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Study of the effectiveness of essential oils of *Mentha aquatica* L. and *Pimpinella anisum* L. in the microbiological stabilization of pasteurized plant extract of *Cyperus esculentus* L. in Mali

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This study evaluates the antimicrobial properties of essential oils of *Mentha aquatica* and *Pimpinella anisum* in the stabilization of pasteurized plant extract of *Cyperus esculentus* in Mali. The extraction of essential oils was carried out by steam distillation of the aerial organs of *M. aquatica* harvested in Bamako and the seeds of *P. anisum* harvested at Diré in Timbuktu Region. Aging tests were carried out by direct incorporation of essential oils into plant extract of *C. esculentus* which is then pasteurized. The plants investigated have an essential oil extraction yield of 1.43% for *M. aquatica* and 2.14% for *P. anisum.* The results of the aging tests showed that the essential oils of *M. aquatica* and *P. anisum* exert a strong antimicrobial activity on the spoilage flora because it made it possible to extend the storage time of vegetable extract up to 32 days. The oils of these plants are therefore an effective alternative to chemical antimicrobials, many of which are harmful to the health of the consumer and it would be interesting to study the biological effect of the combination of these essential oils in the stabilization of this food since they are active on different microbial groups.

Key words: Pasteurized plant extract of *Cyperus esculentus*, essential oils, antimicrobial activities, preservation.

INTRODUCTION

In food technology, controlling the microbiological quality of food is one of the essential bases of its ability to satisfy consumer health safety. The exposure of food to microbial spoilage following the lack of control of this parameter leads to a reduction in its sensory, nutritional, and health characteristics (Bolnot et al., 1985) and its market value. Similarly, losses linked to the production of allergenic compounds and mycotoxins by molds, which are also responsible for the formation of unpleasant tastes in food, are frequently recorded (Ownagh et al., 2010).

Preservation technology, dealing with many problems of oxidation and microbial contamination of foodstuffs, has called on chemistry which has allowed the appearance and use of additives as synthetic food preservatives. These chemicals, used to prevent food spoilage (Nakahara et al., 2003) have proven to be sources of several harms to consumers. Indeed, their application in different industrial sectors (in particular agri-food, insecticide, perfumery, cosmetics, and pharmaceuticals) could be the cause of toxic, carcinogenic, or even mutagenic effects on health; the more reason for consumers to be tempted by a chemical-free diet. This is pushing manufacturers to carry out discussions aimed at reducing the use of additives resulting from chemical synthesis and to develop research and the use of natural products as preservatives with antimicrobial and antioxidant activities in foodstuffs.

Traditionally, empirical knowledge has enabled different civilizations to use plants, particularly aromatic plants, as food additives used as a seasoning or to increase the shelf life of foods (Cheng et al., 2013). Essential oils and aromas constitute in this context the major part of the natural compounds extracted from aromatic plants. These essential oils are now arousing more and more interest for industrialists and researchers because of their strong antibacterial, antifungal, and antioxidant activities (Dung et al., 2008) making them more interesting as natural preservatives in food industries (Gachkar et al., 2007; Rasooli et al., 2008).

It is with this concept in mind that the essential oils of *Mentha aquatica* L. and *Pimpinella anisum* L. belonging, respectively to the Lamiaceae and Apiaceae families were tested in the preservation of a very perishable food, namely the plant extract of *Cyperus esculentus*. These botanical families have been the subject of several investigations by researchers. Also, a search in the scientific literature indicates that there are few reports of scientific studies, particularly on the prospects for extending the shelf life of plant extract of *C. esculentus* in Mali. The plant extract of *C. esculentus* is a milky drink extracted from its tubers after turgidity and grinding. The very sweet *C. esculentus* tubers, called "tigernut" in English, "chufa" in Spanish, "Souchet ou pois sucré" in French, and "tchôgôn" in Bamanankan, are rich in carbohydrates (49%, mainly starch (25%)), lipids (25%), vitamins (C and E), minerals (Ca, Fe, K, P) and crude fiber (Coskuner et al., 2002; Arafat et al., 2009; Ukwuru and Ibeneme, 2011). In Mali, *C. esculentus* is mainly grown in the Sikasso region, which is the main supplier of tiger nut tubers to the Malian populations, who generally use them as snack food.

