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Active and intelligent fibre-based packaging – innovation and market introduction

Design of Active Paper Sheets Through Nanoemulsions and Different Processing Techniques

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PRESERVING FOOD

- ✓ Heating,
- ✓ Cooling,
- ✓ Decreasing Water Activity,
- ✓ Curing,
- ✓ Salting,
- ✓ pH Control,
- ✓ Controlled Atmosphere Packaging
- ✓ Modified Atmosphere Packaging
- ✓ **Additives**

Plant extracts

Essential oils

This talk will include ;

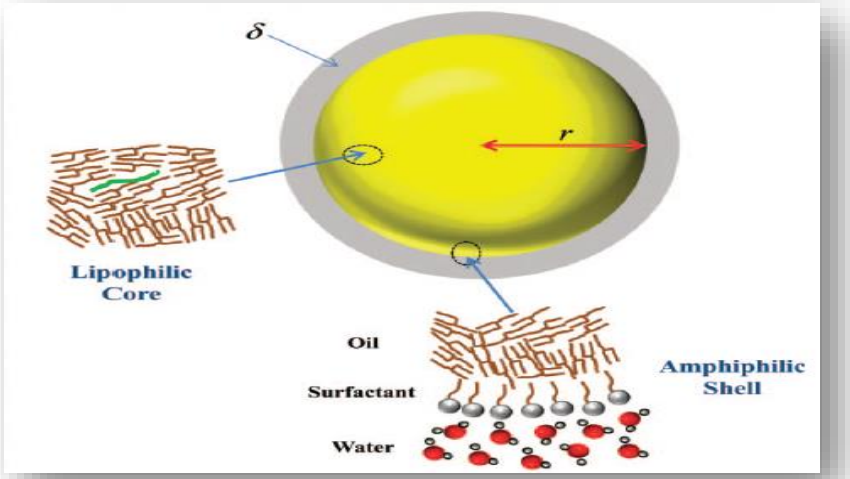
- Formation of cinnamon oil nano emulsions
- Coating of Paper sheets with cinnamon oil
  Effect of processing methods
- Coating of paper sheets with olive leaf extract nanoemulsions
  Effect of processing methods

Essential Oils

Other plant extracts

How can we utilize these plant extracts efficiently with causing undesirable changes on sensory properties?

Could nanoemulsions be an alternative?

