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EFFECT OF SELECTED PRESERVATIVES ON NUTRITIONAL INDICES OF KIDNEY BEANS (*PHASEOLUS VULGARIS*)

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ABSTRACT

Preservatives are most often used to maintain the quality of foods for longer time periods. It has been reported that chemicals, which are used as preservatives have some effects (positive or negative) on the nutritive indices of foods. This research was carried out to investigate the effect of some selected preservatives on the nutritive indices of kidney beans *(Phaseolus vulgaris).* The matured *P. vulgaris* used for this research were collected in bags from the area of cultivation. Following the established research guidelines, the seeds were prepared and sent to laboratory for analysis prior to storage with selected preservatives. The selected preservatives were procured and prepared for the experimental purpose under standard protocols. The kidney beans were divided into airtight containers and labeled in group 1 to 5, which were preserved with selected preservatives; (DDVP) (sniper), birds eye pepper and Aluminium phosphide, while the fourth group was mixed with ash and the fifth group was left without mixing with any preservative to serve as control. The beans mixed with preservative were left to stay on standard research procedure for 6 months. Standard procedures were used to assess the effect of the preservatives on the proximate compositions, mineral contents, and amino acid concentrations, respectively compared with the control group, which was not mixed with the preservatives. Results of the research showed varying effects of the different preservatives on the beans mixed with preservatives when compared with the control group. There was an observed improvement on the proximate composition of the beans preserved with some of the selected preservatives while some beans were negatively affected as the preservative affected the nutritive quality as compared to control groups; same results were observed for the mineral nutrient constituents and amino acid concentrations. The observed improvements in the experimental parameters showed that the chemicals in some groups were more effective for some aspects while natural preservatives were better for other measured criteria. The effectiveness of the preservatives for a longer shelf life and stop or delay the growth of bacteria, suppress the reaction when food comes in contact with oxygen or heat, they also prevent the loss of some essential amino-acids and some vitamins enhance the food flavors and colors. The results of this study showed that some of the selected preservatives used on the kidney beans suggested that kidney beans could improve the shelf life and improve nutritive value.

Key words: Preservatives, Nutrition, Nutritive index, Shelf-life, Storage, proximate analysis, amino acid, minerals

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INTRODUCTION

Legumes are plants belonging to the family Leguminosae, also called Fabaceae that produce seeds within a pod [1, 2]. Common legumes used for human consumption include kidney beans, lima beans, peas, broad beans, lentils, soybeans, lupins, lotus, sprouts, mung bean, green beans and peanuts and are referred to as grain legumes or food legumes [3]. These seeds are valued worldwide as an inexpensive meat alternative and are considered the second most important food source after cereals [4]. Legumes are important especially in terms of human nutrition because they contain essential amino acids that are needed for building proteins, complex carbohydrates, dietary fibre, unsaturated fats, vitamins and minerals needed for human diet [5, 6]. In addition to their nutritional superiority, legumes have also been known to possess economic, cultural, physiological, and medicinal roles owing to their possession of beneficial bioactive compounds [6].

Phaseolus vulgaris, commonly known as kidney beans is a major grain legume consumed worldwide for its edible seeds and pods. It is a highly polymorphic warm-season, herbaceous annual. The kidney bean is a highly variable species with a long history of human consumption. Bush varieties form erect bushes 20-60 cm tall, while pole or running varieties form vines 2-3 m long. All varieties bear alternate, green or purple leaves, which are divided into three oval, smooth-edged leaflets, each 6-15 cm long, and 3-11 cm wide [7]. The leaves of the beans are occasionally used as a vegetable, and the straw can be used for fodder. *Phaseolus vulgaris* is among the common bean species. Its botanical classification, along with other *Phaseolus* species, is as a member of the legume family Fabacae, and must acquire the nitrogen they require through an association with rhizobia, a species of nitrogen-fixing bacteria [8]. It is reddish brown in colour, and it originated from central and South America. Small-seeded and climbing ecotypes are found in the wild in north Argentina and Central America. Kidney bean was independently domesticated in both Central America and in the South America Andes (mainly Peru) [9]. *P. vulgaris* are a rich source of protein and carbohydrates, as well as minerals and vitamins. This diet complements the mainly cereal foods in countries that grow cowpea as a major food crop. Kidney beans are rich in calcium, magnesium, phosphorus, most especially iron. It also has a small amount of sodium, zinc, copper, and manganese [10]. They are also an excellent source of resistant starch, which is broken down by bacteria in the large intestine to produce short-chain fatty acid used by intestinal cells for food energy [11]. It contains calories of 378.9 kcal energy value, 59.0 g total carbohydrates, 1.2 g fat, 25.0 g protein, 1.3 g vitamin c, 40.0 g calcium and 1.8 g iron. It has a better nutritive value

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than cereal straws because it has higher protein content 61% (DM of NDF) vs 51% for a leaf-rich straw [11].

