Essential oil of *Ocimum gratissimum* L. as biopreservative of peanut in post-harvest: Application model and effects on quality of derived products

ABSTRACT

The present study aims to investigate the use of essential oils extracted from leaves of *Ocimum gratissimum* L. in post-harvest preservation of peanut as well as their effects on the physicochemical, technological and organoleptic characteristics of derived products. To do this, preservation tests of peanut with essential oil of *Ocimum gratissimum* at a concentration of 0.6 µl/g were carried out. Control (without essential oil) was made. Evolution of the fungal flora during conservation as well as physicochemical, technological and organoleptic characteristics of preserved peanut seeds were evaluated. Results of microbiological analyzes indicated a significant reduction ($p < 0.005$) of the microbial quantum in peanut samples preserved with essential oils, when compared to the control. The results of physicochemical analyzes revealed that preservation of peanut using essential oil of *Ocimum gratissimum* has very little effect on the physicochemical characteristics of seeds. However, results from evaluation of the technological aptitudes of preserved peanut seeds indicated that the essential oil has modified the functional properties of preserved peanut samples, with a particular impact on the elasticity of the pastes as well as the flavor and the texture of the peanut cakes. organoleptic analyzes revealed that preservation of peanuts with the essential oil of *Ocimum gratissimum* influences the aroma and flavor of derived products.

Keywords: Arachis hypogaea L., Ocimum gratissimum L., essential oil, fungal flora, qualities, Benin

1. INTRODUCTION

Peanut (*Arachis hypogaea* L.) is an important oilseed worldwide [1]. From South-America, the peanut spread to China, Africa, Indian, Japan and United States of America [2]. In the past, peanut was one of Benin's main export products. But nowadays, it is mostly grown for local consumption and as raw material for local peanut oil extraction industries [3]. In west African countries such as Senegal and Nigeria, peanut is a widely consumed food and is also an inexpensive source of protein, fats, minerals and vitamins in the diet of rural populations. It is consumed in boiled or roasted form, but also as peanut pastes or cakes [4]. However, peanut is often contaminated by fungi including *Aspergillus*, *Fusarium* and *Penicillium*. This contamination not only reduces its marketability, but can also lead to the production of mycotoxins [5]. Thus, several studies have reported the contamination of peanut pastes and cakes by mycotoxins, with the co-contamination of aflatoxins and ochratoxin A [6, 7]. Then, strategy to prevent peanut contamination by mycotoxins is necessary because there are few decontamination processes that can eliminate the mycotoxin without denaturing the product [8]. Faced with the numerous nuisances associated with the use of synthetic chemical preservatives, industrialized societies increasingly approve of the trend towards green consumption, desiring fewer synthetic additives [9]. Thus, spices and plant extracts including essential oils, known since antiquity as possessing many virtues (antibacterial, antifungal, antioxidant), are increasingly used in food preservation [10]. Several studies have investigated the use of plant extracts against proliferation of fungi and mycotoxins production in peanut and derived products. Indeed, Kasi et al. [11] reported that the phytoconstituents of ginger extract (*Zingiber officinale*) harvested in India, lead to a substantial reduction in aflatoxin in peanut oil, and also improve its organoleptic characteristics. Moreover, Adjou et al. [12] reported that the essential oil extracted from leaves of *Ocimum gratissimum* harvested in Benin, has *in vitro* antimicrobial properties against fungi isolated from peanut in post-harvest. However, it is not enough to identify a good antifungal agent, but it must also be applicable in the food industry context. Thus, the present study aimed to evaluate conditions of the use of the essential oil of *Ocimum gratissimum* L. (Lamiaceae) in the preservation of peanut in post-harvest as well as its effects on the physicochemical, technological and organoleptic characteristics of derived products.

2. MATERIAL AND METHODS

2.1. Collection of peanut samples

Shelled peanut seeds were collected at *Pahou* (South Benin). In this locality, five different collection sites were chosen. Peanut samples are collected in sterile bags and stored at 4°C in the laboratory.

