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GC-MS Analysis of Clove Essential Oil (*Syzygium aromaticum* L.) as Effective Source of Biomolecules for Traditional Tomato Puree Quality Improvement

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Abstract

The high-water content of tomato predisposes it to spoilage by microorganisms and led producers to carry out traditional processing of tomatoes in the form of pasteurized tomato puree. However, rapid acidification of these traditional tomato purees is often observed. Then, the aim of this study was to evaluate the efficacy of the essential oil extracted from Clove (*Syzygium aromaticum* L.) in the improvement of traditional tomato puree producing technology. Essential oil of Clove (*Syzygium aromaticum* L.) was extracted by hydrodistillation and its chemical composition was determined by GC and GC/MS. Different types of traditional tomato puree were produced by the modification of the traditional processing technology and the addition of the essential oil introduction step, followed by manual stirring during the process. Based on previous studies, two different essential oil concentrations (5.0 and 7.5 $\mu L \cdot g^{-1}$) were investigated. Physicochemical, microbiological and nutritional analyzes were performed in order to evaluate the quality of the traditional tomato puree produced. Results obtained revealed that the essential oil of *Syzygium aromaticum* investigated has a chemical composition characterized by the presence of eugenol (59.11%) and eugenol acetate (33.73%). Good stabilization of the physicochemical, microbiological and nutritional parameters in traditional tomato puree samples preserved with essential oil of *Syzygium aromaticum* were observed when compared to control. The essential oil of Clove, with his biological property, offers a novel approach to the management of traditional tomato puree during storage.

Keywords

Essential Oil, *Syzygium aromaticum*, Tomato Puree Process, Quality, Benin

1. Introduction

According to FAO [1], food security is effectively achieved when the four pillars of food, namely food availability, access, utilization and stability, provide physical and economic access at all times to affordable, safe and nutritious food to meet the needs of an active and healthy life. When any of these four pillars is weakened, the food security of a society is totally compromised, affecting not only human health and well-being, but also the social, economic and political aspects of the society.

In Africa, the losses of agricultural products are estimated on average at 25% for cereals and more than 50% for fruits, vegetables and tubers [2], and access by smallholder farmers to effective and affordable post-harvest conservation techniques is becoming a development priority [3].

Tomato is a crop of great economic and nutritional importance, and approximately one hundred and thirty million tons are produced each year worldwide [4]. In Benin, tomatoes are grown throughout the country depending on the seasons, with an average annual production of 37,648 ha [5]. However, the low valuation and the lack of modern processing technology led producers to carry out traditional processing of tomatoes in the form of pasteurized tomato puree. Unfortunately, rapid acidification of traditional tomato purees is often observed, resulting in post-harvest losses estimated at between 40% and 60% [6].

For several years, the use of chemical synthesis products has been the most widely used technique to combat microbial spoilage of food products [7]. However, the extensive and indiscriminate use of these chemicals has caused contamination of the biosphere and the food chain, eradication of non-target species and the emergence of resistant microorganisms. Their adverse effects on human health range from acute poisoning to chronic diseases including various types of cancer [8].

The increasing consumer demand for natural alternatives to synthetic preservatives in food has therefore made essential oils a natural substitute due to their antioxidant, antibacterial and antifungal properties [9]. Clove (*Syzygium aromaticum* L.) is an aromatic plant widely cultivated in tropical and subtropical countries. Clove essential oil has attracted considerable interest due to its wide application in the perfumery, cosmetics, health, medicine, flavors and food industries.

In Benin, clove is used in culinary preparations. Thus, the present study aims to investigate the efficacy of clove (*Syzygium aromaticum* L.) essential oil in improving the production technology, as well as the quality of the traditional tomato purees produced.

2. Material and Methods

2.1. Plant Materials Collection and Essential Oil Extraction

Plant materials used for essential oil extraction were fresh flower buds of clove (Figure 1), collected at Abomey-Calavi (southern Benin) and identified at the Benin national herbarium, where voucher specimens are deposited. The essential oil was extracted by the hydro-distillation method using Clevenger-type apparatus.

