



# **Influence of *Ocimum gratissimum* (Scent Leaf) on the Organoleptic Acceptability and Shelf Stability of Yoghurt**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors ANI and CEI designed the study. Authors ANI, JIA and CEI managed the analyses of the study and performed the statistical analysis. Authors CEI and ISA handled the supervision of the work. Authors ANI, JIA and ISA wrote the first draft of the manuscript and managed the literature searches, while authors ANI and QCA managed the grammatical checks and final submission of the manuscript. All authors read and approved the final manuscript.*

## **Article Information**

DOI: 10.9734/AFSJ/2020/v16i130163

### Editor(s):

(1) Dr. Marijana Sokolovic, Feed Analysis Laboratory, Croatia.

### Reviewers:

(1) Rohan Rajnikant Vakhariya, Shivaji University, India.

(2) Anderson Luiz Pena da Costa, Brazil.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/57671>

**Original Research Article**

**Received 25 March 2020**

**Accepted 01 June 2020**

**Published 08 June 2020**

## **ABSTRACT**

**Aim:** To investigate the preservative efficiency of *Ocimum gratissimum* or its extracts on yoghurt.  
**Study Design:** Ten yoghurt samples were prepared, furthermore, nine (9) samples were treated with scent leaf in different forms at different concentrations.  
**Place and Duration of Study:** Department of Food Science and Technology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria, between September 2015 and June 2016.  
**Methodology:** Yoghurt was prepared from instant filled milk while Direct Vat Inoculum (DVI) was used as the starter culture. Proximate and phytochemical analyses were carried out following standard procedures. Sensory evaluation was carried out using a 15-man panelist with a seven point hedonic scales and results were statistically analyzed using IBM® SPSS 21.0, at 0.05 probability level. pH and microbial analysis were conducted while bacterial characterization

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involved biochemical tests and isolation of probable microorganisms employed morphological and structural characteristics.

**Results:** Result for proximate analysis indicated differences in the nutritional composition of the raw milk and yoghurt (control). Moisture content increased considerably from 10.00% to 69.75%, while a reduction in protein content was reported (8.7% - 4.95%). The lipid content classified the produced yoghurt as a low-fat yoghurt, and expectedly, the carbohydrate content reduced from 58.20% - 22.30%. Phytochemical screening of the different forms of scent leaf indicated the presence of alkaloids, flavonoids, saponins, cardiac glycosides, and steroids, in different concentrations. Sensory scores showed that the panelists preferred yoghurt formulated with 3 mL of squeezed scent leaf extract. pH values decreased with storage period, while microbial load ranged between  $0.5 \times 10^4$  –  $2.0 \times 10^4$ , where *Klebsiella* spp., *Streptococcus* spp., *E. coli*, *Bacillus*, *S. aureus*, *Pseudomonas* spp., *Enterobacter*, and *Proteus* spp. were isolated.

**Conclusion:** The yoghurt samples formulated with squeezed scent leaf extracts had the best organoleptic acceptance, while the sample treated with 3 mL had the lowest microbial load, hence, it can be inferred that the squeezed scent leaf extracts had the best preservative effect.

**Keywords:** Microbial profile; acceptability; organoleptic; organic preservatives; phytochemicals; yoghurt.

## 1. INTRODUCTION

Yoghurt is a dairy product made through the fermentation of milk and as such serves as a means of preserving the nutrients in the milk [1]. Yoghurt processing is a time-honored procedure, dating back to thousands of years.

In the Middle East, primitive herdsman carried milk in containers made from intestinal gut lining, based on the discovery that it could help extend the life of milk because contact with the intestinal fluids of the containers caused the milk to curdle and sour, preserving it for an extended period. Other than drying, this was historically the only safe method of preserving milk [2].

In yoghurt processing, symbiotic thermophilic starter culture, containing a defined quantity of *Lactobacillus delbrueckii* ssp *bulgaricus* and *Streptococcus salivarius* ssp *thermophilus* is used [3]. These strains are the major bacteria used in milk fermentation. The action of these bacteria strains is what gives yoghurt its characteristic texture and flavor, and the main flavor compound found in yoghurt is acetaldehyde [4].

Milk could be processed into various types of yoghurts namely flavored, set, stirred, or fluid (drinking yoghurt), frozen and dried yoghurt [3]. Usually, yoghurt is prepared from both cow and buffalo's milk.

The conventional method of its preparation involves the standardization of milk to meet legal requirements as stipulated by either the local food regulatory authorities or international regulatory bodies. Standardized milk is

pasteurized (90°C–95°C for 10–20 min) to kill all the pathogenic and almost all the spoilage organisms [5] such as *Escherichia coli*, *Salmonella* spp., and *Campylobacter jejuni* in the milk [2].

After pasteurization, the standardized milk is cooled to about 42°C- 45°C [6] followed by inoculation with yoghurt starter culture at a 2% level. After the addition of the starter culture, milk is maintained at 42°C for about 4–12 h by incubation [7] or up to when the 4.6 pH is attained [3]. The incubation process is followed by cooling to stop the fermentation. Yoghurt obtained in this process is referred to as plain yoghurts and various fruits and flavors can be added in it to enhance its aesthetic appeal.

Flavored yoghurts also known as the liquid or drinkable yoghurts [2], are prepared by adding flavorings either in the form of diced fruit or fruit syrups [8] or by the addition of sweeteners like maple syrup, honey, and so on, added for increased fluidity and flavor. The live lactic acid bacteria (LAB) present in yoghurt by virtue of the inoculation with a starter culture, have some health benefits that include protection against gastrointestinal upsets, enhanced digestion of lactose by mal-digestion, lower blood cholesterol, in addition to increased hormone response, and it also helps the body to assimilate protein, calcium, and iron [9].

The health benefits of yoghurt other than the basic nutritional composition, are responsible for its great consumer acceptability making it one of the most popular fermented dairy products worldwide [10]. Despite these health benefits, yoghurt and most dairy products still have short

shelf lives. Yoghurt is always at risk of proteolytic degradation through proteolysis of milk which may occur during cold storage due to the growth of psychotropic bacteria [11], hence the need for preservation. It was reported that yoghurt from whole cow milk, powdered milk, and soy milk should be kept under refrigeration for no more than 7 days to maintain its freshness [12].

