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Improving food quality and safety by developing innovative antimicrobial packaging activated with natural bioactive compounds

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Background and Aim

As demand grows for **safe** and **eco-friendly food preservation** technologies, **antimicrobial packaging** offers a promising solution for extending food **freshness** and reducing **spoilage**.

This study investigated packaging functionalised with **natural compounds - chitosan, nisin, and carvacrol** at different concentrations- to preserve Italian "*Favette*" **strawberries**.
Five treatments were tested over 12 days to assess their effectiveness in preventing microbial growth and preserving food quality.

Methods

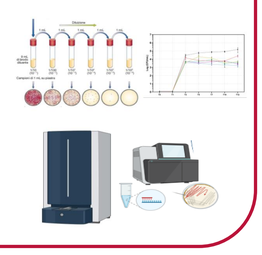
Antimicrobial packaging conditions:

Control: non-functionalised PAD (traditionally used)
AP 1: Chitosan 1% + Nisin 1%
AP 2: Chitosan 1% + Carvacrol 1%
AP 3: Chitosan 1% + Carvacrol 2%
AP 4: Chitosan 1% + Carvacrol 1% + Nisin 1%
AP 5: Company combination

Microbiological analyses

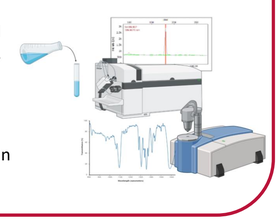
Five different treatments were evaluated over 7 sampling times, extending up to 12 days.

- Determination of the **total bacterial and fungi load** for the **pulp** and **washing water** fractions
- **Isolation** of 95 colonies for the subsequent **identification** using **MALDI-TOF MS** and **molecular techniques** (***identification in progress***)



Chemical characterisation of PADs

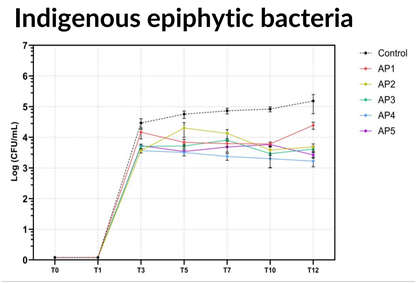
- **Overall migration** of components from the antimicrobial films (absorbent **PADs** and **BOPP**) and trays (**PET**) into aqueous simulants in accordance with EU regulations (OM1 conditions in food simulants A and B).
- **Specific metal migration** and **heavy metal content** (Pb, Cd, and Cr) [Regulation (EU) 10/2011 and Directive 94/62/EC].
- **Specific carvacrol migration** in food simulants by applying **FTIR** technique



Results

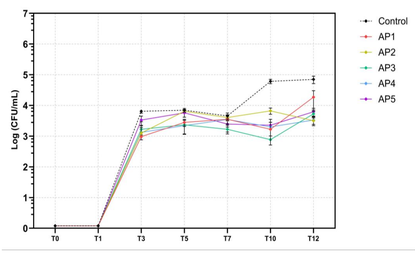
➢ *Microbiological analyses of strawberries' pulp and washing water*

Indigenous epiphytic bacteria



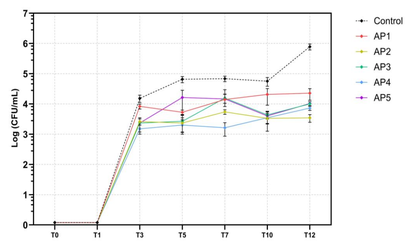
- The control exhibits consistently higher bacterial levels, especially toward T10 and T12, suggesting that **the absence of treatment results in greater bacterial proliferation**.
- Among the treatments, **AP4 and AP5 appear to be more effective** in reducing bacterial growth compared to the other treatments

Indigenous epiphytic fungi



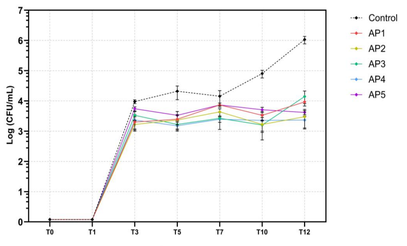
- The control demonstrates a significantly higher fungal load, particularly at T10 and T12.
- The **AP** treatments seem to have an **evident antimicrobial impact**, with **AP2 and AP4 showing better performance** in maintaining lower fungal counts by the final sample time.