Phaseolus vulgaris production is hindered by pest infestation which reduces its quality and also its quantity. To this effect, pesticide use comes into play to kill these pests [12].

The use of pesticides in grain storage is more intensive in the Northern part of Nigeria especially at Doma grains market in Nasarawa State, which is viewed as the largest grain market in Nigeria. The *P. vulgaris* grain farmers and trader's ignorance about pesticide toxicity has led to its misuse such as abusive application, and inadequate usage. Dichlorvos and Aluminium phosphide are part of the classes of insecticides referred to as organophosphates used to control household and stored products, insects. They are effective against mushroom flies, aphids, spider mites, caterpillars, thrips, and white flies in green-houses, outdoor fruits, and vegetable crops [13]. They act against insects as a contact and stomach poison. Concentrates of Dichlorvos is mildly irritating to skin and may cause localized sweating, involuntary muscle contractions, and burning sensations or actual burns. When inhaled, the first effects are usually respiratory, and may include bloody or runny nose, coughing, chest discomfort, difficult or short breath, and wheezing due to constriction or excess fluid in the bronchial tubes. When in contact with eyes, it will cause pain, bleeding, tears, pupil constriction, and blurred vision [2].

Aluminium phosphide (AlP) is the active component as it liberates highly toxic phosphine gas when it comes in contact either with atmospheric moisture or with hydrochloric acid in the stomach [14]. Thus, tablets or pellets gradually lose their potency on exposure to the atmosphere as they release phosphine gas and leave behind a nontoxic residue in the form of aluminum hydroxide. Phosphine, either during inhalation or exhalation after ingestion, directly produces injury to the alveolar capillary membrane in addition to oxidative injury leading to acute lung injury. The exact underlying mechanism of cardiotoxicity and acute circulatory failure caused by phosphine is not well defined.

MATERIALS AND METHODS

The study involved the use of Dichlorvos, Aluminum phosphide, ash, and pepper as preservatives of *P. vulgaris*. This is to determine the effect of the use of these preservatives on the nutritional indices of *P. vulgaris*. In all cases the effects in

preserved groups were compared with that of control for appropriate decisions to be made.

Sample Collection and Processing

Collection of Samples

The matured *P. vulgaris* were collected from the area of cultivation in Doma, Doma Local Government Area, Nasarawa State, Nigeria. The seeds were collected in bags and transported to the laboratory, followed by cleaning and sorting to remove stones and dirt.

Identification of Samples

The seeds were identified and authenticated by an academic staff of the department of Plant science and Biotechnology, Faculty of Science, Nasarawa University Keffi, Nigeria.

Wood-ash preparation

The stem from neem tree *(Azadirachta indica)* was burnt to ash. The cooled ash was then sieved to remove dirt and 150 g weighed, and packed into nylon bags.

Pepper Preparation

Fresh birds eye pepper *(Capsicum frutescens)* purchased from the market was dried in the sun and 150 g of the sun-dried pepper was weighed and packed in nylon bags.

Procurement of Chemicals

Sniper (Dichlorvos or 2, 2-dichlorovinyl dimethyl phosphate (DDVP)) and Aluminium phosphide were purchased from a standard agro-allied store in Keffi, Nasarawa state.

Seed preservation

The cleaned dried-seeds *(P. vulgaris)* were divided into four parts, each having a bucket with tight lids that contained seeds weighing about 200 g. In the first bucket, kidney bean seeds were mixed with DDVP, while the second contained seeds mixed with birds eye pepper, and the third bucket contained seeds mixed with ash. The fourth bucket contained kidney beans with no preservative/treatment, serving as control. The seeds were stored for a period of six (6) months and properly labeled. During the storage period the seeds were checked periodically.

Processing of the kidney beans

Each treatment including the control was milled into powder, packed in a clean polythene bag, labeled, and sealed. The powdered bean was kept for analysis.