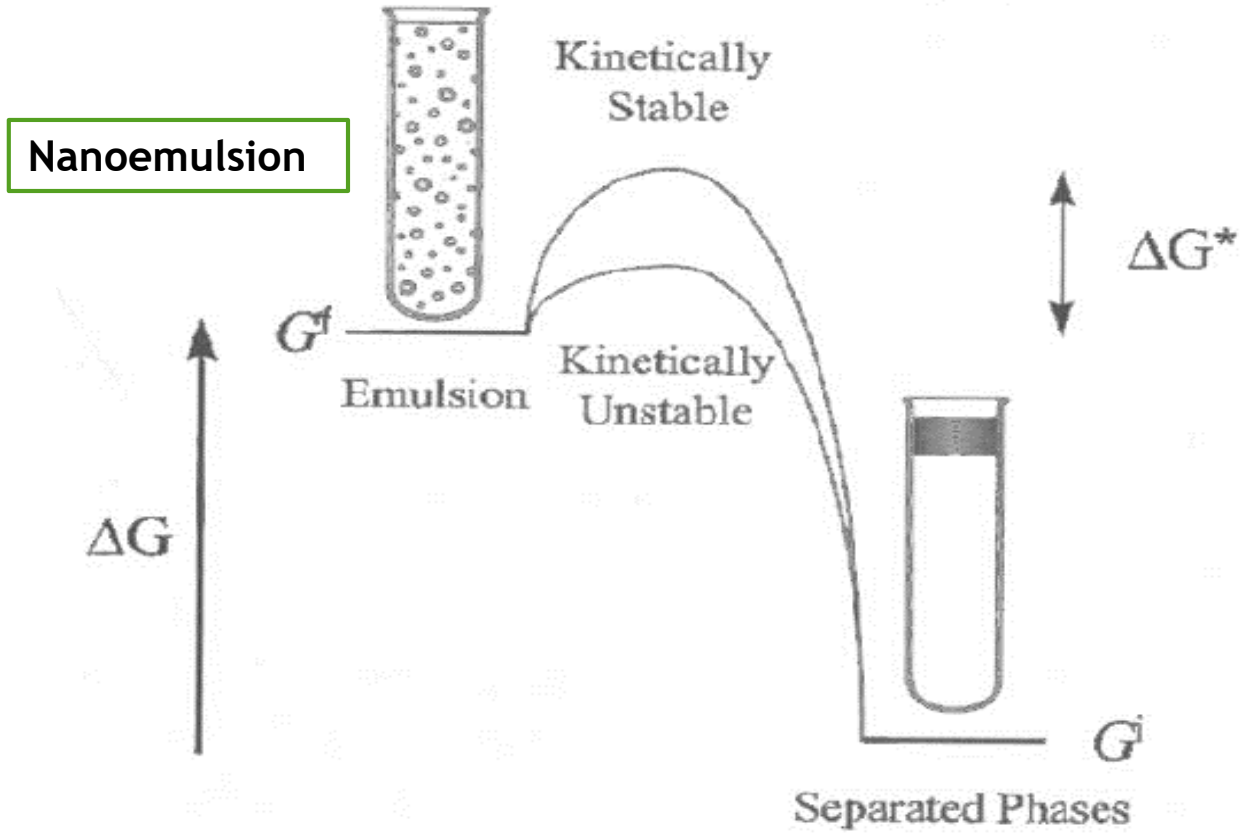


McClements, D. J., & Rao, J. (2011). Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. *Critical Reviews in Food Science and Nutrition, 51(4), 285-330.*

Types of Surfactants

- Ionic Surfactants → CITREM, DATEM, lauric arginate
- Non-ionic Surfactants → **Tweens**, spans, sugar esters
- Zwitterionic Surfactants → **Lecithin**

