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Antioxidant Potentials of Turmeric (*Curcuma longa*) and Clove (*Syzygium aromaticum*) Extracts, Singly and in Synergy on Peanut Butter

Adegoke GO[1]*, Anarado CS[1] and Afolabi MO[2]

[1]Department of Food Technology, University of Ibadan, Ibadan, Nigeria

[2]Department of Food Science and Technology, Bowen University, Iwo, Nigeria

***Corresponding author**: Gabriel Olaniran Adegoke, Department of Food Technology, University of Ibadan, Ibadan, Nigeria; Tel: +2349033296181; Email: goadegoke@yahoo.com

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Abstract

Turmeric and clove are considered to be rich sources of phenolic compounds that can be used to replace synthetic antioxidants in fatty foods. Therefore the use of clove and turmeric extracts as natural antioxidants in peanut butter has future prospects. The main objective of this study was to preserve peanut butter with turmeric and clove ethanol extracts as natural antioxidants. The prepared peanut butter (PB) was preserved at 25˚C with 100 ppm each of turmeric and clove extracts; mixtures of turmeric and clove in ratios 1:1, 1:3 and 3:1 respectively. Butylated hydroxyl anisole (BHA) was used as a control in another sample. Peroxide values (PV) and antioxidant effectiveness (AE) of extracts on the samples were determined on the first day and every 7 days for 28 days. Sensory analysis was carried out on various samples of peanut butter before and at the end of storage period using a 9-point hedonic scale. Peroxide values for all samples at the beginning of storage were 0. At the end of the storage period, untreated peanut butter showed the highest peroxide value (23.33 meq/kg). The PB sample treated with clove extract gave the least PV (8.33meq/kg); however there was no significant difference ($p \leq 0.05$) between this value and those obtained for peanut butter preserved with turmeric extract (11.67meq/kg) Butylated hydroxyl anisole (10.00meq/kg) and sample containing turmeric and clove in ratio 1:3 (15.00meq/kg). Similarly antioxidant effectiveness was highest in peanut butter sample containing 100ppm clove extract (66.68%) and least for peanut butter containing mixtures of turmeric and clove in ratio 1:1 (13.32%). In terms of general acceptability sample with BHA received the highest score and there were no significant differences ($p \leq 0.05$) among the peanut butter samples containing clove extract at 100 ppm and samples with BHA and one with mixture of turmeric and clove extracts in ratio 1:3. It can be concluded that 100ppm clove ethanol extract was the most suitable natural antioxidant and exhibited maximum score in terms of sensory attributes compared to others with added clove and turmeric extracts.

Keywords: Turmeric; Clove; Natural Antioxidants; Synergy; Peanut Butter

Abbreviations: BHA: Butylated Hydroxyl Anisole; TBHQ: Tert-Butyl Hydroquinone; BHT: Butylated Hydroxyl Toluene; PG: Propyl Gallate: OG: Octylgallate; GRAS: Generally Recognized as Safe.