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Proximate Composition Analysis

The powdered samples were analyzed for moisture, protein, fat, ash, fiber, and nitrogen free extract using standard procedures as described by AOAC 2005 methods and Carbohydrate was obtained by subtracting the sum of other proximate parameters from 100.

Determination of Ash Content

When foods and food products are heated to temperatures of 500-600 ^{o}C, the water and other volatile constituents are evaporated and the organic constituents are burned in the presence of oxygen of the air to carbon dioxide and oxides of nitrogen and also eliminated together with hydrogen as water. The mineral constituents remain in the residues as oxides, sulfates, phosphates and chlorides and this inorganic residue constitutes the ash of the food. Ash content was determined using AOAC 2005 method.

Nitrogen determination by micro Kjeldahl method (crude protein)

The nitrogenous compounds of the food material are converted into ammonium sulphate by digesting with concentrated sulphuric acid in presence of digestion mixture, which acts as a catalyst. The clear digest after dilution is made basic with strong alkali, and the liberated ammonia is steam distilled into boric acid.

Fat Determination of Crude Fat

Fats are characterized by the readiness with which they are extracted by ethyl ether, petroleum spirit, carbon disulfide, and n-Hexane. Hence these organic solvents are used for extracting fats from food samples. These solvents also extract small amounts of substances other than the fat and the result is generally designated as crude fat or ether extract.

Crude Fibre Determination

The sample were allowed to boil with 1.25% (w/v) dilute H_2SO_4, washed with water, further boiled with 1.25% dilute sodium hydroxide and the remaining residue after digestion was taken as crude fibre.

Micronutrient Analysis

Atomic absorption spectrometry (AAS) detects elements in either liquid or solid samples through the application of characteristic wavelengths of electromagnetic radiation from a light source. Individual elements will absorb wavelengths differently, and the absorbance are measured against standards. In effect, AAS

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takes advantage of the different radiation wavelengths that are absorbed by different atoms.

Determination of minerals such as Iron (Fe), Zinc (Zn), Calcium (Ca), Potassium (K) and Magnesium (Mg) was carried out using (Atomic Absorption Spectrometry). Bean samples used were analyzed following the methods of the AOAC method. Determination of Amino Acid Profile by AOAC 20005.

Phenylthiohydantoin (PTH) (amino acid Analyzer (HPLC) automatically analyzes phenylthiohydantoin (PTH) amino acids derived from Edman degradation) of proteins and peptides. (Edman degradation, developed by Pehr Edman, is a method of sequencing amino acids in a peptide. In this method, the amino-terminal residue is labeled and cleaved from the peptide without disrupting the peptide bonds between other amino acid residues).

Method of Calculating Amino Acid Values

An integrator attached to the analyzer calculates the peak area proportional to the concentration of each of the amino acids.

Statistical Analysis

The data (collected was analyzed using means, standard deviation, and standard error of the means. One way analysis of variance (Anova) and Duncan's New Multiple Range Test were used to separate and compare differences between means. Significant differences were expressed at $p<0.05$).

RESULTS AND DISCUSSION

The nutritive indices of edible foods or food products are most often determined from the observations and experimental findings obtained from proximate composition screening of such food. Any factor that affects the compositions will likely affect the overall nutritive value of such foods. Improving nutritive value and reduction in spoilage rate of foods requires adopting measures, such as the use of both natural preservatives and other chemicals [4]. The results presented in Table 1, showed the effects of the selected preservatives on proximate composition of kidney beans. The results showed the proximate composition of the kidney beans preserved with sniper to have the lowest moisture content while the wood ash preserved had the highest moisture content. This suggests that sniper possesses dehydration effects on the kidney beans and will therefore regulate microbial activities to prevent spoilage [11]. It was observed also that the seeds treated with aluminium phosphide had the highest protein content compared with other

preservatives and the control. Also, it was observed that the pepper and aluminium phosphide preserved seeds had improved crude fibre contents when compared with control. Same for carbohydrate as all the preservatives caused significant ($p<0.05$) increase in the treatment groups compared with the control.