But refrigeration is not quite practicable in Nigeria due to the epileptic nature of power supply and the inability of some consumers to purchase these refrigerators due to poverty. Therefore, there is a need to use preservatives that will be within the reach of local producers. The most available preservatives in dairy (yoghurt and soymilk) industry are the chemical preservatives such as potassium sorbate and sodium metabisulphite, which are chemical preservatives. In addition to these chemical preservatives, there are natural preservatives that extend the shelf life of dairy products [13].

However, the efficacy of botanical preservatives (that are part of our daily meals) on dairy products such as *Ocimum gratissimum* (scent leaf) could be investigated. This is the goal of this research. It has been reported that extracts of *Ocimum gratissimum* using aqueous extraction method are rich in phytochemicals such as flavonoids, tannins and other phenolic compounds [14].

*Ocimum gratissimum* is an herbaceous plant which belongs to the family *Lamiaceae* [15]. It is widely distributed in tropical and warm temperate regions and is grown in gardens and used as a tea leaf for fever [16].

*Ocimum gratissimum*, locally known in Africa as scent leaf, clove basil, African basil [17], and in Hawaii as wild basil is a species of *Ocimum*. It is native to Africa, Madagascar, southern Asia, and the Bismarck Archipelago, and naturalized in Polynesia, Hawaii, Mexico, Panama, West Indies, Brazil, and Bolivia. The leaves of *Ocimum* has been used as a spice in food and soup preparation, it has a nice minty aroma, hence the common name-scent leaf [18]. *Ocimum gratissimum* is widely cultivated in Nigeria, hence it is abundant in the country. The leaves of *Ocimum gratissimum* contain many bioactive compounds including tannins, saponins, alkaloids, cardiac glycosides, phenols, and flavonoids [19,20]. These bioactive compounds, also known as phytochemicals, have been implicated with many pharmacological properties such as antibacterial, antifungal, antiviral, anti-

inflammatory, cholesterol-lowering, antithrombic and also antioxidant properties [17,18,19,20, 21,22,23].

Following the green movement in Western societies which has stated that natural substances are safer and more desirable than synthetic chemicals [24], The research, was, therefore aimed at harnessing the aromatic property of scent leaf (*Ocimum gratissimum*) as a flavoring in yoghurt, evaluating the acceptance of these yoghurts formulated with scent leaves in different forms at different concentrations and its antimicrobial effect as an organic preservative in Yoghurt.

## 2. MATERIALS AND METHODS

The experiment was conducted between 2015/2016 at the Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. The materials for study and their respective sources are;

1. Powdered milk from Eke-Awka market, Awka, Anambra State, Nigeria.
2. Direct Vat Inoculant (DVI), a culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* obtained from Onitsha main market, Onitsha South Local Government Area, Anambra State, Nigeria.
3. Fresh whole scent leaf from a farmer in Awka, Anambra State, Nigeria.

### 2.1 Research Design

Briefly, the study design included the production of ten (10) yoghurt samples, of which nine (9) was formulated with either powdered scent leaf (PSE), powdered scent leaf extract (PLE) and fresh scent leaf extract (FSE) at three (3) different levels (Table 1).

### 2.2 Sample Preparation

#### 2.2.1 Preparation of squeezed extract from Fresh Scent leaf (FSE)

Obtained freshly harvested whole Scent leaves were processed to get the extract. This was achieved by washing the leaves in running water to remove dirt, then the water was drained out using a muslin cloth. Some part of the washed leaves was taken and, the fresh extract was forced out of the leaves by squeezing.

#### 2.2.2 Preparation of Powdered Scent leaf (PSE)

Some scent leaves that were forcefully drained-off in a muslin cloth were sun-dried for 72 hours

to a moisture content of 3%. The dried leaves were ground in a mortar into fine powdery form. The ground leaves were then sieved with a mesh size of 0.50 mm. The sieved powder was stored in a plastic container at room temperature ( $30^{\circ}\text{C} \pm 0.1$ ) for later use.

### 2.2.3 Preparation of powdered Scent Leaf Extract (SLE)

A modified method of Udochukwu et al. [23] was used. The aqueous extract of powdered scent leaf was gotten by dissolving 50 g of the dried scent leaves in 500 mL of potable water (1:10). The mixture was stirred vigorously and intermittently with a magnetic stirrer and allowed to rest, after which it was allowed to stand for 48 hours, then stirred again and filtered through a Whatman filter paper-lined funnel into a conical flask. The extract was stored in a refrigerator until required for use.

### 2.2.4 Preparation of cow milk

Powdered milk (1.2 kg) was reconstituted in 12 litres of previously boiled warm water ( $40^{\circ}\text{C}$ ). The milk sample was mixed and homogenized properly using an electric blender (Mariam Stainless Steel blender, Model – M2, 1800 W).

### 2.2.5 Preparation of starter culture

A small quantity (100 mL) of lukewarm pasteurized milk at  $43^{\circ}\text{C}$  was used to dissolve 2 g of Direct Vat Inoculant (DVI) starter culture containing defined blends of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, containing cells in the order of  $10^{11} - 10^{13}$  CFU/g, thoroughly in a sterile beaker.

### 2.2.6 Yoghurt processing operation

Yoghurt production was carried out (Fig. 1) as described by Tamine and Robinson [25] with slight modifications. The prepared homogenized milk was pasteurized in a mini-pasteurizer (Model Fj, 15, 115 V, with 14 litres capacity) at  $85^{\circ}\text{C}$  and held at the same temperature for 10 minutes.

The pasteurized milk was left to cool to  $43^{\circ}\text{C}$  and inoculated with the prepared starter culture. The inoculated milk was incubated by holding at  $43^{\circ}\text{C}$  in an incubator for 12 hours under stable (without agitation) conditions. The cultured milk (yoghurt) produced was harvested and stored at  $7^{\circ}\text{C}$ .