2.2. Collection of plant leaves

Plant materials used for essential oil extraction were fresh leaves from *Ocimum gratissimum* L. Plants were collected at Abomey-calavi (south Benin) and identified at the Benin national herbarium, where voucher specimens are deposited.

2.3. Essential oil extraction

The essential oil was extracted by the hydro-distillation method using Clevenger-type apparatus. The oil recovered was dried over anhydrous sodium sulfate and stored at 4 °C until it was used [13].

2.4. Experimental preservation model

Preservation trials of shelled peanuts with essential oil of *Ocimum gratissimum* were carried out using the modified *triple bagging hermetic technology* [14]. As part of this study, the introduction into the package of blotting paper discs impregnated with the essential oil of *Ocimum gratissimum* and the reduction of the number of bags from three (3) to one (1), are the main changes made to the *triple bagging hermetic technology*. Taking into account previous studies, in particular that of Adjou et al. [12], the modified packages are filled with 0.6 µl of the essential oil per gram of shelled peanut. Control (without essential oil) was made following the same procedure. Sampling and periodic inspections are carried out in order to evaluate the evolution of fungi growth during storage.

2.5. Enumeration of fungi

The enumeration of fungi growth in peanut samples was performed using dilution plating method (Nguyen, 2007). 10 g of each peanut samples were added separately to 90 ml of sterile water containing 0.1% peptone water. This was thoroughly mixed to obtain the 10^{-1} dilution. Further, 10-fold serial dilutions up to 10^{-4} were made. 1 ml volume of each dilution were separately placed in Petri dishes, over which, 10 to 15 ml of Yeast Extract Sucrose (YES) agar medium was poured. The plates were incubated at 28 ± 2°C for 7 days.

2.6. Determination of moisture content

Moisture content of samples was determined by desiccation using the method of De Knegt and Brink [15]. A clean platinum dish was dried in an oven and cooled in a desiccator and weighed. From each sample, 5 g was weighed and spread on the dish, the dish containing the sample was weighed. It was then transferred into the air oven at 105°C to dry until a constant weight was obtained and the loss in mass was determined.

2.7. Anti-nutritional factors analysis

Total oxalate was determined as described by Day and Underwood [16]. 1 g of sample was weighed into 100 ml conical flask. 75 ml H_2SO_4 (3 mol. L^{-1}) was added and stirred for 1 h with a magnetic stirrer. This was filtered using a Whatman No 1 filter paper. 25 ml of the filtrate was then taken and titrated while hot against 0.05 mol/L of $KMnO_4$ solution until a faint pink colour persisted for at least 30 s. The oxalate content was then calculated by taking 1 ml of 0.05 mol/L of KMnO4 as equivalent to 2.2 mg oxalate [17]. Phytate was determined using the method of Reddy and Love [18]. 4 g of each sample was soaked in 100 ml of 2% HCl for 5 h and filtered. To 25 ml of the filtrate, 5 ml of 0.3% ammonium thiocyanate solution was added. The mixture was then titrated with Iron (III) chloride solution until a brownish-yellow color that persisted for 5 min was obtained. A 4:6 Fe/P atomic ratio was used to calculate the phytic acid content [19].

2.8. Processing of preserved peanuts

In order to evaluate the effect of the essential oil on the functional properties of peanut, preserved peanuts samples were used for the production of peanut pastes and cakes according to the method described by Adjou et al. [6]. During the processes, all constraints encountered were recorded.

2.9. Organoleptic analyzes

Organoleptic analyzes were performed according to the hedonic test base on a 9-point linear scale [20]. These organoleptic tests concerned peanut pastes and cakes produced from preserved peanut samples, in other to assess the consumer's appreciation on these different peanut derivative products.

2.10. Statistical analysis

Treatments were conducted in three repetitions. Macronutrients, micronutrients and antinutritional factors analysis were performed in triplicate for each treatment repetition. The data generated from these studies were analyzed using Statistical Analysis Software (SAS) and SYSTAT 5.05. The statistical analyses carried out were mean and standard deviation and analysis of variance (ANOVA) [21].

