Use of eugenol-lean clove extract as a flavoring agent and natural antioxidant in mayonnaise: product characterization and storage study

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Abstract Eugenol-lean fraction (98 % lower eugenol content than eugenol-rich fraction) having appreciable phytochemical properties was selectively isolated from clove buds (*Syzygium aromaticum* Linn) using supercritical carbon dioxide extraction at 40 °C and pressure of 25 MPa with static and dynamic time of 120 and 30 min respectively using ground clove powder which was previously subjected to steam distillation. The extract was used as a flavor ingredient (replacing mustard in classical formulation) and also as a source of natural antioxidant in formulating a new mayonnaise product to improve its nutraceutical value and shelf-life. This product was found to be comparable to the mustard formulated sample (experimental control) organoleptically. The sample did not have typical pungency of clove and had improved physical properties such as increased color tonality with higher chroma values, lower thermal and non-thermal creaming, homogenous and compact microstructure and higher consistency index vis-à-vis the control and standard market samples, even at the end of the storage period of 6 months. Mayonnaise formulated with eugenol-lean clove extract had significantly higher antioxidant activity (IC_{50} = 10.85 mg/mL), phenolic content (1.89 mg gallic acid equivalent/g mayonnaise) and reducing power (11.29 mg BHT equivalent/g mayonnaise) than mustard-formulated mayonnaise and the market sample. While, the antioxidant activity and phytochemical properties tend to decrease after 30 days for the reference market sample and after 90 days for the experimental control sample, the mayonnaise formulated with eugenol-lean clove extract was found to be stable beyond 6 months.

Keywords Eugenol-lean clove bud extract · Mayonnaise · Antioxidant activity · Storage study

Introduction

Cloves (*Syzygium aromaticum* Linn) are aromatic dried flower buds of the family Myrtaceae. Clove contains 10 % fixed oil (non-volatile and saponifiable), 15–20 % essential oil (volatile and unsaponifiable), 6–7 % non-essential ether extract (comprising of ethyl caproate, methyl caprylate and methyl stearate, to name a few) and 13 % tannin, besides glycosides (such as acetophenone glucosides) and flavonols (Pathak et al. 2004; Leela and Sapna 2008; Han and Paik 2010). GC/MS analysis of its essential oil identified 36 components, among which eugenol (88.58 %), eugenyl acetate (5.62 %) and β -caryophyllene (1.38 %), were the major ones (Chaieb et al. 2007). It is known that clove oil has considerable antimicrobial (Hernández-Ochoa et al. 2011) and therapeutic activities such as anti-inflammatory (Daniel et al. 2009) and antioxidant properties (Ogata et al. 2000) owing to its significant eugenol ($C_{10}H_{12}O_2$; 4-allyl-2-methoxyphenol) content.

Generally, clove extracts are used as biopreservatives in preventing food spoilage by pathogenic contaminants. Hernández-Ochoa et al. (2011) have used the same in meat samples against pathogenic strains; Ponce et al. (2011) have encapsulated the essential oil and used it for preservation of lettuce leaves; Phoopuritham et al. (2011) have successfully used clove oil as a natural antioxidant and has incorporated the same in designing antioxidant cellulose-based films for enhancing shelf life of soybean oil.

In our previous investigations, we have reported selective isolation of eugenol-rich fraction from clove buds and used the same as a source of natural antioxidant in un-encapsulated and encapsulated forms in food systems (Chatterjee et al. 2012; Chatterjee and Bhattacharjee 2013). However, the usage of this

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eugenol-rich fraction of clove buds as a flavoring agent is limited in foods, owing to its pungent odor. Preliminary study reported that the eugenol-lean fraction which remained after extraction of eugenol has considerable nutraceutical and therapeutic properties with the advantage of reduced pungent smell of eugenol. Therefore, for both holistic utility of clove buds for improved commercial viability of a SC-CO₂ extraction process and to obtain clove bud extract with appreciable phytochemical properties and reduced pungent odor, the extraction of eugenol-lean fraction was carried out on clove matrix which was previously subjected to extraction of eugenol-rich fraction. Alternate extraction methodologies (steam distillation, solvent extraction, liquid CO₂ extraction and subcritical CO₂ extraction) were attempted in combination with SC-CO₂ to obtain eugenol-lean fraction. From several trials, it was found that the clove extracts with minimum amount of eugenol was obtained by using the combined extraction procedures of steam distillation and SC-CO₂ extraction. Therefore, in this work, clove buds were first subjected to steam distillation and the resultant clove matrix was further subjected to SC-CO₂ extraction to obtain eugenol-lean fraction.

This study aims to examine the feasibility of formulating a 'new mayonnaise' using eugenol-lean fraction of clove buds principally as a flavor ingredient (replacing mustard in the classical formulation) and also as a source of natural antioxidant to improve the shelf-life and nutraceutical value of the product. Mayonnaise is an oil-in water emulsion with fat content of 70–80 %. The emulsion consists of finely dispersed droplets of oil (size ranges from $\leq 1 \mu\text{m}$ to $\geq 20 \mu\text{m}$) in a continuous phase of water (Snyder and Kwon 1987). It is one of the most prominent savory dressings prepared by carefully mixing egg yolk, vinegar and oil. Mustard is used as the principal flavor ingredient. Offlate, in commercial dressings, several other spices are also being used; namely dried celery, onion, carrot, garlic, paprika, green and red pepper (Ma and Boye 2013).