## 2.3 Proximate Analyses

### 2.3.1 Moisture content determination

The moisture content of the samples was determined using the oven drying method as described by AOAC [26]. Briefly, the weight of the Petri dish to be used was measured and recorded. The analysis was done in triplicates, where 5g of each of the sample was measured into already washed and dried Petri dishes. The samples were placed in a pre-heated oven at  $110^{\circ}\text{C}$  temperature for 4 hours, after which they were removed, cooled in the desiccator and weighed. The samples were returned to the oven for another 30 minutes, cooled and re-weighed again. This procedure was repeated until a constant weight was obtained. The percentage moisture content was calculated using the formula given below;

$$\% \text{ Moisture} = \frac{\text{Initial weight} - \text{final weight}}{\text{initial weight}} \times 100 / 1$$

### 2.3.2 Determination of crude protein content

The Kjeldahl method was used [26] for protein analyses. This method was carried out in three (3) phases, namely, digestion, distillation, and titration.

### 2.3.3 Lipid content determination

The fat content of the sample was analyzed using the soxhlet extraction method as described by AOAC [26]. The lipid content was obtained by weighing 2 mL of the samples into a filter paper, wrapped carefully and put in the sample holder of the soxhlet apparatus. A clean, dry and initially weighed soxhlet extraction flask was half-filled with n-hexane and the whole apparatus was assembled. The flask was placed on a heating mantle and heated at  $60^{\circ}\text{C}$ . The heating lasted for 3 hours. After heating, the extraction flask was removed, then the weight of the flask and oil was taken and recorded afterwards. The percentage (%) fat was determined as shown below:

$$\% \text{ Crude fat} = \frac{\{(\text{weight of flask} + \text{oil}) - (\text{weight of empty flask})\}}{(\text{initial weight of samples})} \times 100 / 1$$

### 2.3.4 Ash content determination

The AOAC [26] method was used to determine the ash contents of the samples. This was done by weighing Clean, dried crucibles on an electronic balance, after which 5 g of the sample

was weighed into the crucibles. The samples were dried in the oven until constant weights are obtained. Then transferred into the muffle furnace with a pair of tongs and ashed at 550°C for 4 hours until a white ash was obtained. The samples were removed from the furnace and cooled in a desiccator. The percentage (%) ash content will be gotten thus;

$$\% \text{ Ash Content} = \{ \text{Weight of Ash} \} / \{ \text{Weight of sample (after oven drying)} \} \times 100 / 1$$

### 2.3.5 Crude carbohydrate content determination

The carbohydrate content of the sample was obtained by difference that is, as a difference of the total summations of percentage moisture, fat, protein, ash, and 100.

$$\% \text{ Carbohydrate} = 100 - (\% \text{ moisture} + \% \text{ crude fat} + \% \text{ protein} + \% \text{ ash})$$

### 2.3.6 Qualitative phytochemical screening

Qualitative phytochemical screening of the dried leaf powder was carried out on the obtained plant extract, as described by Okwu [27].

#### 2.3.6.1 Test for tannins

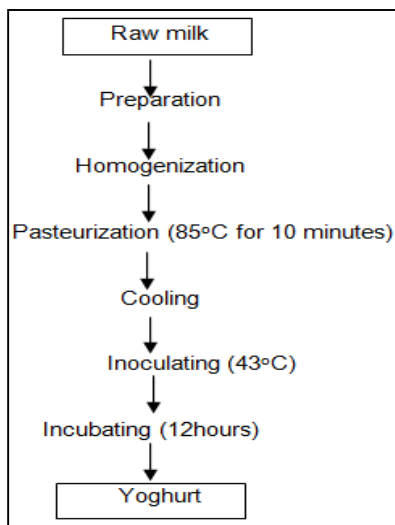
Dried powdered sample (0.5 g) was boiled in 20 mL of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue-black coloration.

#### 2.3.6.2 Test for saponins

The scent leaf sample was boiled in 20 mL of distilled water in a water bath and filtered. 10 mL of the filtrate was then mixed with 5 mL of distilled water and shaken vigorously until a stable froth is obtained. The froth formed was then mixed with 3 drops of olive oil, and eventually shaken vigorously. The mixture was observed for the formation of an emulsion.

**Table 1. Experimental design for the effect of scent leaf on yoghurt**

No.	Codes	Description
1	0-SL	CONTROL
2	1 g-PSL	1 g Powdered Scent leaf in 500 ml of yoghurt
3	2 g-PSL	2 g Powdered Scent leaf in 500 ml of yoghurt
4	3 g-PSL	3 g Powdered Scent leaf in 500 ml of yoghurt
5	1 ml-SLE	1 ml Powdered Scent leaf extract in 500 ml of yoghurt
6	2 ml-SLE	2 ml Powdered Scent leaf extract in 500 ml of yoghurt
7	3 ml-SLE	3 ml Powdered Scent leaf extract in 500 ml of yoghurt
8	1 ml-FSE	1 ml fresh Scent leaf extract in 500 ml of yoghurt
9	2 ml-FSE	2 ml fresh Scent leaf extract in 500 ml of yoghurt
10	3 ml-FSE	3 ml fresh Scent leaf extract in 500 ml of yoghurt



**Fig. 1. Flow chart for the preparation of yoghurt**

Source: Tamine and Robinson, [25]

### 2.3.6.3 Test for flavonoids

A 5 ml of 10% dilute ammonia solution was added to 5 ml of the aqueous filtrate of the plant extract, followed by the addition of concentrated H<sub>2</sub>SO<sub>4</sub>. A yellow coloration observed in the extract indicated the presence of flavonoids.

### 2.3.6.4 Test for cardiac glycosides

A 5 ml of the extract was treated with 2 ml of glacial acetic acid containing 1 drop of ferric chloride solution (0.1%). This was then underlain with 1ml of concentrated H<sub>2</sub>SO<sub>4</sub>. A brown ring of the interface indicated a deoxy-sugar characteristic of cardenolides.

## 2.3.7 Quantitative phytochemicals analyses

### 2.3.7.1 Alkaloid determination

A modified method of Oladoso-Ajayi et al. [28] was used. Here, 5 g of the scent leaf samples were weighed into a 250 ml beaker and 200 ml of distilled water was added, covered, and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate collected and washed with diluted ammonium hydroxide and then filtered. The residue was the alkaloid, which was dried and weighed.