Figure 1. Clove (*Syzigium aromaticum* L.) used for essential oil extraction.

2.2. Gas Chromatography-Mass Spectrometry Analysis

Chemical composition of essential oil from fresh flower buds of clove were determined by gas-chromatography/mass-spectrometry (GC/MS), using An Agilent GC system 7890B (Agilent, Santa Clara, CA, USA) equipped with an Agilent MSD 5977B detector and a split/splitless injector was used. The sources and quadrupole temperatures were fixed at 230˚C and 150˚C, respectively. The carrier gas was helium at a flow rate of 1.2 mL/min. The injection volume is one µL of the essential oil dilution (0.01% in hexane; w/v) in splitless mode. The column temperature was maintained one min at 50˚C, and then increased at a rate of 5˚C/min until 300˚C. The identification of individual compounds is based on their retention times, retention indices relative to C5-C18 n-alkanes, and matching spectral peaks available in the published data [10].

2.3. Production of Traditional Tomato Purees

Ripe tomato fruits from local variety called "Tounvi", collected in the locality of Kpomassè (Southern Benin) are sorted, washed, seeded and then crushed in the millstones to obtain traditional tomato puree. Traditional tomato puree thus obtained are then separated into three (3) parts labelled respectively A, B and C. The two parts of the traditional tomato puree (A, B) are treated with the essential oil of *Syzygium aromaticum* at doses of 5.0 and 7.5 μL·g^{-1}. These doses were chosen because of the strong fragrance of the essential oil. The third part of traditional tomato puree samples (C) are not treated with the essential oil, but was pasteurized at 80˚C during 15 mn, bottled, soaking in boiling water during 10 mn, and is therefore considered as a control. Figure 2 indicated the technological diagram used for traditional tomato puree production. Traditional tomato puree thus produced were observed at room temperature (28˚C ± 2˚C) for 30 days and their physicochemical and microbiological characteristics were then determined.