However, till date, there is no report on use of clove in mayonnaise, possibly owing to its pungent flavor (attributed to the presence of high amount of eugenol therein). We opine that this pungency may be redressed by employing eugenol-lean fraction of clove buds (with reduced pungency) in mayonnaise formulation.

Formulations of mayonnaise with enhanced nutritional quality have been attempted by several authors. While Das et al. (2013) used rice bran oil for the formulation of nutraceutically enhanced mayonnaise; Sathivel et al. (2005) used nutraceutically enriched soluble and insoluble arrow tooth flounder protein powders for its formulation. Abd El-Razik and Mohamed (2013) reported on use of acid casein curd, both with and without *Chlorella vulgaris* biomass as a partial replacement of egg in mayonnaise. Currently, there is a trend towards the production of low fat mayonnaise. Since the distribution of fat and water in an emulsion influences the balance of each flavor, low fat mayonnaise allows proper perception of the added flavor (Ma and Boye 2013). It is opined that incorporation of eugenol-lean clove

fraction as a flavor ingredient would not necessitate a low fat mayonnaise formulation for proper flavor perception. This work reports for the first time on the usage of eugenol-lean fraction of clove buds in mayonnaise as a flavoring agent (replacing mustard) and as a source of natural antioxidant to improve its shelf-life and nutraceutical value.

Materials and methods

Clove buds (*Syzygium aromaticum* Linn) were purchased from a local market of Jadavpur, Kolkata, India. National Research Centre on Seed Spices, Tabiji Farm, Ajmer, have authenticated that only this species of clove is cultivated and commercialized in India. Speciality chemicals such as eugenol (99 % pure), 1, 1-diphenyl-2-picrylhydrazyl (DPPH), sodium nitroprusside, Griess reagent and gallic acid were procured from M/s Sigma, Munich, Germany. All chemicals, solvents and buffers used in the work were of AR grade.

Preparation of clove buds

The clove buds were dried using the procedure followed by Wenqiang et al. (2007) at 30 °C in a tray dryer and stored in self-sealable LDPE bags at ambient temperature (23 ± 2 °C) in dark. The samples were ground by an electric grinder and particle diameter (d_p) was determined by sieve analysis method by screening the powdered clove samples through a set of standard sieves (5, 10, 24, and 35 Tyler meshes) in a sieve shaker and noting the particle size distributions. The powder samples passed through mesh 24 (0.70 mm) and were retained on mesh 35 (0.50 mm). Following approximate estimation of particle diameter of the ground clove powder; their exact diameters were estimated using micrometer screw gauge with an accuracy of 0.01 mm. The mean particle diameter (d_p) of randomly picked 100 such powder particles were determined to be 0.5 mm (Bhattacharjee et al. 2012).

Extraction of eugenol-lean fractions from clove buds

The extraction of eugenol-lean fraction of clove buds was carried out using a combination methodology of steam distillation and SC-CO₂ extraction. Initially, 20 g of ground clove powder ($d_p=0.5$ mm) was subjected to steam distillation under normal atmospheric pressure (0.1 MPa), using 1 L water for 8–10 h. Post extraction of essential oil by steam distillation, the residual clove matrix was strained through a muslin cloth and dried in a tray dryer at 35 °C for 10 h. This powder was then subjected to SC-CO₂ extraction using SPE-ED SFE 2 model of M/s Applied Separations, USA at a temperature of 40 °C and pressure of 25 MPa with static and dynamic time of 120 and 30 min, respectively. The flow rate of CO₂ was maintained constant at 2 L/min. From our

previous investigation (Chatterjee and Bhattacharjee 2013), it was found that these SC-CO₂ extraction conditions were found to yield minimum amount of eugenol. Three independent extraction runs were conducted for extraction of eugenol-lean fractions of clove buds. The clove extract thus obtained was found to contain minimum amount of eugenol. All extracts were weighed and stored in an inert atmosphere of nitrogen, inside screw-capped amber colored glass vials at 4 °C in the dark, until further analyses.

Determination of eugenol content of clove bud extracts

The eugenol content of the eugenol-lean clove bud extracts were determined by densitometric method, considering eugenol as the reference standard in accordance to the method described by Chatterjee and Bhattacharjee (2013), with little modifications. The clove extracts were diluted and spotted on aluminium plates coated with silica gel 60 (F₂₅₄) of dimension 100×100 mm using Camag Linomat V (M/s Camag, Basel, Switzerland). 20 µL of extracts were applied to the plates in the form of bands, each 8 mm wide; spacing between consecutive bands being 11.6 mm. Nitrogen gas was used at a low flow rate at 4 bar during spotting. The plates were developed at (23±2)°C in a glass chamber containing solvent system of toluene and ethyl acetate in the ratio of 3:1, v/v, in which eugenol showed R_f value of 0.6. Scanning of the bands was carried out in Camag HPTLC unit (TLC scanner III) in the wavelength range of 200–350 nm in which eugenol recorded absorbance maximum at 281 nm. Densitometric studies were thus performed at 281 nm and the areas under the curves of different bands were recorded and the amounts of eugenol present in the extracts were determined from the standard curve prepared.

