the moisture content of castor seeds after 180 days of storage
Figure 3. Effect of autoclaving on the crude protein content of castor seeds after 180 days of storage
Figure 4. Effects of autoclaving on total fat content of castor seeds after 180 days of storage
Figure 5. Effects of autoclaving on crude fiber content of castor seeds after 180 days of storage
Figure 6. Effects of autoclaving on the ash content of castor seeds after 180 days of storage
Figure 7. Effects of autoclaving on the nitrogen-free extract content of castor seeds after 180 days of storage
Figure 8. Effects of autoclaving on free fatty acid content of castor seeds after 180 days of storage
Figure 9. Effects of autoclaving on the peroxide value of castor seeds after 180 days of storage

Effects of autoclaving on crude fiber content of castor seeds after 180 days of storage

The trend in the crude fiber content from 0-180 days of storage is presented in Figure 5. In both the un-autoclaved and autoclaved), a similar trend occurred. From the initial value (10.68±2.11%), the level of crude fiber content rose steadily to a higher value (35.60±30.18%), while a gradual rise was recorded in the autoclaved till the end of storage (36.80a±30.18). However, at the end of storage, the difference in the level of crude fiber between the control and the autoclaved was not statistically significant ($P \geq 0.05$).

The non-significantly higher level of crude fiber content (Figure 5.) observed in the autoclaved seeds during this study agreed with the report of Apata (2008) who observed that autoclaving did not cause appreciable changes in the crude fiber content of groundnut meal. The non-significant difference between autoclaved and un-autoclaved seeds with respect to crude fiber content could have been due to the presence of some heat-stable factors in the seeds causing less hydrolysis of the structural carbohydrates. The presence of heat-stable factors in other oilseeds such as *Jatropha curcas* has been reported (Martinez-Herrera et al. (2005).

Effects of autoclaving on the total ash content of castor seeds after 180 days of storage

The trend in the values of ash content from 0-180 days of storage is presented in Figure 6. In un-autoclaved and autoclaved seeds, a similar trend was observed. A gradual rise from the initial value (3.61±1.01%) to higher values

after 180 days (6.20±0.25% and 5.24±0.25% respectively) was observed. After 180 days, the value of the ash content in the un-autoclaved was higher than that in the seeds. However, in both (control and autoclaved), at 180 days of storage, the difference in values between them, were statistically significant ($P \leq 0.05$) with respect to ash content.

The significantly higher level of ash content in the autoclaved seeds than that of un-autoclaved seeds (Figure5.) agreed with the findings of Ezeokonkwo (2005) who obtained similar result from steamed seeds of another oilseed, African almond (*Terminalia catappa*). Salunkhe and Desai (1986) had also reported increased ash content of steamed groundnuts seeds. The higher level of ash in the un-autoclaved seeds could be attributed to non-leaching of the minerals from the seeds which might have occurred in the autoclaved seeds during the autoclaving process. This reasoning is supported by Ataga and Ota-Ibe (2006) who reported leaching of minerals from steamed seeds of Wild Mango (Irvingia gabonensis) leading to decreased total ash content in the autoclaved seeds than in the control.

Effects of autoclaving on the nitrogen-free extract (NFE) content of castor seeds after 180 days of storage

From the trend in the values of the NFE content of castor seed presented in Figure 5.24, the un-autoclaved showed a rise from the initial value (11.79±1.11%) to a higher value (21.31±1.28%) at 60 days and followed by a gradual decline to a lower level (16.29±1.82%). The value gradually rose to a level higher (19.17±0.07%). In the autoclaved seeds, after the initial rise from the pre-storage value (11.79±1.11%) to a higher level (22.44±1.28%), a steady decline followed till end of storage to a lower value (12.55±0.70%). Statistical analysis reveals that at 180 days, the mean values in the level of the NFE, between the autoclaved and controls (un-autoclaved) were significantly different ($P \leq 0.05$).