Total food associated bacteria

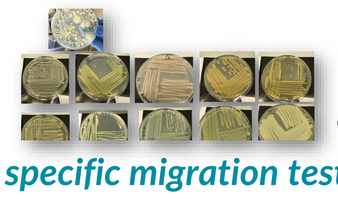


- There is a growth increase from T1, and the control consistently displays higher bacterial loads, particularly toward T10-T12, indicating that the AP treatments are effectively limiting bacterial growth.
- Among the AP treatments, **AP2 and AP4 show greater efficacy**, as they maintain the lowest bacterial counts until T12.

Total food associated fungi



- **The treatments display a more pronounced effect**. The control shows a progressive increase in fungal counts, especially between T7 and T12, highlighting the impact of the AP treatments in controlling fungal proliferation.
- Again, **AP2 and AP4 show the most effective results**, with consistently lower fungal loads compared to the control.



***95 isolates** from strawberries **pulp** and **washing water for the subsequent identification** → 30% v/v glycerol stocks*

➢ *Overall and specific migration tests in food simulants*

I. Overall migration results

Sample	Overall migration Value (± SD)	
	Simulant A	Simulant B
Control AP	2.15 ± 0.21	6.35 ± 0.35
Sample 1	4.55 ± 0.21	7.15 ± 0.21
Sample 2	2.05 ± 0.21	5.90 ± 0.42
Sample 3	1.85 ± 0.21	5.80 ± 0.42
Sample 4	2.10 ± 0.14	7.0 ± 0.42
Sample 5	2.50 ± 0.14	6.60 ± 0.28
BOPP foil	0.55 ± 0.07	0.75 ± 0.07
PET tray	0.63 ± 0.06	1.13 ± 0.06

Plastic materials and articles shall not transfer their constituents to food simulants (A: Ethanol 10% , B: Acetic acid 3%) in quantities exceeding **10 mg/dm²** of food contact surface **(EU Regulation no. 10/2011).**

OM1 testing conditions (10 days at 20°C)

All the results were lower than the overall migration limit of 10 mg/dm² for both food simulants used.

II. Heavy metal migration results

Element	Control AP	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	BOPP foil	PET tray
Ba	0.00056	0.01139	0.00164	0.00051	0.00225	0.00174	0.00379	0.00457
Co	0.00041	< 0.00003	0.00037	0.000375	0.00042	0.00037	0.00015	< 0.00003
Cu	0.00042	0.00233	< 0.00004	< 0.00004	0.00042	0.00013	0.00133	0.00108
Zn	0.00006	0.00070	< 0.00004	< 0.00004	0.00526	0.00820	0.01465	0.00407
Mn	0.00375	0.00235	0.00304	0.00285	0.00419	0.00387	0.00205	0.00296
Ni	0.00205	< 0.00006	0.00186	0.00202	0.00315	0.00245	0.00149	< 0.00006
Li	< 0.00008	0.00052	< 0.00008	< 0.00008	< 0.00008	< 0.00008	0.00014	< 0.00008
Fe	< 0.00006	< 0.00006	< 0.00006	< 0.00006	< 0.00006	< 0.00006	< 0.00006	< 0.00006
Pb	0.00184	0.0054	0.00171	0.00108	0.00284	< 0.00100	< 0.00100	< 0.00100
Cd	< 0.00100	< 0.00100	< 0.00100	< 0.00100	< 0.00100	< 0.00100	< 0.00100	< 0.00100
Cr	< 0.00100	< 0.00100	< 0.00100	< 0.00100	< 0.00100	< 0.00100	< 0.00100	< 0.00100
Sb	0.00022	0.00015	< 0.00003	0.00006	0.00017	< 0.00003	0.00005	0.00217

According to the **(EU) Regulation no. 10/2011 (Annex II),** the imposed limit of the tested metals are:

Ba < 1 mg/kg, **Co** < 0.05 mg/kg, **Cu** < 5 mg/kg,
Zn < 5 mg/kg, **Mn** < 0.6 mg/kg, **Ni** < 0.02 mg/kg,
Li < 0.6 mg/kg, **Fe** < 48 mg/kg, **Sb** < 0.04 mg/kg.