Determination of phytochemical properties of eugenol-lean fraction of clove buds

The clove bud extracts were further subjected for assays of other phytochemical properties such as antioxidant activity, anti-inflammatory activity, total phenolic content, total flavonoid content and reducing power. The antioxidant activity was determined by measuring the radical scavenging activity of DPPH (Aiyegoro and Okoh 2010) and its anti-inflammatory activity by estimating the nitric oxide (NO) scavenging activity (Correa et al. 2009). Both these activities were expressed as IC₅₀ values. Using respective standard curves, the total phenolic compounds was estimated as mg gallic acid equivalent/g dry clove buds with Folin-Ciocalteu reagent (Spanos and Wrolstad 1990); the total flavonoid content as mg quercetin equivalent/g dry clove buds using Dowd method (Meda et al. 2005) and reducing power as mg butylated hydroxytoluene (BHT) equivalent/g dry clove buds, according to the method of Oyaizu (1986).

Formulation of mayonnaise using clove extracts

From the phytochemical analyses, it was found that eugenol-lean fraction of clove extracts has considerable amount of nutraceutical potency. Therefore, food application was carried out on mayonnaise, wherein the eugenol-lean clove fraction was added as a flavor ingredient (substitute of mustard) and as a natural antioxidant to improve its shelf-life. From preliminary trials, it was established that addition of eugenol-lean clove extract at 0.42 % by weight of mayonnaise sample resulted in proper combination of it as a flavoring agent and as a natural source of antioxidant.

Mayonnaise was prepared in accordance to Shen et al. (2011) with little modifications. Fresh egg yolk (12 %), vinegar (5 % (w/v) acetic acid) (9 %), soybean oil (74 %), salt (1.0 %), sugar (1.5 %) and mustard/eugenol-lean clove fraction (0.42 %) (all on w/w basis) were employed in preparation of mayonnaise. At first, egg yolk and vinegar were mixed together and then all other ingredients, except oil were added and stirred homogeneously in a homogenizer (M/s Ika, Germany). Finally, oil was added very slowly, while stirring at 1,500 rpm for 1 min, followed by 2,000 rpm for another 4 min. The mayonnaise thus prepared were stored in screw capped glass jars and stored at 10 °C in a refrigerator until further analyses. Generally mustard is used in mayonnaise as a flavoring agent. Therefore, we have used mayonnaise formulated with mustard (0.42 % of mayonnaise) as control sample; whereas a commercial market sample of mayonnaise (M/s Elmac Agro, India) served as reference standard. The selection of this reference sample was based on the fact that this sample is readily available in local market and its composition is in close agreement with our experimental sample. For each type of mayonnaise, three independent replicate samples were prepared and used for this study.

Sensory evaluation of mayonnaise

Sensory evaluation by a semi-trained panel of 20 members was carried out on freshly prepared mayonnaise samples and 6 month stored samples. The coded samples were randomly presented to each panelist, with a rest period between sample presentations to minimize sensory fatigue. Sensorial sessions were conducted in an air-ventilated room under white light. Nine characteristics such as flavor, thickness, spoonability, body and creaminess, color, taste, appearance, acceptability and aftertaste of mayonnaise were scored on a 9-point hedonic scale (Rahamati et al. 2012).

pH of mayonnaise

The pH of the mayonnaise samples was measured at an periodic interval over a storage period of 6 months, using pH meter (Model-PC 510 pH, M/s Eutech Instruments,

Singapore) with 5 g of mayonnaise samples which were homogenized with 25 ml deionized water (No and Meyers 2004).

Color measurement

Color measurement of mayonnaise samples was carried out at an periodic interval over a storage period of 6 months using the Minolta Chromameter (Model CR-300, Minolta Co., Ltd, Osaka, Japan) at a 10° inclination from light source and reported as L^* , a^* , and b^* values. The color co-ordinates of these mayonnaise samples were calibrated against a standard white plate. Thereafter, chroma and hue angle were calculated using Eqns. 1 and 2 respectively.

$$\text{Chroma} = (a^{*2} + b^{*2})^{1/2} \quad (1)$$

$$\text{Hue angle} = \tan^{-1}(b^*/a^*) \quad (2)$$

Optical microscope observation

Optical microscope observations of the mayonnaise samples were performed to compare the microstructure of the emulsions by using a light microscope (Olympus BX41) of magnification range 0–100x. The system is equipped with a digital camera to prepare micrographs. One drop of each sample was transferred on a glassy flat without dilution and covered by a cover slip to obtain micrographs.