### 2.3.7.2 Saponin determination

The method used was that carried out by Obadoni and Ochuko [29]. A 20 g of ground sample was put into a conical flask and 100 ml of distilled water was added. The sample was heated in a hot water bath at 55°C for 4 hours with continuous stirring. The mixture was filtered and the residue re-extracted with another 200 ml of distilled water. The extracts were reduced to 40 mL over a water bath at about 90°C. The concentrate was transferred into a separation funnel and 20 mL of diethyl ether was added, the mixture was then shaken vigorously. The aqueous layer was collected and the ether layer discarded. The purification process was repeated. Sixty (60) mL of n-butanol was added to the extracts and then washed twice with 10 mL of 55% aqueous sodium chloride (NaCl). The remaining solution was heated. After evaporation, the extracts were dried in the oven

to a constant weight, and percentage saponin content determined.

### 2.3.7.3 Flavonoid determination

As described by Ladipo et al. [30], ten grams of the sample was extracted repeatedly with 100 ml of distilled water at room temperature. The whole mixture was filtered using Whatman filter paper No. 42 (125 mm). The filtrate was transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight.

## 2.4 Sensory Evaluation

A 7-point Hedonic scale, where 7 is "like extremely" and 1 is "dislike extremely", as used by [31] was used to analyze the organoleptic acceptability results of the samples. A 15- man semi-trained panelists consisting of students from the Department of Food Science and Technology, Nnamdi Azikiwe University, Awka, Anambra State was used. The panelists were asked to evaluate the samples for the following attributes; flavor, color, taste, texture and overall acceptability.

## 2.5 Storage Condition

The yoghurts both control and the treated ones were stored on the shelf under ambient condition (30°C ± 0.1 and 50-56% Relative humidity).

## 2.6 pH Analysis

This was determined using a Hand pH meter (Hanna instrument, Waterproof pocket pH tester pHep<sup>®</sup>, HI98107, 0.1 pH resolution). The pH meter was standardized against a known solution of pH (Buffer 7). A sufficient quantity of the yoghurt samples (about 20 mL) was used, the pH meter's electrode inserted in the yoghurt samples, and was rinsed after every reading.

## 2.7 Microbial Analysis

### 2.7.1 Enumeration of gram-negative / enteric bacteria count

The total plate count was determined using the method described by APHA [32]. The yoghurt samples were diluted before being transferred to the agar through the pour plate method.

The samples were first diluted serially by transferring 1 mL of each sample with a sterile micropipette into a test tube containing 9 mL of

water, 1 mL was taken from the first test tube and transferred to a second test tube and the process continued until the fourth test tube. This was done to prepare four-folds dilutions from  $10^{-1}$  –  $10^{-4}$ .

MacConkey Agar, being selective for gram-negative bacteria was used for easy enumeration of the enteric bacteria. Inoculation was done by transferring 1 mL of the serially diluted sample from the fourth test tube and transferred into a pre-labeled sterile Petri dish using sterile pipettes for each sample and repeated in triplicate, the prepared MacConkey agar was poured aseptically into the dishes containing the sample, the medium was rotated gently in the Petri-dishes to ensure proper mixing of the sample and the medium.

### 2.7.2 Enumeration of coliform Count

Enumeration of the total coliform in the samples was carried out on Nutrient agar (OXOID). The growth media was prepared by mixing 23 grams of the NA powder in 1000 mL distilled water. The mixture was heated while mixing vigorously for adequate dissolution of the powder. As in the spread plate method, the dishes were covered and turned upside down after the agar had solidified. After which the media was inoculated with 1 mL of the sample by the use of a sterile micropipette. Flaming of the neck of the conical flask containing the nutrient agar was done after each of the dishes was plated, to ensure sterility. The mixture was incubated at 37°C for 24 hours. The colonies were counted, the result was calculated and expressed as colony-forming unit per ml using the formula shown below;

Colony forming unit (CFU / mL) = {number of colonies} / {volume of inoculum transferred x dilution ratio}

### 2.7.3 Identification and isolation of microorganisms

This was done as a continuation of the colony count. After incubation, the colonies on the plates were sub-cultured on fresh nutrients agar to get the pure cultures of the isolates. The pure cultures were then transferred into nutrient agar slants for biochemical identification.

The pure cultures of the colonies on the nutrient agar were isolated based on their cultural, biochemical, and morphological characteristics. Gram staining, motility test, Urease test,

Catalase test, Methyl Red test, Voges - Proskauer Test (V.P. test), Indole test, Citrate Utilization test, Sugar Fermentation test, Coagulase test, Hydrogen sulfide, Starch hydrolysis and Spore stain were all done using a modified method of Carpenter [33].

## 2.8 Statistical Analyses

Means, Standard deviation, Analysis of Variance (ANOVA), and Mean separation were carried out using the statistical package for social sciences (SPSS) version 21.0.

## 3. RESULTS AND DISCUSSION

### 3.1 Proximate Compositions

Table 2 gives the proximate composition result of powdered milk, untreated yoghurt, and scent leaf. Notably, the yoghurt produced from the milk had a higher moisture content than the powdered milk (10.00% compared to 69.00%). This increment is expected because water was added during the processing of the yoghurt, heat was applied during pasteurization as a result of which most of the bound water must have been released, contributing to the moisture content of the product (yoghurt). The recorded moisture content is considerably lower than that reported by Ahmad [34], he reported that the moisture content of yoghurt should be between 82 – 84%.

The reduction in protein content (8.7% - 5.7%) could probably be as a result of the presence of bacteria strains (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*), which were used as cultures during the production. These cultures act on lactose to produce lactic acid, coagulating milk in the process and giving yoghurt its characteristic texture. The pasteurization of milk at 85°C could also lead to the denaturation of the proteins.

The carbohydrate content reduced greatly from 58.20 – 22.30%, this reduction could be attributed to the fermentation process involved in yoghurt processing. The fermentation process results in the breakdown of lactose by lactic acid bacteria to lactic acid. This conversion of lactose to lactic acid makes yoghurt a good dairy product for people suffering from lactose intolerance [35].