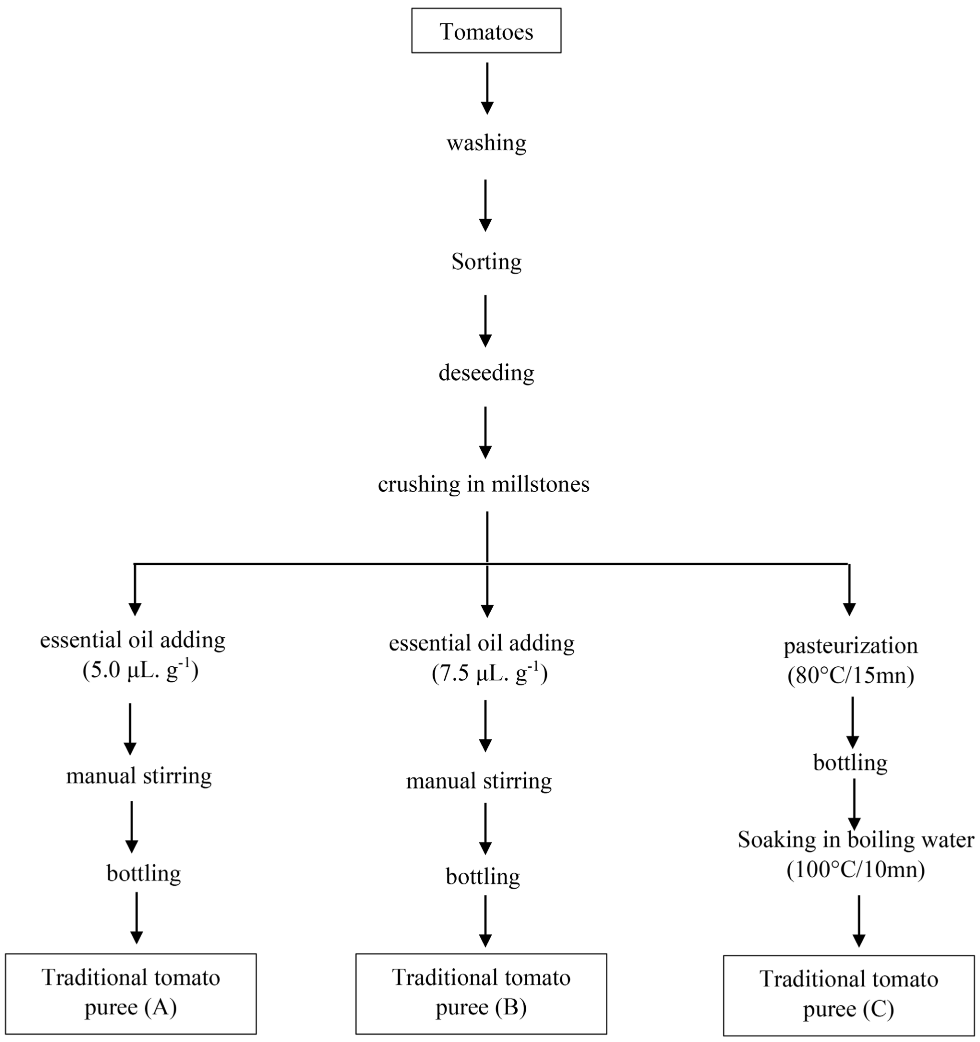

Figure 2. Process diagram of different traditional tomato puree production.

2.4. Physicochemical Analysis

The pH was determined with a digital pH-meter (HANNA HI 98129). Acidity of traditional tomato puree samples was determined by titration with 0.01 mol/L of sodium hydroxide solution, using phenolphthalein as indicator [11]. Brix was determined using a refractometre type Novex Holland. Lycopene content of traditional tomato puree samples was determined according to the method described by Ringeisen *et al.* [12]. *β*-carotene content was determined by the method described by Ishiwu *et al*. [13].

2.5. Microbiological Analysis

Microbiological analysis of traditional tomato puree samples was performed as follow: To 25 g of each sample, 225 ml of peptone water was added and homogenized. Appropriate decimal dilutions were prepared and aliquots were plated in duplicates on various media. For the total bacterial count, Plate count agar medium was used and plates were incubated at 30˚C for 72 h. Total coliforms count in samples were determined using Desoxycholate agar and plates were incubated at 30˚C for 24 h. Desoxycholate agar was also used for the faecal coliforms count, and plates were incubated at 44˚C. The identification of faecal coliforms was made using EMB (Eosine Methylene blue). For performed *Staphylococcus aureus* count, standard NF EN ISO 6888-1 was used. The method used for detection of *Salmonella spp*. is that specified by the standard NF V 08-052. For the detection of yeast and fungi in samples, plating method was performed. Indeed, 10 g of each sample were separately added to 90 ml of sterile water containing 0.1% peptone water, and thoroughly mixed to obtain the 10^{-1} dilution. Further tenfold serial dilutions up to 10^{-4} were made. One milliliter of each dilution was separately placed in Petri dishes, over which 15 ml of Sabouraud media with 60 μg/ml of chloramphenicol was poured. The plates were incubated at 28˚C ± 2˚C for 7 days [14]. A colony counter was used after incubation, to track the number of microbial colonies.

2.6. Statistical Analyses

Statistical Analysis Software (SAS) and SYSTAT 5.05 were used to analyze data generated from these studies. The statistical analyses carried out were mean and standard deviation, and analysis of variance (ANOVA) [15].