Non-thermal and thermal creaming value of mayonnaise

Non-thermal and thermal creaming values of mayonnaise samples were determined at a periodic interval over a storage period of 6 months, in accordance to the method reported by Rahamati et al. (2012) with little modifications. To determine the non-thermal creaming value at room temperature, 8 g of mayonnaise samples were transferred into cylindrical plastic containers and centrifuged at 5,000 g for 20 min. The emulsion creaming at room temperature was determined by the following equation:

$$\%H = H/H_0 \times 100 \quad (3)$$

where, %H is the percentage of creaming, H is the height of the cream separated from emulsion in mm and H_0 is the initial emulsion height in the container in mm. Thermal creaming was determined by a similar procedure except that the containers were incubated at 80 °C for 20 min, prior to centrifugation (Rahamati et al. 2012).

Rheological measurement

Rheological measurement of mayonnaise samples were carried out at a periodic interval over a storage period of 6 months in a Brookfield Digital Viscometer (M/s Brookfield Engineering Company, MA, USA), model LVDV-E with spindle nos. 3 and 4 at 25 ± 1 °C. The fluid behavior was described by consistency index, which was obtained from Power-law model provided below.

$$\tau = k \times D^n \quad (4)$$

where, τ is shear stress (Pa), k is consistency index ($\text{Pa} \cdot \text{s}^n$), n is flow index and D is shear rate (s^{-1}). Taking logarithms on both sides of Eq. 4, a plot of $\log \tau$ versus $\log D$ was constructed, and the magnitude of K and n were determined from the resulting straight line intercepts and slope respectively.

Determination of phytochemical properties of the mayonnaise samples

Phytochemical properties such as antioxidant activity, total phenolic content and reducing power of the mayonnaise samples were carried out at a periodic interval over a storage period of 6 months using 1 g of mayonnaise diluted in 10 mL methanol, in accordance to the method discussed earlier.

Statistical analyses

All the analyses were conducted at least three times ($n=3$). One-way ANOVA has been carried out to study effect of storage period on the physical and phytochemical properties of mayonnaise samples. Significant differences between means were determined by Duncan's multiple-range test. A p value of 0.05 was used to verify the significance of all tests (Chatterjee and Bhattacharjee 2013). All statistical tests of this experiment were conducted using STATISTICA 8.0 software (Statsoft, Tulsa, OK, USA).

Results and discussion

Characterization of eugenol-lean extracts

The eugenol content and phytochemical properties of eugenol-lean fraction of clove buds have been presented in Table 1. The eugenol content of the eugenol-lean fraction was found to be 2.13 mg/g dry clove buds, which is ~98 % lower than the eugenol content of eugenol-rich fraction (130.01 mg/g dry clove buds) (Chatterjee and Bhattacharjee 2013). This result

Table 1 Phytochemical properties of eugenol-rich and eugenol-lean fraction of clove buds

Phytochemical properties ^a	Eugenol-lean fraction
Eugenol content (mg/g of dry clove buds)	2.13±0.69
IC ₅₀ of DPPH assay (μg/mL)	51.94±0.92
IC ₅₀ of NO assay (μg/mL)	799.01±3.22
Total phenol (mg gallic acid equivalent/g dry clove buds)	148.64±2.71
Total flavonoid (mg quercetin equivalent/g dry clove buds)	0.11±0.01
Reducing power (mg BHT equivalent/g dry clove buds)	31.35±1.17

^a Phytochemical properties of eugenol-rich and eugenol-lean fraction of clove buds are mean ± SD of three independent runs

Eugenol content, IC₅₀ of DPPH assay, IC₅₀ of NO assay, total phenol content, total flavonoid content and reducing power of eugenol-rich fractions of clove buds are 130.01±3.89 mg/g of dry clove buds, 3.20±0.12 μg/mL, 4.26±0.12 μg/mL, 287.76±5.71 mg gallic acid equivalent/g dry clove buds, 0.218±0.01 mg quercetin equivalent/g dry clove buds and 900.94±6.24 mg BHT equivalent/g dry clove buds respectively (Chatterjee et al. 2012)

ensures the selective isolation of a eugenol-lean fraction from clove buds. From phytochemical analyses, it was found that the antioxidant activity, anti-inflammatory activity, total phenolic content, flavonoid content and reducing power of the eugenol-lean fraction were significantly lower ($p=0.0000$) than those of the eugenol-rich fraction from clove buds (Chatterjee and Bhattacharjee 2013). However, the residual phytochemical properties in the eugenol-lean fraction (Table 1) were still appreciable to enhance the nutraceutical potency of mayonnaise.

Sensory evaluation of mayonnaise

9-point hedonic scale scores obtained from sensory evaluation of freshly prepared mayonnaise samples (Figs. 1a–b) have been presented as a radar plot (Fig. 2a). It was observed that the panel could not distinguish between thickness, spoonability and appearance of the mayonnaise samples prepared with eugenol-lean clove extracts and that with mustard and the commercial sample. It was found that all the mayonnaise samples have similar organoleptic properties (body and consistency).

Since, both mustard and eugenol-lean clove extract were added as flavoring agents, the flavor of mustard could not be well perceived in the mayonnaise by the panelists; whereas, the gentle flavor of eugenol-lean clove extract (with no pungency of clove) was perceived unabated in the mayonnaise prepared using the same.