The results obtained are in compliance with the EU Regulation

III. Heavy metal content results

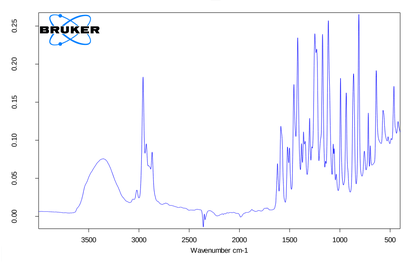
Sample	Pb ± SD	Cd ± SD	Cr (total)
Control AP	0.442 ± 0.014	0.012 ± 0.001	7.156 ± 0.165
Sample 1	2.788 ± 0.003	0.007 ± 0.0002	0.987 ± 0.003
Sample 2	0.364 ± 0.002	0.011 ± 0.0006	6.095 ± 0.110
Sample 3	0.259 ± 0.010	0.011 ± 0.0007	11.937 ± 0.048
Sample 4	< 0.001	< 0.001	< 0.001
Sample 5	0.105 ± 0.002	0.009 ± 0.0002	10.096 ± 0.697
Bopp foil	0.165 ± 0.004	0.007 ± 0.0005	1.005 ± 0.015
PET tray	0.325 ± 0.001	0.007 ± 0.0002	0.704 ± 0.020

Directive 94/62/EC: The sum of concentration levels of **Pb, Cd, Hg and hexavalent Cr** present in packaging or packaging components shall **not exceed 100 ppm**

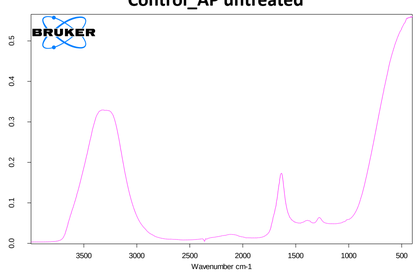
The combined levels of Pb, Cd, and Cr are significantly lower than the imposed limit.

➢ *Specific migration test of carvacrol by FT-IR*

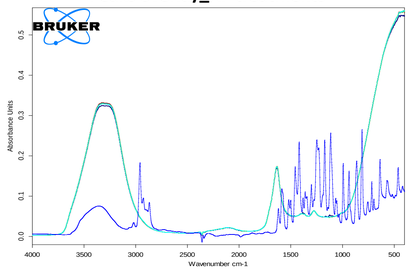
Control_carvacrol



Control_AP untreated



Peaks overlay_AP treatments



- The FT-IR spectrum of pure carvacrol serves as the reference standard, exhibiting characteristic peaks that correspond to the molecular vibrations and bonds specific to carvacrol.
- The FT-IR spectrum of the untreated control shows no signals indicative of carvacrol since this sample was not functionalised.
- The **spectra overlays** of the functionalized samples, which were expected to potentially release carvacrol into the simulant, **showing no significant evidence of the characteristic carvacrol peaks.**

The carvacrol did not migrate into the simulant indicating that the AP retained the carvacrol effectively, preventing its release into the external medium.

Conclusions

The results of the present study underscore the potential of AP4 as a promising active packaging solution, offering both extended shelf-life and compliance with food safety regulations:

- The active **PADs** developed **successfully extended the shelf-life of strawberries up to 12 days** compared to the control
- Among the treatments, **AP2 and AP4 appear more effective** in limiting bacterial and fungi proliferation
- Although microbiological analyses show promising results for the AP2 treatment, the incorporation of **carvacrol alone results in a greater sensory impact** on the product
- **Migration tests further confirmed that both global and specific migration** of metals followed the limits, ensuring the safety of the AP materials for food contact
- **FT-IR analysis demonstrated that no carvacrol migrated into the simulant**, as no characteristic carvacrol peaks were detected in the functionalized samples.

Acknowledgement

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