However, tiger nut tubers are used in Spain for the production of a very attractive milky drink called "horchata de chufa" (Arafat et al., 2009; Ukwuru and Ibeneme, 2011); whose production and marketing are limited by two major constraints:

(1) Its high microbial load (107 CFU/mL) which requires its consumption within 24 h of its production (Aliyu et al., 2022);
(2) The high proportion of starch in this drink makes it difficult to heat treat at the risk of gelatinization of this polysaccharide.

It is in this context that this study takes place to evaluate the possibility of extending the shelf life of pasteurized tiger nut extract using natural food additives such as essential oils.

MATERIALS AND METHODS

Collection of plant material and extraction of essential oils

Essential oils were extracted from the aerial organs of *M. aquatica* and the dried seeds of *Pimpinella*. The aerial parts collected in Bamako were dried in the shade, while the dried anise seeds came to us from Diré in the Timbuktu region. The extraction of essential oils, which lasted 4 h, was carried out by steam distillation using a modified Kaiser-Lang steam circulation device. The essential oils are then dehydrated by crystallization of the residual water in the freezer and stored at +4°C. The extraction yield (Y) is expressed according to the following formula:

$$Yield\ (\%) = \frac{Weight\ (g)\ of\ essential\ oil}{\text{Weight of dry plant material}}$$

Production of pasteurized tiger nut extract

The pasteurized tiger nut extract used as a test food was produced at the quality control laboratory of the University of Ségou from tubers coming directly from a field located in the village of Ifola in the Sikasso region of Mali. The samples are stored under cold conditions at +2°C.

Aging test

The tiger nut extract aging tests were carried out by direct incorporation of each essential oil into 40 mL of tiger nut extract at varying doses. Thus, taking into account the organoleptic parameters (flavor, taste, smell) of the tiger nut extract and essential oil mixture, three doses were tested during the experiment: 10, 20 and 30 µL/40 mL. A control (without essential oil) was also tested. After homogenization by manual rotation, the samples were put under observation and periodic samples of four days followed by analyses were carried out to follow the evolution

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of the microbiological quality of the vegetable extract put in conservation.

Monitoring the evolution of the microbiological quality of plant extract of C. esculentus during storage

A cascade dilution is carried out from the mixture formed by the tiger nut juice and the essential oil. For each quantity of essential oil used, two tubes were used for the decimal dilution in addition to the control. Indeed, in two sterile tubes were introduced 9 mL of physiological water. 1 mL of the product constituting the stock solution or 10° is added to one of the tubes containing 9 mL of physiological saline, this is the 10^{-1} dilution. From this well-stirred mixture, 1 mL is taken, which is then added to 9 mL of physiological water contained in the next tube. This solution corresponds to the 10^{-2} dilution. Microbiological quality parameters were assessed using standard microbiological analysis methods. The germs sought consisted of total aerobic mesophilic flora (total germs; NF V08-051) and fungal flora (ISO 7954). The culture media chosen in this study consisted of Plate Count Agar (PCA) for the total flora and SABOURAUD for the fungal flora.

Statistical analyses

The data obtained was entered in the Excel 2017 spreadsheet and then analyzed by the R software. These data are analyzed by the ANOVA method to determine the differences. For this purpose, the differences are significant if $p < 0.05$; they are not significant if $p > 0.05$.

RESULTS

The plants were investigated to have an essential oil extraction yield of 1.43% for *M. aquatica L.* and 2.14% for *P. anisum*. Tables 1 and 2 present the evolution of the total bacterial flora (TBF) and the fungal flora (FF) in the plant extract of *C. esculentus* supplemented, respectively with essential oil of *M. aquatica* and of *P. anisum* with a view to extend its shelf life. These results show that the antimicrobial activity of essential oils depends on the nature of the essential oil, the dose and even the group of microorganisms targeted.