Introduction

Oxidative deterioration of food products during processing and storage produces off-flavour and compounds such as aldehydes, ketones and organic acids and all these products have been implicated in cardiovascular diseases, mutagenesis, and carcinogenesis [1]. Lipid peroxidation is one of the primary causes of deterioration in quality of food products. It can seriously interfere with the efficiency of processing steps and therefore, leads to potential economic loss. Lipid oxidation produces reactive oxygen species (ROS), which have been implicated in carcinogenesis, inflammation, early aging and cardiovascular diseases [2]. Lipid oxidation also reduces the organoleptic attributes of foods and imparts rancid and unpleasant flavours to the raw and processed oil and fat products, thus making them unacceptable to consumers. Lipid oxidation can however be minimized by adding appropriate amount of antioxidants. Traditionally, synthetic compounds, such as butylated hydroxyl anisole (BHA), tert-butyl hydroquinone (TBHQ), butylated hydroxyl toluene (BHT), propyl gallate (PG) and octylgallate (OG) are used as antioxidants in foods and oil products [3,4]. However, epidemiological studies have shown possible health risks associated with consumption of synthetic antioxidants and strict regulations now govern their use in foods [4]. With adverse reports on the application of BHA and BHT in food matrices therefore, there is an increasing interest in the antioxidant activities of natural compounds [5]. Interestingly, while tert-Butylhydroquinone (TBHQ) is not allowed in Japan, Canada, and Europe, similarly, BHA had been removed from the list of generally recognized as safe (GRAS) compounds [6]. Higher and aromatic plants have been used in traditional medicine as well as to extend the shelf life of foods and most of their properties are due to essential oils produced by secondary metabolism [7]. The use of plant essential oils as antioxidants has been researched in detail with the view of investigating their protective role for food products containing fats and oils [8]. Generally, industrial foods are developed to supply the requirements of consumers in relation to taste, appearance, market value, and practicality in preparation and consumption [9]. Spices are abundant sources of polyphenolic compounds that have strong antioxidant capacities, which promote the protection of important cellular components such as deoxyribonucleic acid (DNA), proteins and lipid membranes against the action of reactive oxygen species and can potentially replace the synthetic antioxidants in food systems [10,11]. Phenolic compounds have redox properties, which may be the result of several mechanisms: free radicals scavenging ability, chelating activity for transition metals and reduction of singlet oxygen. Moreover, these compounds are also known for their roles in preventing lipid peroxidation and inhibiting several types of oxidative enzymes, especially in rosemary and oregano [10]. The indigenous plant species of interest in this present research are the flower buds of *Syzygium aromaticum* (clove) and rhizomes of *Curcuma longa* (turmeric). Apart from their use as additives in food, extracts from some aromatic spice plants have a potential to be used in small amounts in fat containing food systems to prevent or delay some chemical deteriorations occurring during the storage of these products [12-14]. While phenolic compounds can be extracted using polar solvents such as water and ethanol, ethanol was chosen for this present work because of its moderate polarity, inertness and ease of removal from the extract [11].

Materials and Methods

Plant materials

Turmeric, cloves and fresh-shelled peanuts were purchased from local market in Nigeria.

Chemicals and reagents

All chemicals and reagents used were purchased from Sigma Aldrich and Fluka Chemical Co. All chemical and reagents were of analytical grade.

Extraction of spice extract

Turmeric rhizomes and clove buds were washed, sorted, trimmed, peeled, thinly-sliced and sun-dried until brittle. The friable slices were then milled to pass through 100 mesh screen sieve. The powdered spices were extracted using a modified method of If esam, et al. [15]. The turmeric and clove powders were put in two different clean and dry glass containers with lids. Ethanol (96%) was added to each of the containers having the milled spices and covered. The samples were allowed to soak for 72hours with intermittent shaking. The ratio of sample to solvent for the extraction was 1:10 [16]. After the extraction period, the respective solutions were first filtered using clean, dry muslin cloth to remove large particles. The respective filtrates were then carefully filtered using what man filter paper number one. The various extracts were then stored at 4°C pending concentration. Concentration to slurry was done *in vacuo* using a rotary evaporator (Model RE100B, Japan) at 40°c.

The slurry was then evaporated to dryness in a dry pre-sterilised stainless pan, using a water bath at 40°C. The dry mass was scraped off and collected into airtight amber coloured bottles for further analysis and utilisation.

Production of Peanut Butter

Roasting of peanut was done using the method described by Adegoke, et al. [17]. It was then manually dehulled by rubbing in-between the fingers. It was threshed and air aspirated to separate the hulls from the peanuts. The clean nuts were then milled into a smooth paste with a little salt to taste, using a disc attrition mill. The resulting paste (peanut butter) was collected into a pre-sterilised container for immediate use.