Nanoemulsions are thermodynamically unstable systems



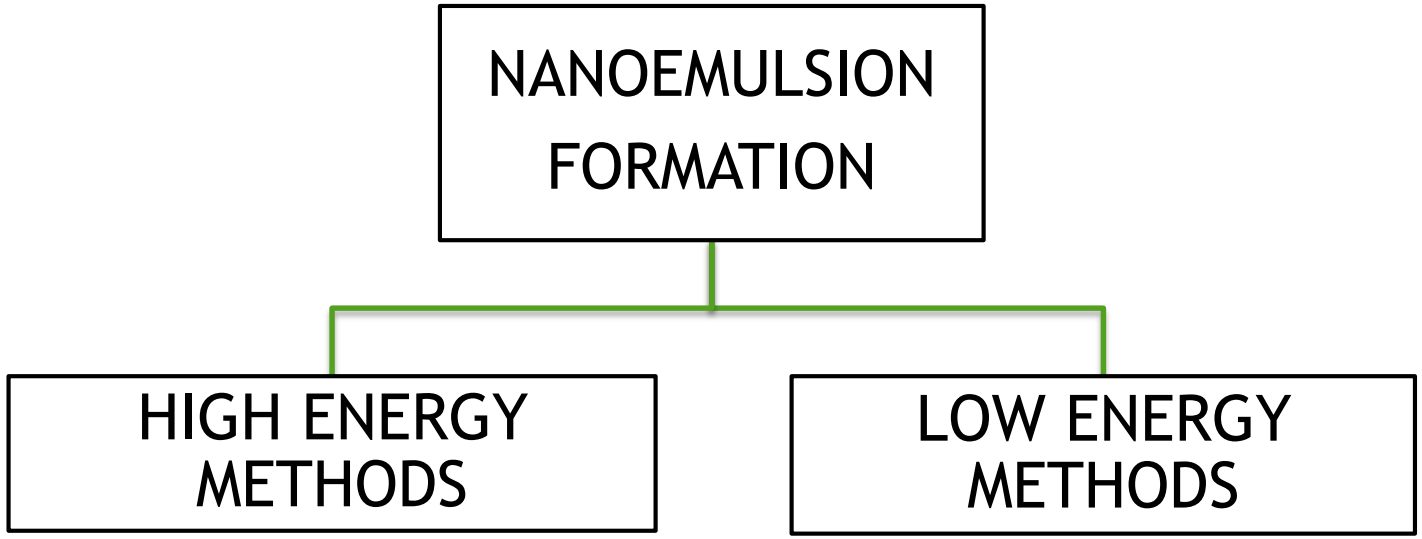
Description of Nanoemulsions

Particle size ?

Nano range ?

Advantages of Nanoemulsions

- The small droplet size and long-term physical stability
- The large surface area
- Delivery of hydrophobic compounds
- Enhance bioavailability
- Encapsulation of functional components and prevent degradation



- High Pressure Homogenizer
- Ultrasonic Homogenization

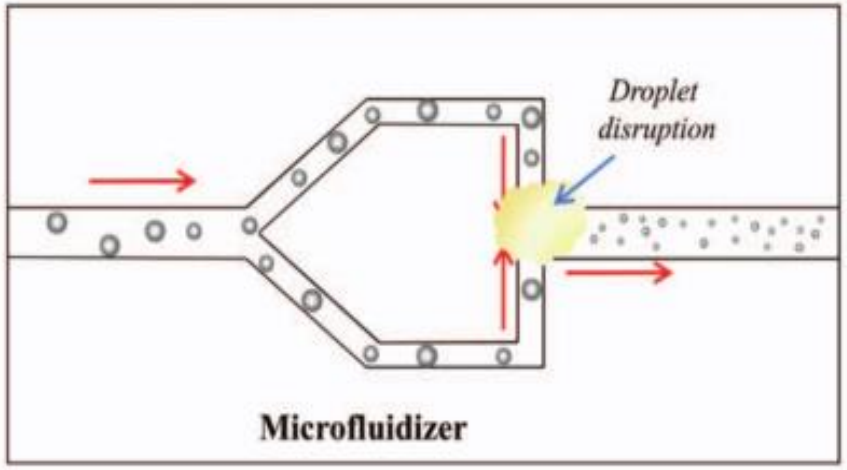
- Spontaneous Emulsification
- Membrane Emulsification
- Solvent Displacement Method
- Emulsion Inversion Point

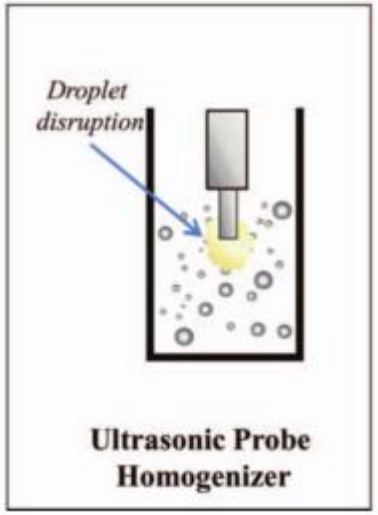
Formation of Nanoemulsion

High Energy Approches

Microfluidization, Ultrasonication

- Well studied in literature
- Capability to scale up
- Suitable for wide range of oil and surfactant types
- Ability to produce small and stable emulsions





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Cinnamon oil nanoemulsions by spontaneous emulsification: Formulation, characterization and antimicrobial activity