The lipid content recorded makes the fresh yoghurt a low – fat yoghurt. This is inferred based on the USDA (2001) classification, which states that yoghurt samples with greater than

3.25% of fat content are to be regarded as normal yoghurt, yoghurt with about 0.5 – 2.0% fat content are labeled low – fat yoghurt [36] and with those with fat content below 0.5% are non-fat yoghurt. [37] reported that the lipid composition of yoghurts has a crucial impact on its consistency (texture) and flavour.

The ash content is an indication of the mineral content of the yoghurt produced. The reduction in ash content could indirectly be related to the loss of some minerals during the processing of yoghurt.

The proximate composition of scent leaf on the average was obtained as 56.0% for moisture, 11.3% for ash, 9.0% for lipid, 17.0% protein, and 6.4% carbohydrate, the ash, and lipid content obtained are close to the results reported by [38].

### 3.2 Phytochemical Results

Qualitative and quantitative phytochemical screening was carried out on the three forms; powdered scent leaf extract (SLE), powdered scent leaf (PSL), and fresh scent leaf (FSE) of scent leaf used as a flavoring agent.

The phytochemical screening of the different forms of the fresh scent leaf (*Ocimum gratissimum*) revealed the presence of alkaloids, phenols, terpenoids, glycosides, Flavonoids, steroids, saponins, and tannins, as shown in Tables 3 and 4. The presence of these phytochemicals is in agreement with the findings of Jumare [20], who reported the aqueous extract of the fresh scent leaf to contain, qualitatively,

phlobatannins, tannins, flavonoids, and other phenolic compounds including steroidal compounds such as saponins. The result confirms the claims of [20,21,23,39]. The Saponins present in the scent leaf have been reported to have good antibacterial properties, anti-inflammatory, and immune-boosting properties [40]. The flavonoid is believed to have good anti-oxidant abilities, anti-viral, anti-cancer, anti-inflammatory, and anti-allergic properties [41] and are good therapeutic agents [42]. The alkaloids are known for their antioxidant, antibacterial [43], anti-insecticidal, and anti-parasitic [44] properties. The tannins present in the scent leaf though have been implicated with decreasing bioavailability and digestibility of protein, also serve as a natural defense in the leaves, by virtue of its anti-microbial properties [45].

### 3.3 Sensory Evaluation Scores

Organoleptic acceptability as judged by the 15-men panelists is shown in Table 5. In all ten samples, there were significant changes (P=0.05) in all sensory attributes evaluated.

Statistically, the control was significantly (P=0.05) as all other samples in terms of flavour. Sample 9 (2 mL- FSE) and 10 (3 mL- FSE) had the same acceptance by the panelist, they both have the same (P=0.05) average score, with sample 2 (1g-PSL) having the least acceptance. With regards to taste as a sensory parameter, all the samples had significantly the same (P=0.05) acceptance as judged by the 15 – men panelist.

**Table 2. Percentage proximate composition of powdered milk, yoghurt, and scent leaf**

No	Sample	Moisture	Protein	CHO	Lipid	Ash
1	Powdered milk (PM)	10.00±0.00	8.70 ± 0.35	58.20±1.45	20.40±0.51	2.70 ± 1.15
2	Yoghurt from (PM)	69.00±2.00	5.70 ± 0.35	22.30±2.31	2.00 ± 0.06	1.00 ± 0.00
3	Scent leaf	56.00±2.00	17.00 ±0.46	6.70 ± 2.71	9.00 ± 2.00	11.30±1.15

NB: values are means and standard deviation (SD) of triplicate

\*CHO is Carbohydrate

**Table 3. Qualitative results of phytochemicals in powdered Scent Leaf extract (SLE), Squeezed Fresh Scent Leaf (FSE) and Powdered Scent Leaf (PSL)**

s/n	Phytochemicals	SLE	FSE	PSL
1	Alkaloids	+	+	ND
2	Flavonoids	+	+	+
3	Glycosides	+	+	ND
4	Saponin	+	+	+
5	Tannin	+	+	+
6	Terpenoids	+	+	ND
7.	Steroid	+	+	ND
8.	Phlobatannins	+	ND	ND

ND is not detected



**Table 4. Quantitative results of phytochemicals in FSE, PSE and SLE\***

Phytochemicals	FSE (mg / 100 g)	PSE (mg / 100 g)	SLE (mg / 100 g)
Flavonoid	4.25 ± 0.01	1.45 ± 0.01	2320.09 ± 0.008
Saponin	2.33 ± 0.02	0.01 ± 0.00	0.91 ± 0.006
Alkaloid	3.73 ± 0.01		1406.57 ± 0.006
Tannin	0.10 ± 0.01	0.11 ± 0.01	1074.94 ± 0.009

NB: values are mean ± Standard deviation (P = 0.05)

\*PSL = Powdered scent leaf; SLE = Powdered scent leaf extract; FSE = Squeezed scent leaf extract

On average, the control's taste had the best acceptance with a value of 4.60, although samples 9 and 10 were significantly (P=0.05) the same as it. The samples (2, 3 and 4) treated with powdered scent leaf had the poorest flavour as judged by the panelists while the samples treated with fresh scent leaf extract (8, 9 and 10) had better acceptances.

In terms of color, the control had the highest mean value, but it is still statistically the same as samples (2, 6, 8 and 10). In general, the samples treated with fresh scent leaf extract still had a better appearance than the samples treated with other forms.

The consistency of the control was judged as being the best by the samples and is significantly (P=0.05) different from the treated samples. The samples treated with powdered scent leaf extract had fair acceptance, but those treated with the powdered scent leaf had the poorest acceptance.

Generally, on average, the panelists judged the samples without any form of scent leaf (control) and sample 10 (3 mL- FSE) were significantly (P=0.05) the same. The samples treated with powdered scent leaf still had the poorest acceptance and significantly (P=0.05) different from other samples (both treated and control) except for sample 2.

### 3.4 Influence of Shelf Storage on pH

The fresh yoghurt had a pH of 4.5 as shown in Table 6. This result confirms the 4.5 pH reported by [46] on milk products fermented with *Lactobacillus bulgaricus* plus *Streptococcus thermophilus*. This pH also coincides with that of [47,48] they reported a pH of 4.00 – 4.90. The pH value confirms that yoghurt is a high – acid food [49] and agrees with the set pH of yoghurt of 4.00 – 4.60 [50]. This decrease in the pH of the milk from 6.7 to 4.5 is due to the fermentation process, during which lactic acid is produced, contributing to the acidity of the product and curdling of casein takes place [51]. The pH is

also as a result of the incubation temperature, which also helps the yoghurt set in the right way.