3. RESULTS AND DISCUSSION

Results of the microbiological analyzes of peanut samples during preservation tests revealed a significant reduction (p<5%) in fungal flora of peanut samples preserved with *Ocimum gratissimum* L. essential oil, when compared to the control (Table 1). After five months of preservation with the essential oil, no fungal growth was observed in peanut samples. However, in control samples, results revealed a rapid growth of fungi. These results show the high antifungal properties of the essential oil of *Ocimum gratissimum* L., and the importance of its use in the post-harvest preservation of food and foodstuff in replacement of synthetic chemical pesticides. Several research studies reported that the efficacy of *Ocimum gratissimum* essential oil is due to the presence of bioactive molecules with proven antimicrobial properties such as terpenoids, which constitute a large group of broad-spectrum antimicrobial compounds [22]. However, taking into account that the composition and structure of foods have a significant effect on the different interactions that take place in food products, application in food industry of essential oils as antimicrobial agents could be influenced by the intrinsic composition of food products.

Table 1. Fungal flora in peanut samples during storage

Shelf life (Days)	Controls	Samples preserved with essential oil
0	2.6.10^{1} ufc.g^{-1}	2.6.10^{1} ufc.g^{-1}
30	3.1.10^{2} ufc.g^{-1}	04 ufc.g^{-1}
60	1.4.10^{3} ufc.g^{-1}	01 ufc.g^{-1}
90	2.9.10^{3} ufc.g^{-1}	-
120	5.4.10^{3} ufc.g^{-1}	-
150	2.3.10^{5} ufc.g^{-1}	-

- : No fungal growth detection

Then, results of moisture and antinutritional factors contents of preserved peanuts samples (Table 2) indicated that the preservation of peanut seeds with essential oils has very low influence on these physicochemical parameters (p<5%). Thus, the risk of alteration of peanut seeds, in particular the loss of the germination potential (due to the reduction of water availability) of the seeds would therefore be greatly reduced.

Table 2. Moisture and antinutrient contents of preserved peanut samples

Peanut samples	Moisture content (%)	Oxalate (%)	Phytate (%)
Control	8.19 ± 0.01^{a}	0.11 ± 0.04^{a}	0.67 ± 0.09^{a}
Samples preserved with essential oil	7.89 ± 0.25^{a}	0.20 ± 0.06^{a}	0.68 ± 0.05^{a}

Values are mean (n = 3). The means followed by different superscript letters in the same column are significantly different according to ANOVA and Tukey's multiple comparison test

Table 3 presents constraints encountered by producers during processing of peanut samples preserved with the essential oil. According to producers, pastes from peanut samples preserved with essential oil are less consistent and required more water during processing than the pastes from control samples. These results indicated that the use of the essential oil of *Ocimum gratissimum* has modified the functional characteristics such as hydratation properties (absorption, retention or swelling) of proteins contained in peanut pastes. From results of organoleptic analyzes of peanut products, it appeared that the preservation of peanut using essential oil of *Ocimum gratissimum* did not modified the texture and the color of different products obtained. However, there is significant difference (p< 5%) on the aroma and taste of different peanut products. These findings are in concordance with the observations made by the producers during the mixing-pressing process of the pasta obtained from peanut samples preserved with the essential oil of *Ocimum gratissimum* (Table 3). Furthermore, results based on the preference of tasters revealed that the majority (75%) of them accepted products derived from peanut seeds preserved with the essential oil due to their flavored characters. Then the constituents of this essential oil (mostly aromatic molecules), have improving the organoleptic characteristics of peanut products. Several studies have confirmed the importance of using essential oils in improving the organoleptic characteristics of food products. Indeed, Tsigarida et al. [23] reported that the addition of 0.8% v/w essential oil improves the flavor of beef meat. According to Harpaz et al., [24], essential oils of *Thyme* and *Oregano* spread throughout Asian sea bass flesh at a ratio of 0.05% v/w gave it herbal smell and allowed it to be stored for 33 days. Furthermore, Santos et al., [25] reported that, above certain concentrations, some essential oils may no longer be viable for food use due to the fact that they become too odoriferous. According to Mejlholm and Dalgaard [26], the concentrations required for several essential oils for extending shelf-life conveyed overly strong flavors, which limited their use. It is therefore for this reason that it is always desirable, after the determination of the Minimal Inhibitory Concentrations, to also evaluate the efficacy of the essential oil by direct application in real food systems. Indeed, the application of essential oils in real food systems as antibacterial agents, despite its many constraints, are now emerging at the lab-scale [26] and several innovative essential oil applications have been proposed to provided effective solutions for the food preservative challenges.