Preparation of Samples

Peanut butter (40g) was weighed into each of the seven dry, pre-sterilised bottles. To six samples were added the following: Tumeric extract: clove extract: synthetic antioxidant (BHA): mixtures of turmeric and clove in the ratios 1:1.1:3 and 3:1 in that order. The proportion of extracts/ mixtures of extracts used were 100ppm (0.01%) according to Code of Federal Regulations CFR [18] for addition of BHA to peanut products. The untreated sample and one with BHA served as negative and positive controls respectively. All the samples were stored at 25°C.

Determination of Peroxide Value (PV) of Peanut Butter

This was determined by using a modified method of the American Oil Chemists' Society (AOCS) [19] official methods. The analysis was carried out in triplicate. One gram of sample (peanut butter) was dissolved in 10ml of the solvent (3:2) mixture of glacial acetic acid and chloroform) and 0.2ml of saturated potassium iodide solution. The mixture obtained was left to stand in the dark for about 5minutes, and thereafter, 0.5ml of saturated freshly prepared starch solution was added. The mixture was shaken to obtain a uniform mixture and then titrated with 0.1N sodium thiosulphate ($Na_2S_2O_3$) solution. The volume of sodium thiosulphate used was recorded. A blank without the sample was also prepared and titrated in the same way.

The peroxide values were expressed as milliequivalent per kilogramme (meq/kg) of butter.
Peroxide value was calculated from equation 2:

$$\text{Peroxide value (PV)} = \frac{(V-Vo)M \times 10^3}{W} \text{ meq/kg} \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where V= Volume of $Na_2S_2O_3$used for the sample
Vo = Volume of $Na_2S_2O_3$used for the blank
M = Normality of $Na_2S_2O_3$
W = weight of sample

Determination of Antioxidant Effectiveness of Clove and Turmeric Extracts in the Peanut Butter

The percentage antioxidant effectiveness during storage test period was monitored using the method described by Adegoke and Gopalakrishna [20]. The method of calculating antioxidant effectiveness was given in equation 3:

$$\text{Antioxidant effectiveness (AE)} = \frac{PV\ of\ control - PV\ of\ test\ sample}{PV\ of\ control} \times 100 \quad \ldots\ldots\ldots (3)$$

Sensory Analysis

Sensory analysis was carried out on samples of peanut butter on the 1st day of production and at the end of storage period. The acceptability of the various samples of peanut butter was evaluated by fifteen (15) panellists drawn randomly from the staff and students of Food Technology Department, University of Ibadan. Each sample was rated on a nine point hedonic scale (9-extremely liked, and 1- extremely disliked)) for taste, colour, aroma, appearance and overall acceptability.

Statistical Analysis

The data obtained from study and sensory characteristics were analysed using the analysis of variance (ANOVA) method at $p \leq 0.05$ least significance difference (LSD). The significance difference was separated using Duncan multiple range test and data was reported as means of triplicate values obtained [21]. The statistical analyses were carried out using statistical package for social sciences (SPSS) version 16.0 computer software.

Results and Discussion

Peroxide Value of Peanut Butter

Data in (Table 1) represented peroxide values of peanut butter samples. The initial PV of samples of PB at day (week) 0 was 0meq/kg values because the samples were still very fresh and devoid of peroxidation products. By the end of the first week, PB sample with 100ppm BHA maintained their zero values while the PV of samples without extract or BHA increased to13.33 meq/kg. Sample containing mixture of turmeric and clove (1:1) gave the second highest value (8.33), while sample with

mixture of turmeric and clove in the ratios 3:1 and 1:3 were not significantly different (p≤0.05) from each other (Table 1). As storage time increased, samples with clove (100ppm), turmeric (100ppm) and BHA (100ppm) consistently showed the least PV in that order while untreated sample (control) consistently gave the highest values respectively. The synergistic effect of mixture of turmeric and clove extracts in the ratio 1:3 was greater than that exhibited by the blend of 3:1 and 1:1 in that order. The results showed that ethanoic extracts of clove was able to retard lipid oxidation better than BHA, turmeric extract and the blends of the extracts. These results are in agreement with the findings of Pokomy [22], Gulcin, et al. [23] and Dipak [23] that plant extracts can serve as substitutes for synthetic antioxidant. According to these authors, spices such as clove have great capacity to give off hydrogen and reduce lipid peroxidation.