Changes in pH were read for three days, at the end of each day shown (Table 7). The readings showed that the pH of the samples reduced with storage days.

The acidity of the samples increased as the day goes, invariably resulting in a decrease in the pH value of the yoghurt samples. The drop in pH values agrees with that reported by the survey of [50]. It was observed in the readings that the pH of the yoghurt samples decreased as the concentration of the different forms of the scent leaf used increased. The decreasing trend is notable and continues in all the samples irrespective of whether there is treatment or not nor the form in which the yoghurt is treated.

### 3.5 Total Enteric and Coliform Count

During the storage period, there was a tremendous increase in the microbial load of the samples in both the control and the treated samples. The microbial load increased from 0.5 x 10<sup>4</sup>CFU / mL (Table 6) for the fresh sample to 1.8 x 10<sup>4</sup> CFU / mL TCC (Table 8). There was no clear pattern to the increment in the microbial load of the samples. The control showed the greatest increase in the microbial count with 1.8x10<sup>4</sup> TPC and 2.0x10<sup>4</sup> TCC. The samples treated with fresh scent leaf extract showed the least microbial growth as compared to others in both TPC and TCC. This ability to reduce the microbial load of the yoghurt when compared to that of the control could be attributed to the antioxidant, anti-bacterial, anti-viral, and antimicrobial activities implicated with scent leaves [20,52,53,54].

The sample treated with 3 ml freshly squeezed scent leaf extract showed the least increment in the microbial count. At the end of the fourth day, sample 10 had the same amount of microorganism as the fresh sample with only an insignificant increase in Total Plate count.

**Table 5. Sensory scores yoghurts with different treatments of scent leaf**

No.	Treatment samples	Flavour	Taste	Colour	Consistency	Gen. Acceptability	Rank (Position)
1	0g-PSL	4.07 ± 0.70 <sup>ab</sup>	4.60 ± 1.55 <sup>d</sup>	4.73 ± 1.28 <sup>c</sup>	5.60 ± 1.06 <sup>e</sup>	4.93 ± 0.88 <sup>d</sup>	1 <sup>st</sup>
2	1g-PSL	3.47 ± 1.46 <sup>a</sup>	2.87 ± 1.36 <sup>ab</sup>	4.00 ± 1.20 <sup>bc</sup>	3.20 ± 1.21 <sup>ab</sup>	3.53 ± 1.30 <sup>b</sup>	8 <sup>th</sup>
3	2g-PSL	3.87 ± 1.30 <sup>ab</sup>	3.06 ± 1.71 <sup>abc</sup>	2.93 ± 1.33 <sup>a</sup>	3.13 ± 1.06 <sup>ab</sup>	2.60 ± 0.99 <sup>a</sup>	9 <sup>th</sup>
4	3g-PSL	3.80 ± 1.14 <sup>ab</sup>	2.13 ± 0.92 <sup>a</sup>	2.73 ± 1.44 <sup>a</sup>	2.67 ± 1.72 <sup>a</sup>	2.33 ± 1.11 <sup>a</sup>	10 <sup>th</sup>
5	1ml-SLE	4.00 ± 1.00 <sup>ab</sup>	4.20 ± 0.94 <sup>d</sup>	3.53 ± 0.74 <sup>ab</sup>	4.60 ± 0.63 <sup>d</sup>	4.07 ± 1.03 <sup>bc</sup>	6 <sup>th</sup>
6	2ml-SLE	3.87 ± 1.30 <sup>ab</sup>	3.20 ± 1.78 <sup>bc</sup>	4.67 ± 1.18 <sup>c</sup>	4.73 ± 0.96 <sup>d</sup>	4.12 ± 1.37 <sup>c</sup>	5 <sup>th</sup>
7	3ml-SLE	4.00 ± 1.50 <sup>ab</sup>	3.07 ± 0.96 <sup>abc</sup>	3.60 ± 0.74 <sup>ab</sup>	3.53 ± 0.92 <sup>abc</sup>	3.60 ± 0.91 <sup>bc</sup>	7 <sup>th</sup>
8	1ml-FSE	4.07 ± 1.03 <sup>ab</sup>	3.93 ± 1.09 <sup>cd</sup>	4.13 ± 0.83 <sup>bc</sup>	4.40 ± 0.83 <sup>cd</sup>	4.45 ± 1.15 <sup>cd</sup>	4 <sup>th</sup>
9	2ml-FSE	4.60 ± 0.74 <sup>b</sup>	4.27 ± 1.09 <sup>d</sup>	3.40 ± 1.24 <sup>ab</sup>	3.87 ± 1.06 <sup>bcd</sup>	4.53 ± 1.25 <sup>cd</sup>	3 <sup>rd</sup>
10	3ml-FSE	4.60 ± 1.06 <sup>b</sup>	4.33 ± 1.11 <sup>d</sup>	4.13 ± 0.83 <sup>bc</sup>	4.13 ± 1.36 <sup>cd</sup>	4.87 ± 0.83 <sup>d</sup>	2 <sup>nd</sup>

NB:

- i. Values are mean ± standard deviation of 15 replicates of the samples for the sensory attributes and pH, values are for three readings
- ii. Means in the same column bearing different superscript differ significantly at ( $P < 0.05$ )
- iii. PSL = Powdered scent leaf; SLE = Powdered scent leaf extract; FSE = Fresh scent leaf extract

