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Extension of the Shelf Life of Attieke (Ivorian Traditional Food) by Essential Oils Incorporation

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ABSTRACT

To study the ability of various essential oils to inhibit spoilage bacteria naturally present in Ivorian traditional food, namely « Attiéké », six different essential oils were tested separately than by pair on the Attiéké matrix storage at 4°C. The results of the investigation showed that only two of the six essential oils were effective in inhibiting of the spoilage flora. Savory and oregano essential oils were the most active separately or together against the spoilage bacteria. In Attiéké matrix, treated with Savory or oregano essential oil, undesirable bacteria counts have declined from 10^9 CFU g^{-1} to 10^6 CFU g^{-1} during the first week of storage before regrowth to reach 10^8 CFU g^{-1} at the end of the experiment. While in those no treated (control) or treated with the other four cloves, coriander, thyme and rosemary essential oil, the CFU count of spoilage bacteria increased from 10^9 CFU g^{-1} to 10^{12} CFU g^{-1} during the first week of storage to reach 10^{15} CFU g^{-1} at the end of the experiment (six weeks). The combination of oregano with savory essential oil allowed a strong decline reaching 4 CFU g^{-1} at the third week before increasing again. It is therefore resulted in a 3-week delay of the growth rebound compared with samples treated with them separately (1-week delay).The addition of oregano or savory essential oil exhibited a synergistic effect to control undesirable bacteria in Attiéké matrix at 4°C. The combination of oregano with savory essential oil may be effectively used in our traditional Attiéké food to enhance its safety and stability.

1. INTRODUCTION

The attiéké is one of the major cassava transformation products developed by Ivorian women for years. It is a fermented food whose preparation requires the use of the fermented cassava (Assanvo, 2002). This traditional Ivorian food plays a vital and strategic role in food safety, it is also exported to Europe and other continents in his dehydrated form (Aboua, 1989). Indeed, Attiéké's manufacturing process combines ferment called "magnan" in Ebrié language or "lidjrou" in Adjoukrou language. It is the main source of microorganisms involved in the early stages of manufacturing (Assanvo et al. 2000). It is certainly from this microbiological niche that provides the strains responsible for Attiéké's alteration. Also, environmental contamination or poor hygiene practice are considered to be another source leading to contamination in this kind of traditional foods.

Improve the safety of this product is a major challenge for the Ivorian authorities. Until now, approaches to seek improved food safety have relied on the search for more efficient chemical preservatives or on the application of more drastic physical treatments (e.g. high temperatures). Nevertheless, these types of solutions have many drawbacks: the proven toxicity of many of the commonest chemical preservatives (e.g. nitrites), the alteration of the organoleptic and nutritional properties of foods, and especially recent consumer trends in purchasing and consumption, with demands for safe but minimally processed products without additives. To harmonize consumer demands with the necessary safety standards, traditional means of controlling microbial spoilage and safety hazards in foods are being replaced by innovative technologies that include biological antimicrobial systems such as essential oils.

An empirical application of various aromatic plants in food preservation has long been known; however, the role of the essential oil fractions of these plants in the inhibition of spoilage and pathogenic micro-organisms has been scientifically demonstrated only by the middle of the last century (Boyle 1955). At present, the antimicrobial activity of essential oils is well established, and their potential as biological adjuncts in food preservation is attracting increased research interest. The multiple hurdles technology has been established as an efficient means to improve the safety and keeping the quality of foods (Leistner & Gould, 2012). This approach is even more attractive that it advocates the combination of natural antimicrobial compounds with

conventional food preservation treatments to minimize the use of chemical additives in response to the growing consumers' demand with respect to their perception of ‘natural foods'.

The effect of various combinations of essential oils or their active constituents with other antimicrobial substances has been extensively investigated (Bassolé et al., 2012; Hyldgaard et al., 2012). A synergetic action between essential oils or their constituents and bacteriocins such as nisin to inhibit spoilage and pathogenic micro-organisms has been demonstrated in vitro (Calo et al. 2015; Nazzaro et al., 2013., Böhme et al., 2014., Moghadam et al., 2016). However, few studies to our knowledge have been carried out on such a synergy in food systems. The present study aimed to investigate the antimicrobial activity of severals essential oils among those commonly used in food industry against Attiéké’s spoilage bacteria by in vitro tests.

MATERIALS AND METHODS

Essential oils

Essential oils used in this study are derived from clove, coriander, oregano, savory, thyme and rosemary. They were obtained from PranaromTM (Horrues, Belgium) and stored at room temperature, protected from light and air.

Antibacterial activity of different essential oils against selected gram-positive and gram-negative foodborne pathogens

Antimicrobial activity of essential oils against *L. monocytogenes* M, *E. coli* ATCC 10536 and *S. Typhi* CWBI-H1 was tested by the good diffusion assay (Tagg and McGiven 1971). Wells (~6 mm in diameter) were punched in plates of M17 agar (Oxoid) seeded with 0.1 ml of an active culture of each indicator strain and filled with 5 µl of each of the essential oils. The plates were then incubated at 37°C for 16–18 h and the diameters of the inhibition zones surrounding the wells were measured.