The significant decrease in the soluble sugar (NFE-nitrogen-free extract) of the autoclaved seeds (Figure 7.) disagreed with the findings of Ezeokonkwo (2005) who reported that roasting or steaming increased the level of soluble carbohydrates (NFE) in another oilseed (*Terminalia catappa*). In addition, Apata (2008) reported that autoclaving did not induce appreciable changes in the composition of cellulose, non-cellulosic polysaccharides and lignin of processed groundnut meal. It has been reported that some seeds may possess heat– stable factors such as lectins and trypsin inhibitors (Martinez–Herrera et al. 2005) which make such seeds more resistant to hydrolysis by heat. The decrease in the level of NFE could be as a result of more stability of some structural carbohydrates of the castor seeds that allowed less hydrolysis of the insoluble sugars (crude fiber) into soluble sugars (NFE).

Effects of autoclaving on free fatty acid (FFA) content of castor seeds after 180 days of storage

The trend in the levels of free fatty acid content of castor seed due to autoclaving is presented in Figure 8. In both autoclaved and control, a similar trend was observed. From the initial value (9.21±0.02%) of the free fatty acid, rise to higher values occurred (31.74±2.34% in the autoclaved and 62.90±2.34% in the control seeds). This was followed by a decline to lower values (21.41±2.98% and 32.94±2.98% in the autoclaved and un-autoclaved respectively) at the end of storage. At the end of the storage period of 180 days, the level of free fatty acid in the autoclaved seeds was lower compared to the un-autoclaved control). Statistical analysis showed that difference in the levels of free fatty acids between the control and the autoclaved was statistically significant ($P \leq 0.05$).

The increase in the level of free fatty acid in the autoclaved seeds (Figure 8.) agreed with Oso (1978), Manji et al. (2006) who reported increased free fatty acid in steamed oil palm fruits. The increase could be due to greater liberation of free fatty acid by the heat process or conversion of the oil into their constituent fatty acids. This is supported by Onyeka et al. (2005) on heated fruits of another oilseed, Black pear (*Dacryodes edulis*). The decline could be as a result of the transformation of the free fatty acid into fatty acid hydroxy peroxides at a rate faster than they were formed, since the peroxides themselves are unstable and decomposed into stable compounds such as aldehydes, ketones, epoxides (Sowunmi 1981).

Effects of autoclaving on the peroxide value of castor seeds after 180 days of storage

Figure 9, presents the trend in the levels of peroxide value of castor seed during storage. An initial rise in the level of peroxide was observed in the autoclaved and un-autoclaved seeds. After 120 days of storage, a decline in the level of peroxide value occurred in the autoclaved and un-autoclaved. The level of peroxide was lower in the autoclaved compared to the un-autoclaved seeds after 180 days of storage period. Statistical analysis showed that between the autoclaved and un-autoclaved seeds, the difference in the level of peroxide value was statistically significant ($p \leq 0.05$).

The significantly higher level of peroxide value in the un-autoclaved seeds (Figure 9) compared to the autoclaved disagreed with Bankole et al. (2005) who reported higher peroxide in the steamed melon seeds. This variance could have been due to the decrease in the peroxide value resulting from the faster rate of decomposition of the hydroperoxy fatty acids (which are themselves unstable) into secondary products such as ketones, aldehydes, epoxides which are more stable and are largely responsible for the off flavors and objectionable odors in deteriorated seeds or oily products. This is supported by the findings of (Going 1968; Arumughan et al. 1984; Amoo and Asoore 2006), they reported faster rate of decomposition of hydroperoxy fatty acids into secondary products.

CONCLUSION

The study has shown that when castor seeds are autoclaved and stored, the total fat content of the seeds increased, with increased free fatty acid level of the seed

oil. The increase in the free fatty acid level of oilseed is not healthy for the economic values of the seed, because increased free fatty acid level will cause rise in cost of processing and also attract reduction in seed price as penalty. In addition, the decreased protein and soluble sugars may not be economically advantageous for those interested in using the seed protein and soluble sugars. Therefore, it is not advisable to autoclave seeds before storage. If storage of seeds is for the purpose of economic end products, such as the oil, protein and soluble sugars, then, autoclaving may not be recommended.

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