Indeed, with the essential oil of *M. aquatica* L., Table 1 shows us a slight slowing down of bacterial growth in the samples containing the 10 and 20 µL doses until the 12th day of storage. While at the level of the sample containing the dose of 30 µL of essential oil of *M. aquatica*, the reduction of the TBF continues until the 20th day of storage of the plant extract of *C. esculentus*. In addition, a gradual and total destruction of the fungal flora is observed throughout the storage period, at all doses of the essential oil. This result shows the effectiveness of the essential oil of *M. aquatica* in controlling the bacterial load and in inhibiting the fungal flora contaminating the plant extract of *C. esculentus*. There is also a start of tiger nut extract fermentation on the 40th day of storage in the sample containing the 20 µL dose and the control. While those containing the 10 and 20 µL doses are fermented on the 44th day of storage. This result shows that in terms of stabilizing the tiger nut extract produced in this study, the 10 and 20 µL doses are more effective than the 30 µL one. Moreover, the multivariate analysis reveals that the doses applied to the samples have statistically identical effects ($p > 0.05$). However, we can also see that the 30 µL doses of the essential oil significantly reduce fungal growth compared to the lower doses. This essential oil therefore shows an effective fungicidal effect and a bacteriostatic effect, respectively against fungi and bacteria contaminating tiger nut extract.

As for the essential oil of *P. anisum* in Table 2, a slowdown was also noticed in the growth of the bacterial flora until the 8th day of storage with the dose of 10 µL, until the 12th day with the dose of 20 µL and until the 16th day with the dose of 30 µL beyond which the bacterial growth resumes but timidly. There is also a reduction in fungal growth up to the total destruction of FF in the tiger nut extract studied at all the doses tested. This result therefore shows the effectiveness of the essential oil of *P. anisum* in the fight against fungi contaminating the plant extract of *C. esculentus*. A start of fermentation can also be observed from the 36th day of storage in the samples containing 10 and 20 µL of essential oil and the control. The last fermented sample on the 40th day of storage is the one containing 30 µL of essential oil. This result shows that among the doses tested, the 30 µL dose is more effective in stabilizing tiger nut extract compared to the lower doses tested. The analysis of variance of these results showed that the differences observed are non-significant ($p > 0.05$) with regard to the counted fungal load, but significant at the level of TBF ($p < 0.05$). In addition, the averages of the colonies counted are lower in the samples containing the essential oil of anise than those not containing it. This means that the added doses of essential oil reduced the fungal load in the pasteurized extract samples. It should also be noted that a decrease in this microbial load is all the more important as the quantity of essential oil increases. This result therefore shows the effectiveness of the essential oil of *P. anisum* in the fight against fungi contaminating the tested pasteurized extract plant of *C. esculentus*.

DISCUSSION

The yields obtained following the extraction of essential oils from the different plants studied showed differences compared to the bibliography. Indeed, the extraction yield of the essential oil of *M. aquatica* is higher than those of the work of Dai et al. (2015) in Cameroon and of Morteza-Semnani et al. (2014) in Iran, and lower to that

Table 1. Effect of essential oil of *Mentha aquatica* L. *1753* on the evolution of total bacterial flora (TBF) and fungal flora (FF) in *Cyperus esculentus* nut extract.

Sampling	TBF (witness)	TBF (10)	TBF (20)	TBF (30)	FF (witness)	FF (10)	FF (20)	FF (30)
Day0	190	210	180	150	140	130	120	110
D+4	210	180	150	110	140	110	100	80
D+8	230	150	110	80	160	90	70	50
D+12	270	160	80	50	190	50	40	40
D+16	340	230	120	30	220	30	20	20
D+20	520	470	180	20	260	20	10	10
D+24	700	580	270	60	270	10	0	0
D+28	980	610	310	100	310	10	0	0
D+32	1450	1150	510	180	390	0	0	0
D+36	5600	1300	750	300	560	0	0	0
D+40	Fermented product	1800	900	Fermented product	Fermented product	0	0	Fermented product
D+44	Fermented product	Fermented product	Fermented product	Fermented product	Fermented product	Fermented product	Fermented product	Fermented product

Table 2. Effect of essential oil of *Pimpinella anisum L.1753* on the evolution of total bacterial flora (TBF) and fungal flora (FF) in *Cyperus esculentus* nut extract.

