GSICA
THE ITALIAN
SCIENTIFIC GROUP
OF FOOD PACKAGING

ISTITUTO ITALIANO PLASTICI SRL
Certificazioni, Ispezioni, Prove

SLIM for young
SHELF LIFE INTERNATIONAL MEETING
2015

SLIM 2015

Shelf-life International Meeting

Vimercate (MB), 21 – 23 October 2015

Edited by

G. Buonocore, F. Licciardello and L. Piergiovanni

Special Issue

ITALIAN JOURNAL
OF
FOOD SCIENCE
Rivista italiana di scienza degli alimenti

CHIRIOTTI EDITORI

This Special Issue of the Italian Journal of Food Science collects the presentations given at the "SLIM 2015, Shelf Life International Meeting" organized by the Italian Scientific Group of Food Packaging (GSICA) and Italian Institute of Plastics (IIP), held at Vimercate (MB), Italy on October 21-23, 2012.
These papers were reviewed by the Scientific Committee of SLIM 2015 before their presentation but they did not undergo the conventional reviewing system of the Italian Journal of Food Science.

Chiriotti Editori S.A.S. - Pinerolo - Italy

ISSN 1120-1770

ACTIVE COATINGS AGAINST DATES FUNGAL DECAY

H. ALOUI[1], K. KHWALDIA*[1], F. LICCIARDELLO[2], A. MAZZAGLIA[2], G. MURATORE[2] and C. RESTUCCIA[2]

[1]Laboratoire des Substances Naturelles (LSN), Institut National de Recherche et d'Analyse Physico-chimique (INRAP), Pôle Technologique de Sidi Thabet, Tunisia

[2]Di3A, University of Catania, Catania, Italy

*Corresponding author: khaoula_khwaldia@yahoo.fr

ABSTRACT

Biodegradable coatings based on chitosan incorporating either bergamot or bitter orange EOs at different concentrations were evaluated against *Aspergillus flavus*, under *in vitro* conditions, in terms of mycelium growth and spore germination inhibition, and under *in vivo* conditions on inoculated dates, stored at 20°C. Sensory analysis was carried out to evaluate the effect of the different coating treatments on the flavour and odour characteristics of the treated fruits. Combined treatments based on CH-2% (v/v) bergamot EO or CH–2% (v/v) bitter orange EO proved to be the most effective coatings to reduce conidial germination resulting in a 87-90% inhibition compared with the control. In fruit decay assays, coatings based on CH incorporating citrus oils were able to reduce fungal decay in the range of 52-62% at day 12.

Keywords: *Aspergillus flavus*, bergamot, bitter orange, chitosan, date, Locust Bean Gum, postharvest decay

1. INTRODUCTION

Date fruit, which is a rich source of carbohydrates, fibres, minerals, vitamins and antioxidant compounds, is one of the most in North Africa, Middle East and South-Asian countries. However during storage, this fruit is susceptible to infection by different spoilage fungi resulting in economic losses, especially for exporting countries. In Tunisia, the highest exporter of "deglet Nour' date variety, more than 50% of dates fruit are lost due to fungal spoilage. *Aspergillus flavus* and *Aspergillus parasiticus* have been reported to be among the most common fungal species infecting dates during storage (AHMED *et al.*, 1997). Under conditions of high humidity and moderate temperature, these postharvest fungi may have the potential to produce aflatoxins which are considered to be among the most significant food contaminants regarding to their negative impact on public health and food security. In recent years, considerable attention has been directed toward natural essential oils (EOs), as a promising approach for controlling fruits' postharvest decay and reducing chemical-based treatments. However, the use of these compounds is often limited due to their intense aroma and their potential toxicity. The incorporation of EOs into edible coating formulations has been investigated as an effective approach to solve some of these problems, by lowering the diffusion processes and maintaining high concentrations of active molecules on the surface of the fruit. Among the polysaccharides used in the edible coating formulations,
chitosan (CH) and Locust Bean Gum (LBG) have been reported to be of interest as potential coating components due to their excellent film forming properties as well as their ability to act as effective matrices for the entrapment of bioactive compounds including EOs (PERDONES *et al.*, 2012).
This study aimed at screening the antifungal activity of five citrus EOs (bergamot, bitter orange, sweet orange, mandarin and lemon) against *A. flavus* in *in vitro* conditions and investigating the potential application of CH and LBG coatings enriched with the most efficient oils in controlling postharvest decay in inoculated dates.

