

Essential oil of *Ocimum gratissimum* L. as biopreservative of peanuts 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. (Basil) in post-harvest preservation of peanuts as well as the their effects on the physicochemical, technological and organoleptic characteristics of derived peanut products. To do this, preservation tests of peanuts with essential oil of *Ocimum gratissimum* at a concentration of 0.6 µl/g were carried out. Control was (without essential oil. Evolution of the fungal flora during conservation as well as physicochemical, technological and organoleptic characteristics of preserved peanuts were evaluated. Results of microbiological analyses 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 analyses revealed that preservation of peanuts using essential oil of *Ocimum gratissimum* had very little effect on the physicochemical characteristics of seeds. However, results from evaluation of the technological aptitudes of preserved peanuts 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 analyses revealed that preservation of peanuts with the essential oil of *Ocimum gratissimum* affected influences the aroma and flavor of derived products.

Comment [PM1]: What does this mean?

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~~South America, the peanut ~~plant~~ spread to China, Africa, Indian, Japan and United States of America [2]. ~~In the past, p~~Peanut was one of Benin's main export products ~~but~~ 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, peanuts ~~are is~~ often contaminated by fungi, including *Aspergillus*, *Fusarium* and *Penicillium*. This contamination not only reduces its ~~marketability, but~~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, sA~~ strategy to prevent peanut contamination by mycotoxins is ~~therefore~~ necessary because there are few decontamination processes that can eliminate the mycotoxin without denaturing the product [8]. Faced with the numerous ~~challenges 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, ~~and~~ antioxidants), are increasingly used in food preservation [10]. Several studies have investigated the use of plant extracts against proliferation of fungi and mycotoxins production in peanuts 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~~and improved its organoleptic characteristics. Moreover, Adjou et al. [12] reported that the essential oil extracted from leaves of *Ocimum gratissimum* (~~Basil~~) harvested in Benin, has *in vitro* antimicrobial properties against fungi isolated from peanuts in post-harvest. However, it is not ~~sufficient enough~~ to identify ~~an effective good~~ antifungal agent, ~~and but it~~ must also be applicable in the food industry context. Thus, the present study aimed to evaluate conditions ~~for of~~ the use of the essential oil of *Ocimum gratissimum* L. (Lamiaceae) in the preservation of peanuts 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. ~~The collected p~~ Peanut ~~seed~~ samples ~~were are transferred collected in to~~ sterile bags and stored at 4°C in the laboratory.

2.2. Collection of plant leaves

Comment [PM2]: How much was collected? 1 kg, or 5 grams etc. Please include the quantity of peanut collected. How many peanut samples were used? n = ?. Were their replicates? The methodology regarding the peanut samples must be elaborated on.

Comment [PM3]: How many grams/kilograms of plant materials were collected?

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 a voucher specimen ~~was are~~ deposited.

Comment [PM4]: How was the plant material prepared to extract the oil?

2.3. Essential oil extraction

The essential oil was extracted by the hydro-distillation method using a Clevenger-type apparatus. The oil recovered was dried over anhydrous sodium sulfate and stored at 4°C until ~~required for use~~ ~~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), ~~were are~~ the main changes made to the triple bagging hermetic technology. ~~Taking into account~~ Considering previous studies, ~~in particular~~ ~~that that~~ of Adjou et al. [12], the modified packages ~~were are~~ filled with 0.6 µL of the essential oil per gram of shelled peanuts. Control ~~(without essential oil)~~ was made following the same procedure ~~but~~ ~~without the essential oil~~. Sampling and periodic inspections ~~were 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 a 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 was~~ 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.

Comment [PM5]: It what form was the peanut sample? Whole, ground crushed etc?

Comment [PM6]: ? Is this 10:1 dilution?

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2.6. Determination of moisture content

~~The m~~Moisture content of ~~samples~~ was determined by desiccation using the method of De Knecht 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.

Comment [PM8]: What samples? Peanut samples?

Comment [PM9]: 5 g of what?

Comment [PM10]: Please give the brand name of the oven, supplier name, city and country.

2.7. Anti-nutritional factors analysis

Total oxalate ~~of peanut samples~~ was determined as described by Day and Underwood [16]. 1 g of sample was weighed into 100 mL conical flask. 75 ml H₂SO₄ (3 mol/L⁻⁴) was added and stirred ~~at~~

~~room temperature?~~ for 1 h with a magnetic stirrer. ~~The suspension (sample) -This~~ was filtered ~~through a 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 KMnO₄ 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 ~~at room temperature?~~ and filtered ~~through....~~ 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].

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Comment [PM12]: How many samples in total? See previous comment.

Comment [PM13]: American or British spelling?

2.8. Processing of preserved peanuts

~~In order to~~To evaluate the effect of the essential oil on the functional properties of peanuts, preserved peanut samples were used ~~for the production of to produce~~ 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 analyses

Organoleptic ~~analyzes~~analyses 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 ~~order other~~ to assess the consumer ~~preference 's appreciation~~ on ~~the these~~ different peanut derivative products.