The organoleptic properties of mayonnaise samples evaluated after 6 month storage period were found to be appreciable (Fig. 2b). Although, overall there was no significant difference

Fig. 1 **a** Mayonnaise formulated with eugenol-lean clove fraction. **b** Mayonnaise formulated with mustard

($p=0.0611$) between the mayonnaises formulated with mustard and eugenol-lean clove extract, the latter had slight edge in flavor, thickness, taste and acceptability over the former. However, both these mayonnaise samples were found to have better organoleptic properties than the commercial market sample at the end of storage period.

pH of mayonnaise

The pH values of mayonnaise samples recorded over a storage period of 6 months have been presented in Table 2. It was found that pH of freshly prepared mayonnaise samples were in the range of 3.6 to 4.1, indicating the mayonnaise to be acidic in nature. ANOVA study reveals that pH of all the mayonnaise samples changed insignificantly ($p=0.08203$) during the storage period and the final pH at the end of 6 months was found to be ~4.5, which supposedly prevented the growth of food spoilage microorganisms.

Physical properties of the mayonnaise samples

The color analysis of all the mayonnaise samples was represented by L*, a*, and b*, chroma and hue angles (Table 3). It was found that the commercial market sample was lighter in color (higher L* values) and less yellowish (lower b* values) than mayonnaises prepared with mustard and eugenol-lean clove extract. The latter samples have significantly higher ($p=0.0001$) chroma values, indicating higher color intensities

Fig. 2 **a** Radar plot of hedonic scores of fresh mayonnaise samples. **b** Radar plot of hedonic scores of 6 month stored mayonnaise samples

than the market sample. Mayonnaise prepared with eugenol-lean clove extract is most yellowish with highest chroma values and color intensities, indicating that addition of clove extract improved the color profile of mayonnaise. The hue angle values of all mayonnaise samples ranged

from 80 to 103, indicating that the mayonnaise samples were light yellowish in color. From the storage study, it was found that color parameters of all the mayonnaise samples did not change significantly ($p=0.0611$) and remained stable after 6 months.

Table 2 pH of mayonnaise samples over storage period of 6 months

Storage period (Days)	pH of mayonnaise samples at 25 °C		
	With eugenol-lean clove extract [*]	With mustard [*]	Market sample [*]
0	4.10±0.00 ^a	3.80±0.02 ^a	3.60±0.00 ^a
15	4.11±0.01 ^a	3.91±0.03 ^a	3.65±0.01 ^a
30	4.14±0.01 ^a	4.07±0.01 ^a	3.72±0.01 ^a
60	4.22±0.02 ^a	4.17±0.01 ^a	3.88±0.03 ^a
90	4.30±0.01 ^a	4.29±0.02 ^a	4.09±0.04 ^a
120	4.41±0.02 ^a	4.35±0.01 ^a	4.28±0.04 ^a
150	4.47±0.00 ^a	4.47±0.03 ^a	4.45±0.06 ^a
180	4.50±0.01 ^a	4.52±0.01 ^a	4.49±0.02 ^a

^{*} pH of mayonnaise samples are mean ± SD of three independent samples. Different letters in a column indicate significant difference at $p < 0.05$ level by Duncan's multiple range test

The microstructure of mayonnaise prepared with eugenol-lean clove extract and mustard, observed by optical microscope at 100x magnification are shown in Figs. 3 (a-b). Small fat globules were found to be distributed in both the samples. Eugenol-lean mayonnaise sample had homogenous and compact microstructures, while the mayonnaise formulated with mustard had less homogenous structure with scattered distribution of mustard powder.

The other physical properties of mayonnaise samples such as creaming and rheological measurement of mayonnaise samples have been presented in Tables 4 and 5 respectively. Mayonnaise is an oil in water emulsion. Creaming refers to phase separation owing to the upward rising of fat globules due to the differences in the density between dispersed and continuous phases (McClements 2009). During thermal creaming, addition of heat resulted in accelerated flocculation which caused more creaming. In non-thermal creaming study, it was found that all the mayonnaise samples (with eugenol-lean clove extract, mustard and commercial market sample) had creaming lower than 1 % (Table 4). This indicates that the mayonnaise samples prepared with eugenol-lean clove extract and mustard had shown comparable emulsion properties with the commercial sample. ANOVA study reveals that the non-thermal creaming for all the mayonnaise samples does not change significantly ($p=0.0841$) over a storage period and final creaming is found to be less than 1 %, even at the end of 6 months. However, under thermal conditions, the mayonnaise with eugenol-lean clove fraction produced less cream (36 %) than the mayonnaise with mustard (54 %) and the commercial sample (51.69 %) (Table 4). From the storage study, it was found that thermal creaming of both mustard sample ($p=0.0212$) and commercial sample ($p=0.0135$) increased significantly during the storage period; while for eugenol-lean sample, it increased insignificantly ($p=0.0618$) to 42.21 % until 6 months. Therefore, it could be reasonably