Sample	Week 0	Week 1	Week 2	Week 3	Week 4
Untreated sample	0	13.33a	16.67a	18.33a	23.33a
Mixture ratio:					
Tumeric: Clove					
1:00	0	0c	5d	8.33cd	11.67cd
0:01	0	0c	3.33d	6.67d	8.33d
3:01	0	3.33c	10bc	13.33bc	16.67bc
1:01	0	8.33b	13.33ab	16.67ab	21.67ab
1:03	0	1.67c	6.67cd	11.67bcd	15cd
Butylated Hydroxyl Anisole (BHA)	0	0c	3.33d	8.33cd	10cd

Table 1: Peroxide values (meg/kg) of peanut butter samples during storage.

*Mean values followed by different superscript along the column are significantly different (P ≤0.05).

** Each value represents mean of 3 readings.

Antioxidant Effectiveness (AE) of Spice Extracts

The results obtained for antioxidant effectiveness (AE) of peanut butter samples are shown in Table 2. For day 0, no activity was recorded for all the samples because no PV value was got. At day 7, the AE of sample with turmeric, clove extracts and BHA were 100% while that of sample containing mixture of turmeric and clove extract gave the lowest AE of 37.51%. For sample containing only turmeric extract, there was a progressive decease in AE as the days increased from 100% at day 7 to 53.32% at day 28. Other samples showed decreasing antioxidant effectiveness (AE) up to day 21 and increased on day 28. Sample with only clove extract showed better antioxidant effectiveness than other samples at 21 and 28 days with the values of 63.61 and 66.8% respectively. These values showed that clove extract were more potent than BHA which had AE values of 54.56 and 60.0% at 21 and 28 days in that order. This might be due to clove being a better hydrogen donor than either of BHA or turmeric as a result of solvent used for extraction [22]. Clove oil contained eugenol, 2-Heptanone and Methyl salicylate as phenolic compounds. Sample with turmeric showed good AE on peanut butter, although not as good as those of clove and BHA. From the results obtained in this present study, the combination of turmeric and clove was not as effective as either of them being used singly.

Sample	0	7	14	21	28
Untreated	-	-	-	-	-
Tumeric: Clove ratio					
1:0	0	100.0	70.01	54.56	53.2
0:1	0	100.0	80.02	63.61	66.68
3:1	0	75.02	40.01	27.28	33.32
1:1	0	37.51	20.04	9.06	13.32
1:3	0	87.47	60	36.33	40.00
Butylated Hydroxyl Anisole (BHA)	0	100	80.01	54.56	60.00

Table 2: Antioxidant Effectiveness (AE) of Turmeric and Clove on peanut butter samples over a period of 28 days.

Antioxidant Effectiveness (AE) (%)