Comment [PM14]: Where are the organoleptic results for peanut cakes and pastes (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~~analyses of peanut samples during ~~treatment preservation~~ tests ~~showed revealed~~a significant reduction ($p < 5\%$) in fungal flora of peanut samples ~~treated preserved~~ with *Ocimum gratissimum* L. essential oil, when compared to the control (Table 1). ~~After three five months (90 days) of treatment preservation~~ with the essential oil, no fungal growth was observed in ~~the~~ peanut samples. However, in ~~the~~ control samples, ~~results revealed~~ a ~~progressive rapid~~ growth of fungi ~~was observed~~. 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

Comment [PM15]: According to table 1

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proven antimicrobial properties such as terpenoids, which constitute a large group of broad-spectrum antimicrobial compounds [22]. However, ~~taking into account~~ considering that the composition and structure of foods have an significant effect on the different interactions that take place in food products, application in food industry of essential oils as antimicrobial agents could be affected influenced by the intrinsic composition of food products.

Table 1. Fungal flora in peanut samples during storage

| Shelf life (Days) | Controls | Samples <u>treated preserved</u> with essential oil |
|-------------------|--|---|
| 0 | 2.6.10 ¹ ufc/g ⁺ | 2.6.10 ¹ ufc/g ⁺ |
| 30 | 3.1.10 ² ufc/g ⁺ | 04 ufc/g ⁺ |
| 60 | 1.4.10 ³ ufc/g ⁺ | 01 ufc/g ⁺ |
| 90 | 2.9.10 ³ ufc/g ⁺ | No fungal growth - |
| 120 | 5.4.10 ³ ufc/g ⁺ | No fungal growth - |
| 150 | 2.3.10 ⁵ ufc/g ⁺ | No fungal growth - |

~~∴ No fungal growth detection~~

Comment [PM17]: Is this 4?

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~~The Then,~~ results of moisture, oxalate and phytate ~~and antinutritional~~ factors contents of treated preserved peanuts samples (Table 2) indicated that the treatment preservation of peanuts seeds with essential oils has a minimal very low effect 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 |
| <u>Treated samples</u> | 7.89 ±0.25 ^a | 0.20 ±0.06 ^a | 0.68±0.05 ^a |

Values are means (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

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Table 3 presents constraints encountered by producers during processing of peanut samples treated preserved with Ocimum gratissimum ~~the~~ essential oil. According to producers, pastes from peanut samples treated 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 ~~hydration~~ properties (absorption, retention or swelling) of proteins contained in peanut pastes. From results of the organoleptic analyzes ~~analyses~~ of peanut products, it appeared that the preservation of peanuts 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%) in en the aroma and taste of the 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 treated 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

Comment [PM19]: Where are these results?

seeds ~~treated preserved~~ with the essential oil due to their flavored ~~characteristic characters~~. ~~The Then~~ ~~the~~ constituents of this essential oil (mostly aromatic molecules), have ~~improved~~ ~~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 a herbal ~~aroma 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 because~~ they become ~~too~~ odoriferous. According to Mejlholm and Dalgaard [26], the concentrations required for several essential oils for extending shelf-life ~~resulted conveyed in~~ 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~~ laboratory-scale [26] and several innovative essential oil applications have been proposed to ~~provided provide~~ effective solutions for the food preservative challenges.

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Table 3. Observations made by producers during processing of [treated/preserved](#) peanut samples

| Process stages | Findings | Rendering |
|------------------------------|---|---|
| Reception- Sorting- Roasting | Absence of major technological constraints | Treatment Preservation of peanuts with the essential oil has no effect influence on these stages of the process when compared to control samples |
| Peeling, Milling | Absence of major technological constraints | Treatment Preservation of peanuts with the essential oil has no effect influence on these stages of the process when compared to control samples |
| Mixing-Pressing | Pastes from peanuts samples treated/preserved with essential oils of <i>Ocimum gratissimum</i> are less consistent than pastes from control samples. | The essential oil of <i>Ocimum gratissimum</i> 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 p eanuts samples treated/preserved with essential oils requires more water than pastes from control samples | The essential oil of <i>Ocimum gratissimum</i> 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

Treatment Preservation of peanuts with the essential oil has no effect influence on these stages of the process when compared to control samples

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4. CONCLUSION

Comment [PM22]: The conclusion must be rephrase to address the results of this paper.

The use of essential oil with techniques involving ~~quality treatment preservation~~ and food safety has nowadays a great interest for food ~~industry because~~ it is not ~~sufficient 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 its ~~treatment biopreservative~~ potential in post-harvest preservation of peanuts against spoilage factors from fungi origin. ~~Ocimum gratissimum~~ ~~This plant has the potential could therefore be prospected for the production of to produce a~~ 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.

Comment [PM23]: This paper did not investigate the efficacy of essential oil of *Ocimum gratissimum*. The effect of the essential oil on fungal flora was investigated.

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