Table 3 Color parameters of mayonnaise samples over 6 month storage period

Storage period (Days)	L*			a*			b*			Chroma			Hue angle		
	With eugenol-lean clove extract ^y	With mustard ^y	Market sample ^y	With eugenol-lean clove extract ^y	With mustard ^y	Market sample ^y	With eugenol-lean clove extract ^y	With mustard ^y	Market sample ^y	With eugenol-lean clove extract ^y	With mustard ^y	Market sample ^y	With eugenol-lean clove extract ^y	With mustard ^y	Market sample ^y
0	83.23±0.00 ^a	83.26±0.00 ^a	88.09±0.00 ^a	2.32±0.00 ^a	2.30±0.00 ^a	-1.40±0.00 ^a	22.91±0.00 ^a	22.81±0.00 ^a	6.96±0.03 ^a	23.03±0.00 ^a	22.93±0.00 ^a	7.10±0.00 ^a	84.21±0.00 ^a	84.24±0.00 ^a	101.37±0.05 ^a
15	83.23±0.00 ^a	83.26±0.00 ^a	88.11±0.00 ^a	2.32±0.00 ^a	2.30±0.00 ^a	-1.41±0.00 ^a	22.91±0.00 ^a	22.81±0.00 ^a	6.90±0.01 ^a	23.03±0.00 ^a	22.93±0.00 ^a	7.04±0.01 ^a	84.21±0.00 ^a	84.24±0.00 ^a	101.56±0.03 ^a
30	83.23±0.00 ^a	83.28±0.00 ^a	88.11±0.00 ^a	2.32±0.00 ^a	2.29±0.00 ^a	-1.41±0.00 ^a	22.91±0.00 ^a	22.75±0.02 ^a	6.89±0.02 ^a	23.02±0.00 ^a	22.86±0.09 ^a	7.03±0.02 ^a	84.21±0.00 ^a	84.25±0.00 ^a	101.58±0.05 ^a
60	83.25±0.00 ^a	83.29±0.01 ^a	88.13±0.01 ^a	2.31±0.00 ^a	2.28±0.00 ^a	-1.43±0.01 ^a	22.85±0.02 ^a	22.71±0.02 ^a	6.83±0.03 ^a	22.97±0.06 ^a	22.82±0.15 ^a	6.98±0.02 ^a	84.23±0.00 ^a	84.27±0.00 ^a	101.82±0.13 ^a
90	83.27±0.01 ^a	83.34±0.01 ^a	88.15±0.01 ^a	2.29±0.01 ^a	2.24±0.02 ^a	-1.44±0.01 ^a	22.78±0.02 ^a	22.55±0.04 ^a	6.77±0.05 ^a	22.89±0.02 ^a	22.66±0.04 ^a	6.92±0.04 ^a	84.26±0.02 ^a	84.32±0.04 ^a	102.01±0.61 ^a
120	83.30±0.01 ^a	83.37±0.01 ^a	88.19±0.02 ^a	2.27±0.01 ^a	2.22±0.01 ^a	-1.47±0.02 ^a	22.68±0.04 ^a	22.45±0.04 ^a	6.63±0.02 ^a	22.79±0.04 ^a	22.56±0.04 ^a	6.79±0.01 ^a	84.28±0.02 ^a	84.35±0.02 ^a	102.50±0.20 ^a
150	83.32±0.01 ^a	83.38±0.01 ^a	88.21±0.01 ^a	2.26±0.01 ^a	2.21±0.01 ^a	-1.49±0.01 ^a	22.62±0.03 ^a	22.42±0.02 ^a	6.57±0.01 ^a	22.73±0.03 ^a	22.53±0.01 ^a	6.74±0.00 ^a	84.29±0.02 ^a	84.37±0.02 ^a	102.78±0.11 ^a
180	83.32±0.01 ^a	83.41±0.02 ^a	88.22±0.00 ^a	2.26±0.00 ^a	2.19±0.01 ^a	-1.50±0.01 ^a	22.62±0.00 ^a	22.32±0.05 ^a	6.54±0.02 ^a	22.73±0.00 ^a	22.43±0.04 ^a	6.71±0.01 ^a	84.29±0.61 ^a	84.39±0.01 ^a	102.92±0.13 ^a

^y L*, a*, b*, Chroma and Hue angle of mayonnaise samples are mean ± SD of three independent samples. Different letters in a column indicate significant difference at $p < 0.05$ level by Duncan's multiple range test

Fig. 3 **a** Optical microscopic image of mayonnaise formulated with eugenol-lean clove fraction at 100x magnification. **b** Optical microscopic image of mayonnaise formulated with mustard at 100x magnification

concluded that addition of clove extract resulted in formation of better emulsion in mayonnaise, which was stable over a wide range of temperature (23 to 80 °C).