Table 3. Observations made by producers during processing of preserved peanut samples

Process stages	Findings	Rendering
Reception- Sorting- Roasting	Absence of major technological constraints	Preservation of peanut with the essential oil has no influence on these stages of the process when compared to control samples
Peeling, Milling	Absence of major technological constraints	Preservation of peanut with the essential oil has no influence on these stages of the process when compared to control samples
Mixing-Pressing	Pastes from peanuts samples preserved with essential oils of *Ocimum gratissimum* are less consistent than pastes from control samples.	The essential oil of *Ocimum gratissimum* modifies the functional characteristics of proteins contained in peanut pastes, in particular the structuring properties involving protein-protein type relationships.
	The mixing process pastes from peanuts samples preserved with essential oils requires more water than pastes from control samples	The essential oil of *Ocimum gratissimum* modifies the functional characteristics of the proteins contained in peanut pastes, in particular the properties of hydration (relations of the protein with water)
Seasoning-Homogenization-Shaping-Frying-Draining-Cooling	Absence of major technological constraints	Preservation of peanut with the essential oil has no influence on these stages of the process when compared to control samples

4. CONCLUSION

The use of essential oil with techniques involving quality preservation and food safety has nowadays a great interest for food industry, because it is not enough to identify a good antifungal agent, but it must also be applicable in the food industry context. This study underlined the efficacy of essential oil of *Ocimum gratissimum* and it biopreservative potential in post-harvest preservation of peanut against spoilage factors from fungi origin. This plant could therefore be prospected for the production of biopesticides in large post-harvest systems. Future research should therefore focus on other technologically innovative applications of essential oils in food industry, using scientific knowledge-based information.

REFERENCES

1- Zhou X, Ren X, Luo H, Huang L, Liu N, Chen W, Lei Y, Liao B, Jiang H. Safe conservation and utilization of peanut germplasm resources in the Oil Crops Middle-term Genebank of China**.** *Oil Crop Science.* 2022; 7(1): 9-13.

2- Zhao X, Chen J, Du F. Potential use of peanut by-products in food processing: a review. *J Food Sci Technol.* 2012); 49(5): 521–529

3- Honfo FG, Hell K, Akissoe N, Dossa RAM, Hounhouigan JD. Diversity and nutritional value of foods consumed by children in two agro-ecological zones of Benin. Afr J Food Sci. 2010; 4: 184-191.

4- Bankole SA, Ogunsanwo BM, Eseigbe DA. Aflatoxins in Nigerian dry-roasted groundnuts. Food Chemistry. 2005; 89: 503–506.

5- Sultan Y, Magan N. Mycotoxigenic fungi in peanuts from different geographic regions of Egypt. Mycotox. Res. 2010; 26 :133–140.

6- Adjou ES, Yehouenou B, Sossou CM, Soumanou MM, de Souza CA. Occurrence of mycotoxins and associated mycoflora in peanut cake product (kulikuli) marketed in Benin. African Journal of Biotechnology. 2012 ; 11(78) : 14354-14360.

7- Boli ZIA, Kambire O, Zoue LT, Nevry RK. Fungal variation during peanut paste storage. Hindawi International Journal of Microbiology. 2020. doi.org/10.1155/2020/8836726.

8- Atanda SA, Aina JA, Agoda SA, Usanga OE, Pessu PO. Mycotoxin Management in Agriculture : a Review. *J Anim Sci. Adv*. 2012; 2: 250-260.

9- Burt S. Essential oils: their antibacterial properties and potential applications in foods-a review. International Journal of Food Microbiology. 2004; 94: 223–253.