**Table 6. pH and TPC of fresh yoghurt**

S/N	Samples	Ph	TPC (Cfu / ml)
1	Fresh yoghurt	4.5	$0.50 \times 10^4$

NB: TPC is the total plate count  
Cfu is colony-forming unit

**Table 7. pH of yoghurt samples for three days**

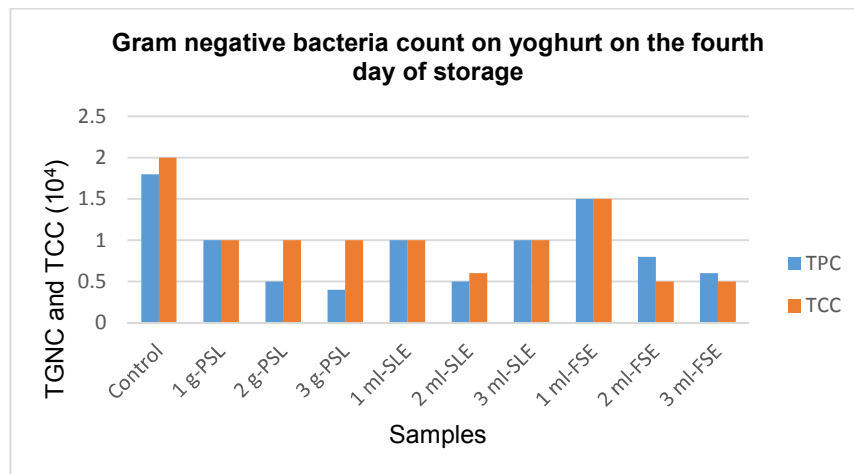
S/N.	Samples	Day 1	Day 2	Day 3
1	Control	$4.33 \pm 0.33$	$4.10 \pm 0.00$	$3.50 \pm 0.00$
2	1 g-PSL	$4.27 \pm 0.33$	$4.07 \pm 0.33$	$3.43 \pm 0.33$
3	2 g-PSL	$4.17 \pm 0.33$	$3.97 \pm 0.33$	$3.67 \pm 0.33$
4	3 g-PSL	$4.03 \pm 0.33$	$3.97 \pm 0.33$	$3.73 \pm 0.33$
5	1 ml-SLE	$4.33 \pm 0.33$	$3.80 \pm 0.58$	$3.57 \pm 0.33$
6	2 ml-SLE	$4.17 \pm 0.33$	$3.67 \pm 0.33$	$3.53 \pm 0.33$
7	3 ml-SLE	$4.33 \pm 0.33$	$3.40 \pm 0.58$	$3.13 \pm 0.33$
8	1 ml-FSE	$4.37 \pm 0.33$	$3.77 \pm 0.33$	$3.53 \pm 0.33$
9	2 ml-FSE	$4.17 \pm 0.33$	$3.63 \pm 0.33$	$3.47 \pm 0.33$
10	3 ml-FSE	$4.07 \pm 0.33$	$3.53 \pm 0.33$	$3.37 \pm 0.33$

NB: Values are mean  $\pm$  standard deviations of triplicate readings

**Table 8. Bacterial count on the treated and untreated yoghurt samples on the fourth day of storage**

S/N.	Samples	TGNC	TCC
1	Control	$1.8 \times 10^4$	$2.0 \times 10^4$
2	1 g-PSL	$1.0 \times 10^4$	$1.0 \times 10^4$
3	2 g-PSL	$0.5 \times 10^4$	$1.0 \times 10^4$
4	3 g-PSL	$0.4 \times 10^4$	$1.0 \times 10^4$
5	1 ml-SLE	$1.0 \times 10^4$	$1.0 \times 10^4$
6	2 ml-SLE	$0.5 \times 10^4$	$0.6 \times 10^4$
7	3 ml-SLE	$1.0 \times 10^4$	$1.0 \times 10^4$
8	1 ml-FSE	$1.5 \times 10^4$	$1.5 \times 10^4$
9	2 ml-FSE	$0.8 \times 10^4$	$0.5 \times 10^4$
10	3 ml-FSE	$0.6 \times 10^4$	$0.5 \times 10^4$

NB: \*TGNC = Total Gram Negative Bacteria Count, \*TCC = Total Coliform Count and \*Cfu = Colony Forming Unit

**Fig. 2. Bar chart showing microbial load of yoghurt samples**

**Table 9. Characterization of bacterial isolates from yoghurts after three days of production**

	Isolates									
	1	2	3	4	5	6	7	8	9	
Gram Reaction	-RODS	+COCCI (In chain)	-RODS	+RODS	+COCCI	-RODS	-RODS	+RODS	-RODS	
Citrate	+	-	-	+	+	-	+	-	-	
Methyl Red	-	+	+	+	-	+	+	-	-	
VP Test	-	-	-	-	-	+	+	-	+	
Hydrogen Sulphide	-	-	-	+	+	-	+	-	-	
Mortality	-	-	-	+	-	+	-	+	+	
Coagulase	-	-	-	-	+	-	-	-	-	
Indole	+	-	+	-	+	-	-	-	-	
Catalase	-	-	-	+	+	-	+	-	+	
Urease	-	-	-	+	-	-	-	+	+	
Spore strain	-	ND	-	+	-	ND	ND	-	+	
Starch Hydrolysis	+	-	+	-	+	+	+	+	+	
SUGAR	Lactose	AG	-	AG	-	A	A	-	AG	-
	Glucose	AG	A	A	A	A	A	AG	AG	AG
	Fructose	AG	A	AG	AG	A	A	-	AG	-
FERMEN-TATION	Sucrose	AG	-	AG	AG	A	A	-	AG	-
	Mannitol	AG	-	AG	AG	AG	A	AG	AG	-
	Maltose	AG	-	A	AG	A	A	AG	AG	-
Possible Organism	<i>Klebsiella</i> spp.	<i>Streptococcus</i> spp.	<i>Escherichia coli</i>	<i>Bacillus</i> spp.	<i>Staphylococcus</i> spp.	<i>Pseudomonas</i> spp.	<i>Enterobacter</i> spp.	<i>Lactobacillus bulgaricus</i>	<i>Proteus</i> spp.	