The above conclusion was further affirmed by the rheological data which are presented in Table 5. The flow indices of all the mayonnaise samples (both freshly prepared and 6 month stored) were found to be less than 1, establishing shear-thinning pseudoplastic fluid behavior. From the

consistency indices determined by Power-law model, it was found that at 25 °C, the mayonnaise with eugenol-lean clove extract has significantly higher ($p=0.0021$) consistency index than the experimental control and the reference standard, affirming the formation of better emulsion in the clove formulated mayonnaise sample. From the ANOVA study, it was found that consistency indices of both mayonnaise formulated with eugenol-lean extract ($p=0.0944$) and mustard ($p=0.0653$) did not change significantly with storage period, while consistency index of market sample changed significantly ($p=0.0494$) at the end of 6 months. Therefore, from the microstructure, creaming and rheological study, it could be concluded that the addition of eugenol-lean clove extract improved emulsification in the mayonnaise sample. Therefore, the above studies confirmed that the mayonnaise formulated with clove extract had better physical properties than the experimental control and the market sample.

Phytochemical properties of mayonnaise samples

From the phytochemical analyses of freshly prepared mayonnaise samples (Table 6), it was found that the mayonnaise formulated with eugenol-lean clove extract had significantly higher antioxidant activity ($IC_{50}=10.85$ mg/mL) ($p=0.0494$), phenolic content (1.89 mg gallic acid equivalent/g mayonnaise) ($p=0.0068$) and reducing power (11.29 mg BHT equivalent/g mayonnaise) ($p=0.0019$) than mayonnaise sample formulated with mustard and commercial market sample. Both these samples had significantly higher ($p=0.0055$) phytochemical properties than the commercial market sample. From the storage study (Table 6), it was found that antioxidant activity and phytochemical properties tend to decrease after 30 days for reference market sample and 90 days for experimental control sample. However, no such decreasing trend for phytochemical properties was observed for mayonnaise

Table 4 Thermal and non-thermal creaming of mayonnaise samples over 6 month storage period

Storage period (Days)	Thermal creaming fraction (%)			Non-thermal creaming fraction (%)		
	With eugenol-lean clove extract	With mustard	Market sample	With eugenol-lean clove extract	With mustard	Market sample
0	36.36±0.61 ^a	54.55±0.27 ^a	51.69±0.86 ^a	0.85±0.01 ^a	0.92±0.01 ^a	0.89±0.01 ^a
15	36.38±0.88 ^a	54.59±0.79 ^a	52.81±0.72 ^a	0.85±0.02 ^a	0.93±0.01 ^a	0.90±0.01 ^a
30	37.01±0.91 ^{ab}	55.82±0.81 ^a	53.27±0.34 ^a	0.85±0.02 ^a	0.94±0.02 ^a	0.91±0.02 ^a
60	39.11±0.93 ^{ab}	58.71±0.98 ^{ab}	56.76±1.79 ^{ab}	0.85±0.01 ^a	0.94±0.01 ^a	0.91±0.02 ^a
90	40.02±0.58 ^{ab}	61.88±1.07 ^{bc}	59.13±1.91 ^{bc}	0.86±0.01 ^a	0.95±0.02 ^a	0.92±0.02 ^a
120	40.98±0.64 ^{ab}	64.23±1.11 ^{cd}	62.87±1.75 ^{cd}	0.87±0.02 ^a	0.96±0.02 ^a	0.92±0.01 ^a
150	41.56±0.87 ^{ab}	67.11±1.05 ^{de}	65.48±1.28 ^{de}	0.88±0.01 ^a	0.98±0.01 ^a	0.92±0.01 ^a
180	42.21±0.51 ^b	71.07±1.67 ^e	69.58±2.01 ^e	0.88±0.01 ^a	0.98±0.01 ^a	0.94±0.02 ^a

* Thermal and non-thermal creaming of mayonnaise samples are mean ± SD of three independent samples

Different letters in a column indicate significant difference at $p < 0.05$ level by Duncan's multiple range test

Table 5 Flow parameters of mayonnaise samples over 6 month storage period

Storage period (Days)	k (Pa·s) ^a			n ^a		
	With eugenol-lean clove extract	With mustard	Market sample	With eugenol-lean clove extract	With mustard	Market sample
0	1.44±0.00 ^a	0.96±0.00 ^a	0.93±0.00 ^a	0.25±0.00 ^a	0.28±0.00 ^a	0.80±0.00 ^a
15	1.44±0.00 ^a	0.95±0.00 ^a	0.93±0.00 ^a	0.25±0.00 ^a	0.28±0.00 ^a	0.80±0.00 ^a
30	1.44±0.00 ^a	0.95±0.00 ^a	0.92±0.00 ^a	0.25±0.00 ^a	0.28±0.00 ^a	0.80±0.00 ^a
60	1.43±0.00 ^a	0.95±0.00 ^a	0.91±0.00 ^a	0.25±0.00 ^a	0.28±0.00 ^a	0.80±0.00 ^a
90	1.42±0.00 ^a	0.93±0.00 ^a	0.89±0.01 ^a	0.25±0.00 ^a	0.28±0.00 ^a	0.79±0.00 ^a
120	1.41±0.01 ^a	0.93±0.00 ^a	0.88±0.01 ^a	0.25±0.00 ^a	0.28±0.00 ^a	0.79±0.00 ^a
150	1.39±0.01 ^a	0.92±0.01 ^a	0.86±0.01 ^a	0.26±0.00 ^a	0.29±0.00 ^a	0.78±0.00 ^a
180	1.38±0.01 ^a	0.91±0.01 ^a	0.85±0.01 ^a	0.26±0.00 ^a	0.30±0.00 ^a	0.78±0.00 ^a