10- Bounatirou S, Smiti S, Miguel MG, Faleiro L, Rejeb MN, Neffati M, Costa MM, Figueiredo AC, Barroso JG, Pedro LG. Chemical composition, antioxidant and antimicrobial activities of the essential oils isolated from Tunisian *Thymus capitus* Hoff. et Link. Food Chemistry. 2007; 105: 146–155.

11- Kasi M, Anandaraj K, Hatamleh AA, Albaqami FS, AL-Sohaibani S. Groundnut oil biopreservation: bioactive components, nutritional value and anti-aflatoxigenic effects of traditional ginger seasoning. Journal of Food Processing and Preservation. 2016. doi:10.1111/jfpp.12984.

12- Adjou ES, Kouton S, Dahouenon-Ahoussi E, Sohounhloue DCK, Soumanou MM. Antifungal activity of *Ocimum canum* Essential oil against Toxinogenic Fungi isolated from Peanut Seeds in post-harvest in Benin. International Research Journal of Biological Sciences. 2012 ; 1(7) : 20-26.

13- Adjou ES, Chougourou D, Soumanou MM. Insecticidal and repellent effects of essential oils from leaves of *Hyptis suaveolens* and *Ocimum canum* against *Tenebroides mauritanicus* (L.) isolated from peanut in post-harvest. Journal of Consumer Protection and Food Safety. 2019 ; 14 (1) : 25-30.

14- Baoua IB, Margam V, Amadou L, Murdock LL. Performance of triple bagging hermetic technology for postharvest storage of cowpea grain in Niger. Journal of Stored Products Research. 2012; 51: 81-85.

15- De Knegt RJ, Brink H V D. Improvement of the drying oven method for the determination of the moisture content of milk powder. International Dairy Journal. 1998; 8: 733-738.

16- Day RA, Underwood AL. Quantitative analysis. 5th ed. Prentice- Hall publication. 1986, p.701

17- Chinma CE, Igyor MA. Micronutrients and anti-nutritional contents of selected tropical vegetables grown in Southeast, Nigeria. Nigerian Food Journal. 2007; 25 (1): 111-116.

18- Reddy MB, Love M. The impacts of food processing on the nutritional quality of vitamins and minerals. *Advances in Experimental Medicine and Biology*.1999; 459: 99-106.

19- Okon EU, Akpanyung EO. Nutrients and Antinutrients in selected Brands of Malt Drinks Produced in Nigeria. Pakistan Journal of Nutrition. 2005 ; 4(5) : 352- 355.
20- AFNOR. Directives générales pour la réalisation d'épreuves hédoniques en laboratoire d'évaluation sensorielle ou en salle en conditions contrôlées impliquant des consommateurs. Recueil de normes. 2000 ; 6: 1-60.
21- Ogbeibu AE. Biostatistics, a Practical Approach to Researchand DataHandling. Mindex Publishing Company Ltd. Benin City, Nigeria, 2005. 264 p.
22- Soumanou MM, Adjou ES. Sweet fennel (*Ocimum gratissimum* L.) oil: botanical aspects and uses in food preservation. In: Preedy V.R. (Editor). Essential oils in food preservation, flavour and safety. Academic Press. 2016, 765-773.
23- Tsigarida E, Skandamis P, Nychas G-JE. Behaviour of *Listeria monocytogenes* and autochthonous flora on meat stored under aerobic, vacuum and modified atmosphere packaging conditions with or without the presence of oregano essential oil at 5 °C. Journal of Applied Microbiology. 2000; 89: 901–909.
24- Harpaz S, Glatman L, Drabkin V, Gelman A. Effects of herbal essential oils used to extend the shelf life of fresh water reared Asian sea bass fish (*Lates calcarifer*). Journal of Food Protection. 2003; 66 (3): 410–417.
25- Santos MIS, Marques C, Mota J, Pedroso L, Lima A. Applications of Essential Oils as Antibacterial Agents in Minimally Processed Fruits and Vegetables—A Review, Microorganisms. 2022; 10: 1-24.
26- Mejlholm O, Dalgaard P. Antimicrobial effect of essential oils on the seafood spoilage micro-organism Photobacterium phosphoreum in liquid media and fish products. Lett Appl Microbiol. 2002; 34: 27–31.