For the effect of selected preservatives on mineral composition of the kidney beans as shown in Table 2, it was observed that the kidney beans preserved with wood ash, sniper and pepper improved the mineral elements concentrations while others had an opposite effect. Calcium is the most abundant mineral in the body as it regulates many cellular processes and has other vital roles in living organisms [7]. The Dietary Reference Intake (DRI) value for calcium is 1000 mg/day (21). The results of this work showed that calcium content, was improved with a concentration of 12.92 ± 0.6 mg/ 100 g for groups preserved with sniper when compared with the control which had 10.58 ± 0.20 mg/ 100 g for sniper. Zinc plays a vital role in cellular membrane structure and function, and helps to maintain adequate levels of vitamin A in the body [21]. It acts as a potent antioxidant and is essential for growth and development of body tissues, proper immune function, and regulation of insulin.

Amino acids are important biomolecules that both serve as building blocks of proteins and are intermediates in various metabolic pathways. They serve as precursors for synthesis of a wide range of biologically important substances including nucleotides, peptide hormones, and neurotransmitters. Moreover, amino acids play important roles in cell signaling and act as regulators of gene expression and protein phosphorylation cascade nutrient transport and metabolism in animal cells as well as innate and cell-mediated immune responses [27].

The effect of some selected preservatives on kidney beans amino acid composition is presented in Table 3 for its essential and nonessential composition. The results showed that seeds treated with preservatives led to significant ($p<0.05$) reductions in the essential amino acid compositions of both the essential and non-essential amino acids. However, glutamic acid content was the highest followed by aspartic acid while cysteine and methionine were the lowest. It was observed that the each of selected preservatives had separate effects on each group when compared with the control. It was observed that some preservatives (pepper and wood ash) helped in preventing spoilage and enhancing nutritive indices while some (aluminium phosphide and sniper) compromised the nutritive value.

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CONCLUSION, AND RECOMMENDATIONS FOR DEVELOPMENT

Even though natural preservatives have been used for centuries, the synthetic chemicals sniper and aluminium phosphide were comparatively studied and compared to natural preservatives like pepper and wood ash. It was observed that the chemicals were as well effective with respect to some measured quality attributes when compared the natural preservatives.
However, it is recommended that the health implication of these selected preservatives as well as storage time should be assessed in future studies. It is also important to undertake further studies on residue levels of the preservatives in foods after the preservation period.

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Table 1: Effect of selected preservatives on proximate composition of kidney beans (%)

	Crude protein	Crude fat	Crude fibre	Moisture	Ash	Carbohydrate
KA	21.31 ± 0.07^{c}	4.35 ± 0.02^{a}	5.15 ± 0.04^{b}	1.21 ± 0.09a	5.11 ± 0.03^{e}	62.87 ± 0.12^{d}
KB	19.14 ± 0.03^{a}	4.56 ± 0.02^{b}	9.92 ± 0.21^{d}	2.28 ± 0.09^{b}	4.42 ± 0.05^{d}	59.68 ± 0.16^{b}
KC	21.52 ± 0.05^{d}	4.64 ± 0.04^{b}	8.38 ± 0.25^{c}	2.89 ± 0.07^{c}	4.07 ± 0.04^{c}	58.50 ± 0.21^{a}
KD	20.82 ± 0.04^{b}	5.82 ± 0.10^{c}	3.43 ± 0.04^{a}	8.06 ± 0.14^{e}	3.15 ± 0.03^{a}	58.72 ± 0.16^{a}
KE	21.49 ± 0.09^{c}	4.57 ± 0.03^{b}	3.83 ± 0.06^{a}	4.75 ± 0.12^{d}	3.43 ± 0.02^{b}	61.93 ± 0.21^{c}

Mean ± SEM of three replications
KA - Kidney beans preserved with sniper
KB - Kidney beans preserved with pepper
KC - Kidney beans preserved with aluminium phosphate
KD - Kidney beans preserved with wood ash
KE - Kidney beans preserved with control
Note: Mean values with different superscripts down the column are considered significantly different at ($p < 0.05$)

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Table 2: Effect of selected preservatives on mineral composition of kidney beans (mg/1000 g)

	Ca	Fe	Zn	K	Mg
KA	12.92 ± .06^{e}	4.37 ± 0.03^{c}	0.63 ±0.002^{b}	104.89 ± 0.33^{a}	73.46 ± 0.04^{d}
KB	9.74 ± .02^{c}	3.98 ± .07^{b}	0.55 ± 0.01^{a}	192.23 ± 0.10^{d}	55.71 ± 0.05^{a}
KC	7.42 ± .03^{a}	3.78 ± .02^{a}	1.48 ± 0.01^{d}	126.54 ± 0.17^{c}	137.93 ± 0.55^{e}
KD	7.75 ± .04^{b}	5.24 ± .02^{e}	0.63 ± 0.02^{b}	107.61 ± 0.12^{b}	64.44 ± 0.10^{b}
KE	10.58 ± .02^{d}	4.08 ± .02^{b}	0.71 ± 0.01c	104.38 ± 0.09^{a}	69.35 ± 0.06^{c}