^a Consistency index and flow index of mayonnaise samples are mean ± SD of three independent samples

Different letters in a column indicate significant difference at $p < 0.05$ level by Duncan's multiple range test

formulated with eugenol-lean clove extract, even at the end of storage period. ANOVA study reveals that the phytochemical properties of both market sample ($p=0.0000$) and experimental control sample ($p=0.0011$) decreased significantly with storage period, while mayonnaise with eugenol-lean clove extract was stable even until 6 months of storage with insignificant decrease in phytochemical properties ($p=0.0914$). Therefore, it could be concluded that the addition of eugenol-lean clove extract enhances nutraceutical properties and provided '90 days lead' to the shelf-life of standard mayonnaise.

Conclusions

Eugenol-lean fraction of clove buds was obtained by combined extraction methodology of steam distillation, followed by SC-CO₂ extraction. Successful application of eugenol-lean

fraction of clove buds was carried out in mayonnaise as a replacement of mustard. Sensorically, this sample was found to be comparable with classical mayonnaise formulated with mustard and with commercial market sample and all the mayonnaise samples were found to retain appreciable organoleptic properties until 6 months. Mayonnaise formulated with eugenol-lean clove extract had improved physical properties such as higher color intensity, lower thermal and non-thermal creaming, homogenous and compact microstructure and higher consistency index, vis-à-vis the experimental control and commercial market samples. These physical properties did not change significantly during the storage period. From phytochemical analyses, it was found that mayonnaise formulated with eugenol-lean clove extract had significantly higher antioxidant activity, phenolic content and reducing power than the mayonnaise sample formulated with mustard and the market sample. Storage study also established that

Table 6 Phytochemical properties of mayonnaise samples over storage period of 6 months

Storage period (Days)	IC ₅₀ values of DPPH radical scavenging assay (μg ml ⁻¹) [*]			Total phenolic content (mg gallic acid equivalent g ⁻¹ mayonnaise) [*]			Reducing power (mg BHT equivalent g ⁻¹ mayonnaise) [*]		
	With eugenol-lean clove extract	With mustard	Market sample	With eugenol-lean clove extract	With mustard	Market sample	With eugenol-lean clove extract	With mustard	Market sample
0	10.85±1.05 ^a	11.86±1.21 ^a	29.69±1.63 ^a	1.89±0.01 ^a	1.63±0.04 ^a	0.46±0.01 ^a	11.29±0.09 ^a	9.31±0.08 ^a	6.67±0.03 ^a
15	16.22±1.26 ^b	24.75±2.11 ^b	76.96±2.76 ^b	1.81±0.01 ^a	1.56±0.03 ^a	0.42±0.02 ^a	11.14±0.06 ^a	6.31±0.09 ^{ab}	6.45±0.02 ^a
30	18.42±1.78 ^{bc}	32.69±2.56 ^c	136.20±4.17 ^c	1.44±0.09 ^a	1.36±0.03 ^a	0.31±0.01 ^a	11.07±0.07 ^a	5.65±0.09 ^{abc}	6.31±0.02 ^a
60	19.31±1.51 ^{bc}	40.01±3.17 ^d	187.59±4.23 ^d	1.32±0.08 ^a	1.12±0.09 ^a	0.19±0.01 ^a	10.32±0.09 ^a	4.18±0.08 ^{bcd}	6.01±0.04 ^a
90	22.07±2.01 ^{cd}	55.17±4.11 ^e	244.61±5.79 ^e	1.18±0.09 ^a	0.99±0.01 ^a	0.08±0.00 ^a	9.11±0.08 ^a	3.22±0.04 ^{bcd}	5.25±0.08 ^a
120	25.61±2.31 ^{de}	78.21±4.45 ^f	320.18±7.21 ^f	1.07±0.01 ^a	0.67±0.01 ^a	0.01±0.00 ^a	8.52±0.06 ^a	1.56±0.03 ^{bcd}	5.11±0.06 ^a
150	27.29±2.89 ^e	97.62±3.76 ^g	410.22±7.58 ^g	1.03±0.02 ^a	0.32±0.02 ^a	0.01±0.00 ^a	7.98±0.05 ^a	0.45±0.02 ^{cd}	4.76±0.09 ^a
180	29.98±1.37 ^e	111.31±3.19 ^h	501.08±6.29 ^h	0.98±0.01 ^a	0.24±0.01 ^a	0.01±0.00 ^a	7.41±0.07 ^a	0.18±0.01 ^d	4.22±0.11 ^a

^{*} IC₅₀ of DPPH radical scavenging assay, total phenolic content and reducing power of mayonnaise samples are mean ± SD of three independent samples

Different letters in a column indicate significant difference at $p < 0.05$ level by Duncan's multiple range test

addition of eugenol-lean clove extract to mayonnaise provided it a lead of 90 days compared to the mustard formulated sample. Thus, mayonnaise prepared with eugenol-lean clove extract was enhanced nutraceutically owing to its appreciable antioxidant activity and phytochemical potency.

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