3. Results and Discussion

The yield of essential oil of *Syzygium aromaticum* was 14.2%, (v/w) during hydrodistillation, and oil was yellow in colour. Chemical analysis by GC/MS of the components of the oil led to identification of six components (**Table 1**) representing 96.9%. The major components of the *Syzygium aromaticum* oil were eugenol (59.11%) and eugenol acetate (33.73%). **Table 2** presented the results of the physicochemical characteristics of the different groups of traditional tomato puree produced. These results indicated that the pH of traditional tomato puree produced was between 2.06 ± 0.04 and 4.38 ± 0.02, with an acidity varying from 3.21 ± 0.03% and 4.76 ± 0.11%, and a Brix varying from 11.8˚Bx and 24.8˚Bx. The results obtained during the evaluation of the nutritional characteristics of the different groups of traditional tomato puree produced (**Table 3**) indicated that the high contents of *β*-carotene and Lycopene in the traditional tomato purees are

1.78 ± 0.01 µg/100g and 27.16 ± 0.04 µg/100g respectively. Statistical analyzes revealed that there is no significant difference ($p < 0.05$) between samples preserved using essential oil at concentrations of 5.0 µl/g and 7.5 µl/g. However, there are significant differences ($p < 0.05$) in physicochemical and nutritional contents of traditional tomato puree samples preserved using essential oil, when compared to the control. These results therefore indicated the influence of the addition of *Syzygium aromaticum* essential oil on the physicochemical and nutritional parameters of traditional tomato purees. Results obtained during the evaluation of the microbiological characteristics of the different group of traditional tomato puree (Table 4) indicated that the total bacteria count in analyzed sampled was between 1.0×10^1 ufc/g and 9.0×10^3 ufc/g. Total coliform, feacal coliform, *Staphylococcus aureus*, yeast and mould are also detected, with the absence of *Salmonella spp* in all analyzed sampled. Statistical analyses indicated that there is significant difference ($p < 0.05$) between microbiological characteristic of samples produced by addition of essential oil of *Syzygium aromaticum*, when compared to the control.

Table 1. Chemical composition of the essential oil of *Syzygium aromaticum* investigated.

Components	Kovats index (KI)	Percentage (%)
Eugenol	**19.09**	**59.11**
Caryophyllene	20.304	5.51
Alpha-humulene	20.994	0.79
Eugenol Acetate	**22.561**	**33.73**
Caryophyllene Oxide	23.647	0.54
Caryophylle-4(12),8(13)-dien-5-beta-ol	24.637	0.31

Table 2. Physicochemical characteristics of different types of traditional tomato puree investigated.

Traditional tomato purees	pH	Acidity (%)	˚Brix
A	4.31 ± 0.07a	3.21 ± 0.03a	24.7a
B	4.38 ± 0.02a	3.26 ± 0.06a	24.8a
C (Control)	2.06 ± 0.04b	4.76 ± 0.11b	11.8b

Values are mean (n = 3) ± SE. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests.

Table 3. Nutritional characteristics of different types of traditional tomato puree investigated.

Traditional tomato purees	*Β*-carotene	Lycopene
A	1.72 ± 0.04a	27.13 ± 0.08a
B	1.78 ± 0.01a	27.16 ± 0.06a
C (Control)	0.91 ± 0.07b	6.16 ± 0.11b

Values are mean (n = 3) ± SE. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests.

Table 4. Microbiological characteristics (UFC/g) of different types of traditional tomato puree investigated.

Traditional tomato purees	Total bacteria count	Total coliform count	Faecal coliform count	*Staphylococcus aureus count*	*Salmonella spp*	Yeast and mold count
A	1.2×10^1	<10	<10	Absence	Absence	<10
B	1.0×10^1	<10	<10	Absence	Absence	<10
C (Control)	9.0×10^3	3.0×10^2	1.6×10^1	7.0×10^1	Absence	5.7×10^1

In Benin, the efforts of industrial-scale tomato processing initiated by the government, with the installation in Natitingou (northern Benin) of a tomato concentrate production plant having failed, and small-scale tomato processing remains the probable outcome [16]. Thus, according to Dossou *et al.* [17], it remains only to produce scientific knowledge to support this objective. In this context, results obtained during this study, focused on the conservation trials of traditional tomato purees using essential oil of *Syzygium aromaticum*, indicated an acceleration of microbial activity in control samples, characterized by a decrease of the pH, Brix degree, carotenoid content, as well as an increase of the acidity of control samples. This fermentation observed in the control tomato puree samples not only leads to changes in their physicochemical, biochemical and nutritional characteristics, but also their acceptability by the consumer, who prefers low-acidity tomato purees, depending on the needs for culinary preparations.