Determination of minimal bactericidal concentrations against *Listeria monocytogenes*

The minimal bactericidal concentrations (MBC) of two essential oils (i.e. savory and oregano) that gave the largest diameter of inhibition zones against *L. monocytogenes* M by the good diffusion assay (Table 1) were determined in liquid media as described by Wan et al. (1998). In a

series of test tubes each containing 10 ml of MRS broth, savory or oregano essential oil was added to different concentrations ranging from 0 to 5.0 µl ml^{-1}.

Tubes were then inoculated with 100 µl of an overnight culture of *L. monocytogenes* M and incubated aerobically at 37°C for 48 h and under agitation (120 rev min^{-1}) to keep the medium homogeneous. A 1-ml sample was withdrawn at 0 h and at 48 h, to determine the numbers of *L. monocytogenes* on PALCAM agar after incubation at 37°C for 48 h. The MBC was defined as the lowest concentration required for complete inhibition of the test organism after 48 h of incubation in the presence of the essential oil.

Antibacterial activity of oregano or savory essential oil in Attiéké

Two independent trials were conducted to study the effectiveness of the oregano or savory essential oils to control spoilage bacteria in Attiéké. In each trial, an Attiéké block of 200 g was divided aseptically into portions of approximately 50 g each to make four different batches (B1 to B4). The batches were placed individually in sterile aluminum foil under a laminar flow hood (Clean Air, VWR, Belgium) for subsequent treatments. The essential oil (oregano or savory) was added to batches B2 and B3, when (oregano + savory) was added to batch B4 to a final concentration of 50 µl 100 g^{-1} of Attiéké. The batch B1 was not treated and served as a negative control. For practical reasons (i.e. low volume of the essential oil to be applied), the essential oil was mixed with attiéké manually. All samples were placed in separate sterile plastic bags, which were then heat-sealed and held at room temperature during the course of the experiments.

Microbiological analyses and arbitrary unit determinations

Samples (15 g) were aseptically taken at 0 h weekly from each batch for bacteria enumerations. The samples were placed in sterile plastic bags containing 180 ml of sterile saline solution (0.85% NaCl) and thoroughly homogenized by hand massaging and shaking for 1 min. Suspensions were then serially diluted and a volume of 0.1 ml from each dilution was spread-plated in duplicate onto MRS agar (Oxoid). The CFU of spoilage bacteria were determined after incubation at 37°C for 24h.

Statistical analysis

Each trial was repeated twice and each determination was performed in duplicate. Statistical analysis was made by analysis of variance $\alpha = 0.05\%$ and Student's t-test

RESULTS

Antibacterial activity of various essential oils

Table 1 summarizes the results of the antibacterial activity of the selected essential oils against *L. monocytogenes* M, *E. coli* ATCC 10536 and *S. Typhi* CWBI-H1.

Table 1. Diameter of the zones of inhibition (mm ± SD)* of lyophilized cell adsorbed bacteriocin (LCAB) of *Lactobacillus curvatus* CWBI-B28 and selected essential oils (EO) against *Listeria monocytogenes* M, *Salmonella* serotype Typhi and *Escherichia coli* strains

Antimicrobial agent agent	*L. monocytogenes* M	*S. Typhi* CWBI-H1	*E. coli* ATCC10536
Coriander EO	7 ± 1	14 ± 0	7 ± 0
Savory EO	23 ± 1	23 ± 0	20 ± 1
Clove EO	10 ± 1	13 ± 0	9 ± 0
Oregano EO	23 ± 0	21 ± 0	21 ± 1
Rosemary EO	NI	9 ± 1	10 ± 2
Thyme EO	10 ± 0	11 ± 2	13 ± 1

*Results include the diameter of the well (6 mm).

- No inhibition.

As expected, different performances of the essential oils were recorded depending on the bacterial strain. The antibacterial activity of coriander, clove, rosemary and thyme varied greatly depending on the indicator strain, but their antibacterial activity was weak. Oregano and savory essential oils had the highest inhibitory activity against all bacteria tested and were, therefore, retained for the next experiments. The MBC of each of the latter essential oils was determined against *L. monocytogenes* which was completely inactivated after 48 h of incubation in presence

of savory or oregano essential oil at concentrations ranging from 0.5 to 5.0 µl ml^{-1}. In contrast, the pathogen grew well in the positive control (i.e. no added essential oil) to reach $1.7x10^7$ CFU ml^{-1} after 48 h of incubation. The level of 0.5 µl ml^{-1} was, therefore, considered as the MBC for both savory and oregano essential oils.