Key: +=positive, -=negative, ND=not determine, A=acid production, AG= acid and gas production

**Table 10. Bacterial Isolates from on samples on the fourth-day**

S/No	Treatments	<i>Klebsiella</i> spp.	<i>Streptococcus</i> spp.	<i>E. coli</i>	<i>Bacillus</i> spp.	<i>S. aureus</i>	<i>Pseudomonas</i> spp.	<i>Enterobacter</i> spp.	<i>Proteus</i> spp.
1	0 g-SL	-	+	-	+	-	-	-	-
2	1g-PSE	+	+	+	+	+	-	-	+
3	2g-PSE	-	+	+	-	+	+	+	-
4	3g-PSE	+	+	-	+	-	+	+	-
5	1ml-SLE	+	+	+	-	+	-	+	-
6	2ml-SLE	+	-	-	-	+	-	+	-
7	3ml-SLE	+	+	+	+	-	-	+	-
8	1ml-FSE	+	-	-	-	+	-	+	-
9	2ml-FSE	+	-	-	-	+	-	+	-
10	3ml-FSE	-	-	-	-	-	-	+	-

This increase agrees with [55] observations that time for spoilage to occur depends on the number and composition of the initial microflora and the storage temperature. The general bacterial count obtained for every sample analyzed were discovered to be lower than the acceptable limit for pasteurized milk ( $3 \times 10^4$ ) CFU / mL [56]. The total coliform count of the samples was higher than that reported by Matin et al. [57], they reported a total coliform count ranging from  $1.02 \times 10^2 - 4.51 \times 10^2$ .

### 3.6 Isolated Bacteria Species

The characterization of the microbial content of the yoghurt formulated with scent leaf resulted in the identification of nine (9) strains of bacteria in the ten (10) samples, control inclusive. *Klebsiella* spp., *Streptococcus* spp., *Escherichia* spp., *Bacillus* spp., *Staphylococcus* spp. And *Enterobacter* spp. (Table 9). The bacteria isolated from the control sample (without treatment) is similar to that of Moh et al. [58]. The sample treated with 3 mL squeezed scent leaf was able to suppress the growth of most of the bacteria except *Enterobacter* spp. The forms of bacteria isolated from the yoghurt samples included both gram -ve and gram +ve, some were non-motile, others tested -ve with methyl red, while some were ROD like in shape, few produced both acid and gas with sugar fermentation, some were also ROD like in shape but motile, spore formers, catalase +ve, and also produced both acid and gas in sugar fermentation. The gram-negative bacteria isolated with the MacConkey agar appeared pink on the agar, in both rod-like and cocci shapes. The gram-negative bacteria isolated include *E. coli*, *Proteus* spp., *Klebsiella* spp., *Pseudomonas* spp.

These morphological and biochemical characteristics of the microorganisms provided the basis for the identification of probable strains of microorganisms in the shelf stored yoghurt samples. The MacConkey agar used made the isolation of the gram-negative bacteria easier while the nutrient agar allowed for a general view of the microbial profile of the yoghurt samples.

These organisms isolated from the samples are important food spoilage organisms and some are pathogenic [59,60]. The isolation of *E. coli* and *S. aureus* in the sample is a possible indication of lack of asepsis during the production of the yoghurts that could have stemmed from

unsanitary production condition [61]. The presence of these organisms could prompt abdominal cramps in humans. These microorganisms also lead to quality deterioration of the yoghurt samples [60].

Though the yoghurts are high acid foods (pH 4.6), the chemical and nutritional profile, specifically, the pH and high moisture content of the yoghurt influences the growth of these bacteria [62]. The presence of the *Bacillus* spp. is an implication of the pH and these could also initiate spoilage of the yoghurt making the environment conducive for other microorganisms and the yoghurt prone to spoilage.

The spoilage of the yoghurt was first perceived by the change in color of the products, the presence of mold on the surface of the drinks. These were the first indicators of spoilage in the yogurt samples. These visible mold growths on the samples could have been favored by the yoghurt's low pH and they are a very crucial indicator of yoghurt spoilage [63,64]. Their presence was highly undesirable, their consequence is depreciation in the acceptability of the yoghurt samples, objectionable changes that lower the yoghurt quality [65]. The molds just as reported by Foschino et al. [66] were responsible for the off flavour, shrinkage, swelling, poor appearance of the yoghurts. The molds in the sample also made the yoghurt more susceptible to protein break down (proteolysis) and bacteria invasion [67,68].

## 4. CONCLUSION

*Ocimum gratissimum* is a very important plant and its usage in Nigeria cannot be overemphasized, especially in the South-Eastern part of the country. The anti-microbial properties of the leaves have also led to its application in traditional medicine amongst the locals of Nigeria, where its extracts are in use as a cure for malaria, fever, inflammation, gout, and so on.

The local scent leaf plant of different forms and concentration had a good organoleptic acceptance as flavoring agents in yoghurt as judged by the sensory panelist. The squeezed extract of the *Ocimum* leaves had the best acceptance amongst all used forms. The sensory result of the research depicts that usage of scent leaf as a flavoring agent in yoghurt could be accepted but the formulation should be in a minute ratio to the dairy drink itself, and the

squeezed extract of the scent leaf was able to compete with the plain yoghurt.

In terms of preservation potency, the scent leaves could also act as an organic preservative. Yoghurt samples treated with powdered scent leaf and the powdered scent leaf extract could only keep the samples on the shelf for about 60 hours  $\pm$  2, however, a greater outcome was recorded in the yoghurt samples treated with squeezed scent leaf extract, as the samples were found to be shelf-stable for 96 hours (4 days), making the squeezed extract the best form in terms of preservation. This result indicated that scent leaf (squeezed extract), in addition to being an alternative for synthetic preservatives, which could have a long term adverse effect, could also serve as a form of preservative to low-income segments of the population. Furthermore, extracts of the scent leaf could also be used together with some approved and edible preservative like sodium metabisulphite industrially, in which case, there would be a consequential uplift in the economic importance of the leaves and consequentially the full potential of the leaves could be harnessed.

Chemical changes undergone by the yoghurt during shelf storage could have led to the color deterioration, changes in flavor, taste, and a decrease in pH. The low pH also allowed the growth of yeast which could have contributed to the development of an undesirable odor of the products on the fourth day.

Following the report of Kabiru et al. [60], the life span of the scent leaf treated yoghurt could be increased by ensuring aseptic production and refrigerating the products after production and formulation. Irrespective of the wide use of the plant, the toxicity of its usage needs to be checked because some polyphenols it contains are toxic like tannins and the alkaloid because it is another class of phytochemicals and it has a huge diversity of chemical structures.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but the advancement of knowledge. Also, the research was not funded by the producing

company rather it was funded by the personal efforts of the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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