2. MATERIALS AND METHODS

2.1. Screening for the most effective EOs against *A. flavus*

The Poison food medium method was used in order to screen the most effective citrus oil against *A. flavus* as previously described by ALOUI *et al.* (2014). The most effective EOs were selected for all the further experiments.

2.2. Determination of selected EO effective concentration

Based on Poison Food medium method, bergamot and bitter orange EOs were identified as the most effective against *A. flavus*. In order to determine the minimum effective concentration of the selected oils, an in vitro conidial germination inhibition assay was carried out, using the "cavity slide" technique as previously described by ALOUI *et al.* (2014). A concentration of 2% (v/v) EO was selected for the evaluation of the *in vitro* and the *in vivo* antifungal activity of the combined treatments based on CH or LBG enriched with either bergamot or bitter orange EOs.

2.3. Preparation of the film forming dispersions

CH solutions (1%, w/v) were prepared by dissolving CH powder in an aqueous solution of glacial acetic acid (1%, v/v), while that of LBG (0.5%, w/v) were obtained by dispersing LBG powder in heated distilled water with constant agitation. The selected amount of either bergamot or bitter orange EOs (2%, v/v) was then added to CH and LBG film forming solutions, before being homogenized at 13,500 rpm for 4 min, using an Ultra-Turrax T25 (IKA, Labortechnik GmbH., Munich, Germany).

2.4. *In vitro* antifungal activity of combined treatments

Conidial germination inhibition assays was carried out in order to evaluate the antifungal potential of CH and LBG either alone or in combination with either bergamot or bitter orange EOs at a concentration of 2% (v/v).

2.5. In *vivo* antifungal assay: Fruit decay

The antifungal activity of the most effective coating treatments was evaluated according to ALOUI *et al*. (2014). Briefly, dates previously washed with sodium hypochlorite (0.01%) were injured and dipped in a conidial suspension of *A. flavus* at a concentration of 10^5 conidia/mL for 1 min and dried at room temperature for 2 h. Inoculated fruits were then immersed in the different coating solutions (30 dates for each treatment) for 1 min and air-dried at room temperature before being stored at 25°C, 75% RH for 12 days. Disease incidence, expressed as the number of infected dates out of the total number of fruits per treatment, was daily evaluated.

2.6. Sensorial analysis

The effect of the different coating treatments on the sensory characters of dates has been evaluated 24 h after coating application, using the sensory profile method (UNI, 10957, 2003).

3. RESULTS AND DISCUSSIONS

3.1. Effect of combined treatments on conidial germination

As it can be inferred from Table 1, pure CH inhibited conidial germination by 17% compared to the control and the pure LBG treatment. Combined treatments based on CH–2% bergamot EO and CH–2% bitter orange EO were the most effective in reducing conidial germination allowing an inhibition in the range of 87-90% compared with the control ($p < 0.05$), followed by those based on LBG enriched with either bergamot or bitter orange for which conidial inhibition was in the range of 73-82%. In agreement with our results, DOS SANTOS and AGUIAR (2012) reported an inhibition by more than 90% of conidial germination of *Rhizopus stolonifer* and *A. niger* when CH was assayed in combination with origanum EO. According to these authors CH and origanum EO inhibited germination through an interaction with the cell wall of conidia.

Table 1: Effect of different treatments on conidial germination inhibition of *Aspergillus flavus*. Mean values and standard deviation.

Treatments	Conidial Germination Inhibition (%)
Control	0.00±0.00[a]
LBG	0.00±0.00[a]
CH	17.50±2.50[b]
LBG-2% bitter orange EO	73.57±1.24[c]
LBG-2% bergamot EO	82.20±2.31[d]
CH-2% bitter orange EO	87.12±0.82[e]
CH-2% bergamot EO	93.61±1.20[f]

[a-f]: different superscripts indicate significant differences among treatments ($p < 0.05$).

3.2. Fruit decay

As shown in Table 2, pure CH coatings led to a significant delay in the rate of fungal decay. At day 11, although all the uncoated dates were infected, only 55% of those treated with pure CH were decayed compared to the control and those treated with pure LBG ($p < 0.05$). This result seems to be related to antifungal activity of CH previously proved against postharvest fungi including *Aspergillus*. Statistical analysis revealed CH coatings enriched with either bergamot or bitter orange to be the most effective in controlling fungal decay of dates allowing a reduction in the range of 52-62% compared with the control at day 12 ($p < 0.05$).

Table 2: Effect of coating treatments on the decay percentage of inoculated dates during storage at 25°C. Mean values and standard deviation.