Antibacterial activity of essential oil in Attiéké matrix

The results of the antibacterial activity of the oregano or savory essential oil during cold storage of Attiéké food are summarized in Table 2

Table 2 Enumeration of spoilage bacteria as function of time in Attiéké treated with oregano essential oil (OEO) or/and savory essential oil (SEO)

Time (weeks)	log10 CFU g-1 ± SD			
	Control	OEO	SEO	SEO + OEO
0	9.1± 0.2	9.2± 0.12	9.4± 0.15	9.1± 0.21
1	12.2± 0.18	6.1± 0.02	6.5± 0.08	6.0± 0.2
2	13.3± 0.15	6.8± 0.3	7.0± 0.2	5.1± 0.12
3	13.9± 0.2	7.1± 0.1	7.5± 0.16	4.5± 0.2
4	14.1± 0.32	7.5± 0.3	7.9± 0.32	5.5± 0.3
5	14.7± 0.2	8.1± 0.16	8.0± 0.2	6.1± 0.22
6	15.1± 0.15	8.5± 0.15	8.1± 0.16	6.7± 0.32

A significant ($P< 0.05$) reduction in undesirable bacteria counts was observed in the first week of storage in all test samples as compared with the positive control (i.e. no added essential oil). However, the extent of this inhibition and the subsequent protection of the Attiéké product from the growth of spoilage bacteria varied according to the treatment applied. In batches treated with oregano or savory or both essential oil the counts of spoilage bacteria have declined from 9 to 6 and 4 in a 1-g sample during the first and third week of storage. While in nontreated samples, the growth of spoilage bacteria was observed throughout the experience (Table 2). In all test samples, an increase in spoilage bacteria counts was noted after they have reached their lowest values. Such an increase (i.e. rebound phenomenon) was observed a week earlier in nontreated

samples compared with those treated with oregano or savory essential oil alone or with the combination of both essential oils where rebound phenomenon was observed at the second and the fourth week respectively.

DISCUSSION

In vitro, antimicrobial activity assays showed that the six different essential oils inhibited various bacterial strains of health and spoilage significance. The essential oils inhibited the indicator strains to various degrees depending on the essential oil and the bacterial strain (Table 1). No evidence for the difference in sensitivity among gram-positive and gram-negative bacteria to the essential oils could be noted in agreement with earlier reports (Hyldgaard et al., 2012; Nazzaro et al. 2013; Prakash et al. 2015). In contrast, other studies suggested that essential oils are generally more active against gram-positive than gram-negative bacteria (Cherrat et al. 2014; Langeveld et al. 2014). The high inconsistencies in the chemical composition of an essential oil depending on physiological and ecological conditions of the producing plants (Prakash et al. 2015) may explain the variability in the susceptibility of sensitive strains regardless of their Gram staining. Yet, essential oils containing high levels of carvacrol were reported to be the most inhibitory to microbial growth (Lang & Buchbauer 2012). This is consistent with the fact that oregano and savory essential oils had the highest antimicrobial activity against all bacteria tested in this study, as carvacrol represents 80% (Kordali et al., 2008) and 57% (Weerakkody et al., 2010) of these essential oils, respectively. Determinations of the MBC of savory and oregano essential oils showed that low levels of these essential oils effectively inactivate sensitive bacteria. In vitro bactericidal effect of essential oils at low concentrations is well documented (Savoia, 2012; Gyawali & Ibrahim, 2014). However, the concentrations to be used in actual food systems for preservation were shown to be as high as 100-fold of those recorded in in vitro studies (Calo et al. 2015), which may affect the sensory attributes of foods. Therefore, the search for adequate combinations of essential oils with other hurdles to microbial growth has been the focus of tremendous work in recent years aiming to improve their overall effectiveness in food preservation and, hence, reduce the amount to be added. The study of the in situ effects of savory or oregano essential oil on the growth of spoilage bacteria in Attiéké matrix showed different performances with overall tendency to reduce the counts of the undesirable bacteria. However, after an initial decrease in spoilage bacteria counts to their lowest levels in all the treated

samples, the growth of these bacteria was re-initiated in all of them. Such a rebound could be explained by the essential oil inactivation, adaptation of an undesirable bacteria to the stressful environment (Kouakou et al. 2009; Verraes et al. 2013), spontaneous development of resistant mutants (Ryall et al., 2012; Verraes et al. 2013), interactions with food constituents (Hyldgaard et al. 2012; Bassolé & Juliani 2012) and the ability of some bacteria to repair damaged cells (Cadet et al. 2012; Budden et al. 2013) may also account for such a phenomenon. In this study, the rebound was observed in samples treated with the combination of oregano and savory essential so much later suggesting a strong synergistic action between these antimicrobial compounds. However, this combination failed to prevent the growth rebound which was only delayed from the first to the second (samples treated with oregano or savory essential oil) or the fourth (samples treated with both together) week of storage.

CONCLUSION

The combination of oregano and savory essential oil has improved the control of spoilage bacteria in Attieke food as compared with their utilization separately. The synergistic action of oregano and savory essential oil provided more efficient and a longer protection of Attiéké food from the resuscitation of an undesirable bacteria. Therefore, the combination oregano and savory essential oil may be a useful means to enhance the safety and stability of our Ivoirian traditional Attiéké food.

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