However, the results obtained in the tomato puree samples preserved using essential oil, indicated stability in the physicochemical and nutritional parameters; which could be due to the absence of fermentative activity of the microorganisms, confirmed by the results of the microbiological analysis in the samples of traditional tomato purees produced.

As reported by Nogueira *et al.* [18], the antimicrobial potential of this essential oil could also be due to its ability to cross the cell wall, and to damage the cellular enzymatic system, including that related to energy production. Similarly, thinning of hyphae of fungi cultured in the presence of essential oil was also reported by de Billerbeck *et al.* [19]. Irreversible damage to the cell wall, cell membrane and organelles of fungi cultured in the presence of Thymus essential oil was also reported by Rasooli and Owlia [20].

Tang *et al.* [21] also explored the antibacterial mechanism of essential oils against methicillin-resistant *Staphylococcus aureus* (MRSA), and reported that *Amomum villosum* essential oil caused significant damage to the cell membrane, that resulted in loss of membrane integrity, leading to leakage of intracellular macromolecular substances, protein inhibition, and biofilm synthesis.

However, given the large number of different groups of chemical compounds present in essentials oils, it is highly likely that its antimicrobial properties are not attributable to a specific mechanism, but to a synergy action on multiple targets in the cell [22]. Several research have reported the antimicrobial effect exerted by eugenol presented in **Figure 3** [23], on main foodborne pathogens. Indeed, Mith *et al.* [24] evaluated the antimicrobial activity of commercial eugenol (Sigma-

Aldrich) against foodborne bacteria, and reported that *Listeria monocytogenes* and *Escherichia coli* O157:H7 were inhibited with a concentration of 0.5 µl/ml of commercial eugenol, while *Salmonella thyphimurium* was inhibited with a concentration of 1 µl/ml of commercial eugenol. Abbaszadeh *et al.* [25] indicated that the fungal strains most affected by eugenol were *Aspergillus ochraceus*, *Cladosporium spp*., *Penicillium citrinum* and *Rhizopus oryzae*. Simovic *et al.* [26], focused on the in vitro antifungal activity of eugenol against fungi foodborne pathogenic such as *Aspergillus carbonarius* and *Penicillium roqueforti*, indicated that the minimal inhibitory concentration (MIC) of eugenol against *A. carbonarius* and *P. roqueforti* were 2 µl/ml and 1 µl/ml respectively. They therefore also concluded that eugenol could be a natural alternative for the food preservation.

The presence of eugenol in high proportion in the essential oil of *Syzygium aromaricum* could therefore justify its efficacy in the preservation of traditional tomato puree. Ju *et al.* [27], reported that presence of eugenol gives essential oils, a broad-spectrum activity against food spoilage, but also against foodborne pathogenic microorganisms. According to these authors, its addition can extend the shelf life without affecting the original taste, flavor, texture, appearance or sensory acceptability.

These results therefore indicate that the essential oil of *Syzygium aromaticum* used in the present study, contains a valuable phytochemical constituent. However, according to Miladinović *et al.* [28], it would also be interesting to specifically investigate the role that the minor compounds of the essential oil could also play in its antimicrobial potential.

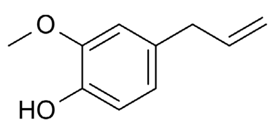

Figure 3. Molecular structure of eugenol.

4. Conclusion

This study underlined the nutritional potential of traditional tomato puree and the importance of the use of essential oil of *Syzigium aromaticum* in the improvement of the microbiological quality of this traditional product. Nevertheless, due to the possible interaction between the composition of traditional tomato puree and the essential oil, further investigations are necessary to identify the conditions that maximize the activity of the essential oil, without detrimental effects on the organoleptic properties of the food product.

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Conflicts of Interest

There is no conflict of interest in the study.

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