Storage time (days)	Fungal decay (%)					
	Control	LBG	CH	LBG-2% bergamot EO	CH-2% bitter orange EO	CH-2% bergamot EO
1	0.00±0.00 [a,u]	0.00±0.00[a,u]	0.00±0.00[a,u]	0.00±0.00[a,u]	0.00±0.00[a,u]	0.00±0.00[a,u]
7	0.00±0.00[a,u]	0.00±0.00[a,u]	0.00±0.00[a,u]	0.00±0.00[a,u]	0.00±0.00[a,u]	0.00±0.00[a,u]
8	43.33±4.71[b,u]	41.66±2.35[b,u]	8.33±2.35[b,v]	10±0.00[b,vw]	3.33±0.00[b,wx]	0.00±0.00[a,x]
9	66.66±4.71[c,u]	63.33±4.71[c,u]	16.66±0.00[c,v]	18.33±2.35[c,v]	3.33±0.00[b,w]	0.00±0.00[a,w]
10	81.66±2.35[d,u]	78.33±2.35[d,u]	31.66±2.35[d,v]	36.66±4.71[d,v]	11.66±2.35[c,w]	6.66±0.00[b,w]
11	100±0.00[e,u]	100±0.00[e,u]	54.99±2.35[e,v]	59.99±4.71[e,v]	36.66±0.00[d,w]	24.99±2.35[c,x]
12	100±0.00[e,u]	100±0.00[e,u]	73.33±0.00[f,v]	78.33±2.35[f,v]	48.33±2.35[e,w]	38.33±7.07[d,x]

[a-f] : different superscripts within a column indicate significant differences among storage time ($p < 0.05$).
[u-x]: different superscripts within a file indicate significant differences among treatments ($p < 0.05$).

Moreover these coatings were able to delay the onset of disease symptoms and slow down mould growth during storage period. After 7 days of storage, more than 40% of dates were decayed; no signs of fungal decay, however, were observed for fruits treated with CH coatings enriched with both citrus oils. In agreement with our results, a further reduction in the fungal decay of inoculated dates has been observed by PERDONES *et al*. (2012) when lemon EO was added to CH coatings.

3.3. Sensorial analysis

Sensory evaluation revealed significant differences in four descriptors including colour, gloss, citrus odour and flavour. A significant decrease in both glossiness and colour of dates was observed after coating application ($p < 0.05$). This decrease was more pronounced in samples treated with formulations containing citrus EOs. A significant loss of surface glossiness has been also observed by PERDONES *et al*. (2012) in CH-coated strawberries when lemon EO was added to CH matrix. Such behavior was ascribed to the increase in the opacity of the film as a result of oil droplet aggregation during drying process. On the other hand, a relatively high intensity of citrus odour and flavour was detected by the panel in dates treated with formulations containing either bergamot or bitter orange EOs. However, none of the judges revealed the presence of off-flavours and/or off-odour.

4. CONCLUSIONS

Treatments based on CH or LBG incorporating citrus EOs were proven effective in inhibiting *A. flavus* conidial germination and in controlling postharvest decay in infected dates. CH coatings enriched with citrus oils were the most effective in reducing fungal decay of inoculated dates (52-62% at day 12). These results and the complete absence of off-flavours and off-odours demonstrate the potential of CH coatings carrying citrus EOs as a promising alternative to synthetic antifungal agents for controlling postharvest growth of *A. flavus* in dates.

REFERENCES

Ahmed I.A., Ahmed A. and Robinson R.K. 1997. Susceptibility of date fruits (Phoenix dactylifera) to aflatoxin production. J. Sci. Food Agric. 74: 64-68.

Aloui H., Khwaldia K., Licciardello F., Mazzaglia A., Muratore G. Hamdi M. and Restuccia C. 2014b. Efficacy of the combined application of chitosan and Locust Bean Gum with different citrus essential oils to control postharvest spoilage caused by *Aspergillus flavus* in dates. Int. J. Food Microbiol. 170: 21-28.

Dos Santos N.S.T. and Aguiar A.J.A.A. 2012. Efficacy of the application of a coating composed of chitosan and *Origanum vulgare* L. essential oil to control *Rhizopus stolonifer* and *Aspergillus niger* in grapes (*Vitis labrusca* L.). Food Microbiol. 32: 345-353.

Perdones A., Sánchez-González L., Chiralt A. and Vargas M., 2012. Effect of chitosan-lemon essential oil coatings on storage-keeping quality of strawberry. Postharvest Biol. Technol. 70, 32-41.

UNI 10957. 2003. Sensory analysis-method for establishing a sensory profile in foodstuffs and beverages. UNI, Ente Nazionale Italiano di Unificazione, Milan, Italy.