* Quantity of antioxidant mixture added in each sample was 100ppm

Sensory Evaluation

The sensory analysis was carried out at the start of the storage period (day 0) and at the end of 28 days. The results of the sensory analysis on day 0 are shown in Table 3 & 4. The taste and aroma of all the samples showed no significant differences ($p \leq 0.05$) among the replicates. There were varying responses in their colour, appearance and general acceptability. Samples with turmeric and clove in ratios 3:1 and 1:1 gave a very noticeable yellow colouration while the sample with turmeric and clove in ratio 1:3 had a slight yellow tinge. The reddish yellow pigment accounted for low values obtained for samples with higher level of turmeric. Samples with clove extract retain the colour of peanut butter. In terms of general acceptability there were no significant differences ($p \leq 0.05$) among the samples containing clove extract alone, BHA, turmeric and clove in the ratio 1:3 and untreated sample respectively. At the end of the storage period of 28 days, in terms of taste, samples preserved with clove alone and BHA had the highest values (6.60 and 6.40 respectively). The untreated sample was slightly disliked by the panelists with the least value of 3.80 and the same trend was followed by aroma. The colours of samples containing only turmeric and mixtures of turmeric and clove in ratio 3:1 were rated lowest (3.00 and 4.20 respectively) while the one containing only clove was rated highest (7.60). At the end of 28 days, sample preserved with only clove extract was rated highest rating (7.13), followed by sample preserved with BHA (6.80). The result was in agreement with the findings of Mohd-Rozalli, et al. [24,25], who reported 4 weeks of oxidative stability for stabilizer- free natural peanut butter at temperature of 25°C. According to the authors, storage temperature had the most significant effect on quality changes of peanut butter.

Sample	Taste	Colour	Aroma	Appearance	General acceptability
Untreated sample	7.33^{a}	7.07^{a}	7.60^{a}	7.07^{a}	7.40^{ab}
Tumeric/ clove ratio					
1:0	7.33^{a}	4.60^{b}	7.13^{a}	4.80^{b}	5.80^{c}
0:1	7.80^{a}	7.40^{a}	7.53^{a}	7.07^{a}	7.27^{ab}
3:1	7.53^{a}	4.93^{b}	7.27^{a}	5.07^{b}	5.80^{c}
1:1	7.13^{a}	5.93^{b}	7.33^{a}	5.37^{b}	6.47^{bc}
1:3	7.67^{a}	7.00^{a}	7.53^{a}	7.13^{a}	7.47^{a}
Buty lated Hydroxyl Anisole (BHA)	7.67^{a}	6.80^{ab}	7.73^{a}	6.87^{ab}	7.40^{ab}

Table 3: Sensory evaluation of peanut butter treated with Tumeric and clove extracts at day 0.
*Means in the same column, followed by the same letter are not significantly difference ($P \leq 0.05$).

Sample	Taste	Colour	Aroma	Appearance	General Acceptability
Untreated sample	3.8^{c}	7.2^{ab}	4.4^{b}	7.2^{a}	5.8^{cd}
Tumeric/ clove ratio					
1:0	5.8^{ab}	3.00^{e}	6.20^{a}	4.20^{c}	5.00^{d}
0:1	6.60^{a}	7.60^{a}	7.20^{a}	7.20^{a}	7.13^{a}
3:1	5.60^{ab}	4.20^{d}	6.00^{a}	4.40^{c}	5.07^{d}
1:1	4.80^{bc}	5.40^{c}	4.80^{b}	5.00^{bc}	5.00^{d}
1:3	5.60^{ab}	6.40^{bc}	6.80^{a}	5.80^{b}	6.20^{bc}
Butylated Hydroxyl Anisole (BHA)	6.40^{a}	6.80^{ab}	6.60^{a}	7.20^{a}	6.80^{ab}

Table 4: Sensory evaluation of peanut butter treated with Turmeric and clove extracts at day 28.
Means in the same column, followed by the same letter are not significantly difference ($P \leq 0.05$) Quantity of antioxidant mixture added in each sample was 100p.

Conclusion

The crude ethanoic extracts of clove and turmeric exhibited varying degrees of prevention of oxidative deterioration in peanut butter. Clove extracts showed better potential for the prevention of oxidation and flavour deterioration than the butylated hydroxylanisole (BHA) and turmeric. The extracts used singly showed greater ability in the prevention of oxidative deterioration than when the extracts were mixed.

Significant Statements

This present study utilized natural preservatives from plant materials to prevent lipid peroxidation in foods with ability to scavenge free radicals and prevent flavour deteriorations. Hence these plant materials are suitable for use as antioxidants in processed foods containing fats and oil.

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