Mean ± SEM of three replications
KA - Kidney beans preserved with sniper
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Table 3: Effect of selected preservatives on amino acid composition of Kidney beans (g/100 g)

Amin acids	KA	KB	KC	KD	KE
Leucine	4.56 ± 0.09^{ab}	5.25 ± 0.17^{c}	4.93 ± 0.10^{bc}	4.25 ± 0.12^{a}	4.41 ± 0.21^{a}
Lysine	7.65 ± 0.20^{b}	8.17 ± 0.17^{b}	7.40 ± 0.18^{ab}	6.72 ± 0,11^{a}	7.77 ± 0.45^{b}
Isoleucine	5.12 ± 0.12^{b}	5.56 ±0.13^{c}	5.23 ± 0.09^{b}	4.63 ± 0.10^{a}	5.07 ± 0.02^{b}
Phenyalanine	5.21 ± 0.15^{a}	6.08 ± 0.13^{c}	5.64 ± 0.10^{b}	5.52 ± 0.10^{ab}	5.15 ± 0.02^{a}
Tryptophan	1.09 ± 0.13^{a}	1.24 ± 0.11^{a}	1.13 ± 0.16^{a}	0.91 ± 0.11^{a}	1.05 ± 0.01^{a}
Histidine	2.74 ± 0.11^{a}	3.61 ± 0.13^{b}	2.62 ± 0.15^{a}	2.27± 0.14^{a}	2.65 ± 0.15^{a}
Threonine	4.25 ± 0.12^{ab}	5.03 ± 0.15^{c}	4.62 ± 0.12^{b}	4.17 ± 0.11^{a}	4.51 ± 0.12$_{ab}$
Valine	5.82 ± 0.10^{bc}	6.19 ± 0.15^{c}	5.62 ± 0.15^{b}	4.97 ± 0.14^{a}	5.88 ± 0.06^{bc}
Methionine	1.11 ± 0.12^{a}	1.16 ± 0.14^{a}	1.00 ± 0.10^{a}	0.78 ± 0.12^{a}	1.06 ± 0.01^{a}
Proline	3.12 ± 0.14^{bc}	3.46 ± 0.20^{c}	2.75 ± 0.16^{ab}	2.46 ± 0.15^{a}	3.07 ± 0.01^{bc}
Arginine	6.18 ± 0.09^{b}	6.78 ± 0.14^{c}	6.07 ± 0.15^{b}	5.64 ± 0.16^{a}	6.15 ± 0.02^{b}
Tyrosine	4.80 ± 0.09^{b}	5.53± 0.13^{c}	4.32 ± 0.16^{a}	3.91 ± 0.15^{a}	4.77 ± 0.10^{b}
Cystine	0.82 ± 0.15^{a}	0.97 ± 0.14^{a}	0.77 ± 0.13^{a}	0.66 ± 0.15^{a}	0.77± 0.06^{a}
Alanine	4.42 ± 0.13^{b}	4.94 ± 0.17^{c}	4.42 ± 0.18^{b}	3.84 ± 0.12^{a}	4.41 ± 0.15^{b}
Glutamic acid	12.07 ± 0.13^{a}	13.07 ± 0.13^{b}	12.77 ± 0.16^{b}	11.56 ± 0.16^{a}	11.86 ± 0.17^{a}
Glycine	2.33 ± 0.16^{a}	2.91 ± 0.14^{b}	2.53 ± 0.11^{ab}	2.18 ± 0.13^{a}	2.38 ± 0.17^{a}
Serine	2.55 ± 0.17^{ab}	3.09 ± 0.13^{b}	2.83 ± 0.12^{ab}	2.33 ± 0.18^{a}	2.52 ± 0.18^{a}
Aspartic acid	11.62 ± 0.12^{b}	12.32 ± 0.17^{c}	11.78 ± 0.13^{b}	10.94 ± 0.11^{a}	11.45 ± 0.12^{b}

Mean ± SEM of three replications.
KA - Kidney beans preserved with sniper,
KB - Kidney beans preserved with pepper
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