Sampling	TBF (witness)	TBF (10)	TBF (20)	TBF (30)	FF (witness)	FF (10)	FF (20)	FF (30)
Day0	10	40	40	40	10	30	20	60
D+4	10	30	30	40	10	20	10	30
D+8	30	20	20	30	10	10	10	10
D+12	60	40	10	10	10	10	0	0
D+16	70	80	20	10	10	0	0	0
D+20	110	180	30	70	10	0	0	0
D+24	130	200	50	110	10	0	0	0
D+28	140	800	910	200	10	0	0	0
D+32	230	2900	2100	380	20	0	0	0
D+36	Fermented product	Fermented product	Fermented product	1100	Fermented product	Fermented product	Fermented product	0
D+40	Fermented product	Fermented product	Fermented product	Fermented product	Fermented product	Fermented product	Fermented product	Fermented product

of Pavela et al. (2014) in the Czech Republic. That of the essential oil obtained from the seeds of *P. anisum* is lower than those obtained by Ullah et al. (2015) in Pakistan, Saibi et al. (2012) in Algeria and Arslan et al. (2004) in Turkey.

However, the differences observed could be affected by several factors, namely the harvest period, the stage of development of the plant, the nature of the soil, the geography, the cultural practices or even the extraction techniques (Marzouki, 2009; Olle and Bender, 2010).

In addition, this study focused on the evaluation of the antimicrobial activities of essential oils of two (2) aromatic plants in the stabilization of pasteurized plant extract of *C. esculentus L.* in

Mali. The results obtained confirm their antimicrobial properties and the importance of their use in the conservation of plant extract of *C. esculentus*. Indeed, the bacterial and fungal flora studied have shown themselves to be sensitive to the antimicrobial actions of the essential oils tested. Thus, Tables 1 and 2 allow us to better understand the behavior of microbes in the plant extract of *C. esculentus* studied and to estimate its storage time. The shelf life obtained with the essential oils tested explains their significant interest in being used as a preservative in the food industry. This result confirms the data of Degnon et al. (2016) which stipulate that the use of the essential oil of *M. piperita* is possible in the agri-food industry with a view to stabilizing cow's milk in southern Benin. While maintaining the microbial load below the Spanish standard which is $2.5×10^5$ CFU/mL (Mosquera et al., 1996), the essential oils of these aromatic plants of the Lamiaceae and Apiaceae families have demonstrated their ability to delay the process of oxidation in the tiger nut extract tested. These results confirm the work of Getahun et al. (2008) and Barakat et al. (2016) and who have all demonstrated the antimicrobial and antioxidant powers of the essential oils of these species.

It should be noted that all of the results obtained are superior to those obtained by Selma-royo et al. (2022) who estimated the shelf life of tiger nut extract at +2°C for up to 3 weeks if it is processed at a temperature of 70-75°C for 40-60 s. Also, the maintenance of all the essential oils of the microbial load below the Spanish standard which is $2.5×10^5$ CFU/mL confirms their ability to retard microbial growth in tiger nut extract. All of these results confirm not only the antimicrobial activity of essential oils but also their ability to delay the oxidation of foodstuffs. They therefore indicate that the industrial use of these essential oils could be envisaged with a view to stabilizing tiger nut extract. This is also the case with other products in the agri-food sector where essential oils are used in many sectors, particularly in bakery and cheese (Vazquez et al., 2001), in charcuterie (Quintavalla and Vicini, 2002) and in confectionery (Lanciotti et al., 2004). This trend is increasingly motivated by the attraction of industrial companies towards green consumption, which are increasingly resistant to synthetic additives because of the many harmful effects associated with the use of these antimicrobials and food preservatives chemical synthesis (Burt, 2004). Similarly, Degnon et al. (2016) state that essential oils possess the advantage of being bioactive in the vapor phase, a characteristic that makes them useful as possible fumigants for the protection of stored products.

Also, spices and plant extracts including essential oils, known since antiquity as having many virtues (antibacterial, antifungal and antioxidant) are increasingly used in food preservation (Bounatirou et al., 2007) due to their relatively safe status, wide consumer acceptance and exploitation for potentially versatile functional use (Lan-phi et al., 2009).

Conclusion

This study made it possible to evaluate the antimicrobial activities of essential oils of seven aromatic plants tested in the microbiological stabilization of pasteurized plant extract of *C. esculentus L.* in Mali. In view of the results obtained, we can conclude that these plants could constitute an effective alternative to replace synthetic antimicrobials, several of which have been harmful to the health of the consumer and to the environment.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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