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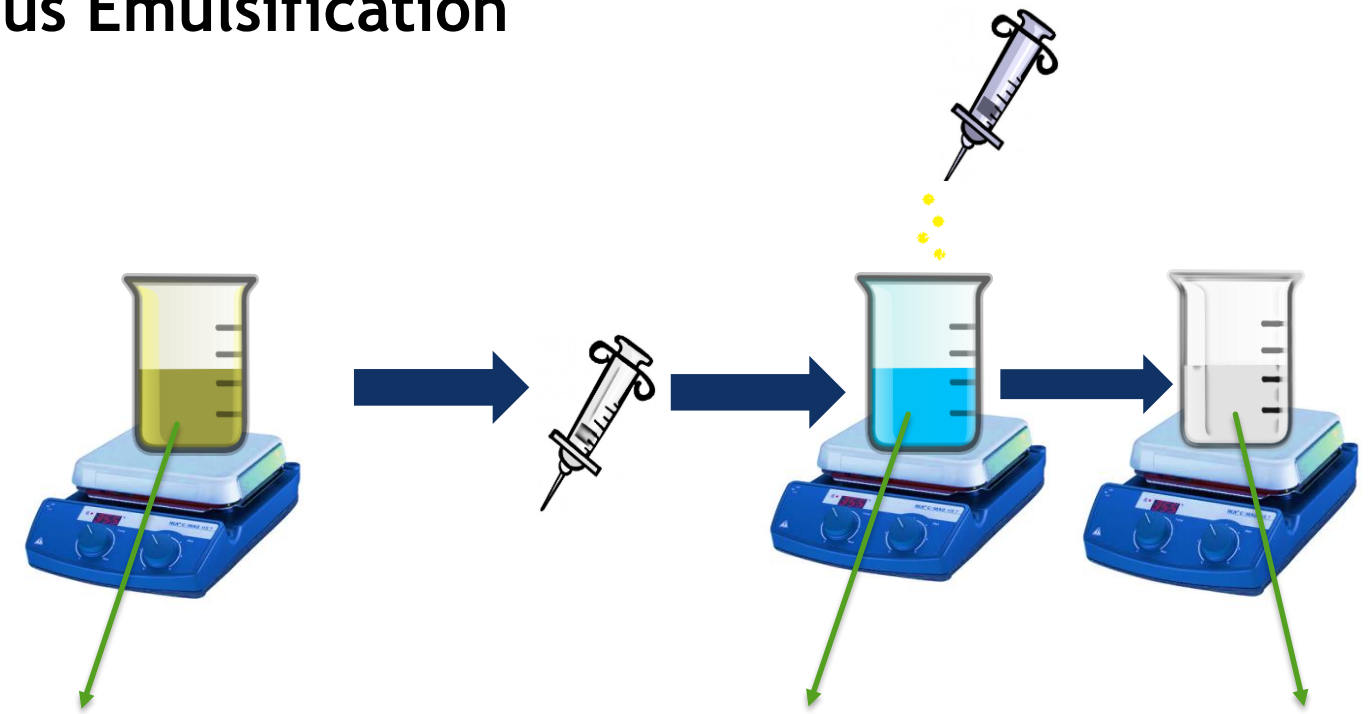
ABSTRACT

The goal of this study was to formulate stable cinnamon oil nanoemulsions (NEs) exhibiting high antimicrobial activity by using the low-energy approach: spontaneous emulsification (SE) and compare it with two high-energy methods. To prepare the nanoemulsions by SE, oil phase containing cinnamon oil (CO) and carrier oil (coconut oil (CNO)) at different ratios (2:8–10:0) and surfactant (Tween 80) at 10% (w/w) was titrated into an aqueous phase (distilled water). For antimicrobial activity, agar disc diffusion method with *E. coli* as the model microorganism was used. NEs were characterized by Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM). Both DLS and TEM gave parallel results and mean particle size were found as ~ 100 nm for 6:4 (CO: CNO) oil phase composition. These NEs also showed high physical stability during one-month storage. NEs were also prepared by using two high-energy homogenization methods: microfluidization and ultrasonication. Ultrasonication and SE showed similar trends for mean particle size and microbial activity. Microfluidization resulted in the smallest mean particle size ($p < 0.05$) and antimicrobial activity was not effected from cinnamon oil concentration ($p > 0.05$).

Spontaneous Emulsification



Organic phase (Oil phase)

Oil + Surfactant

Cinnamon oil + Carrier Oil

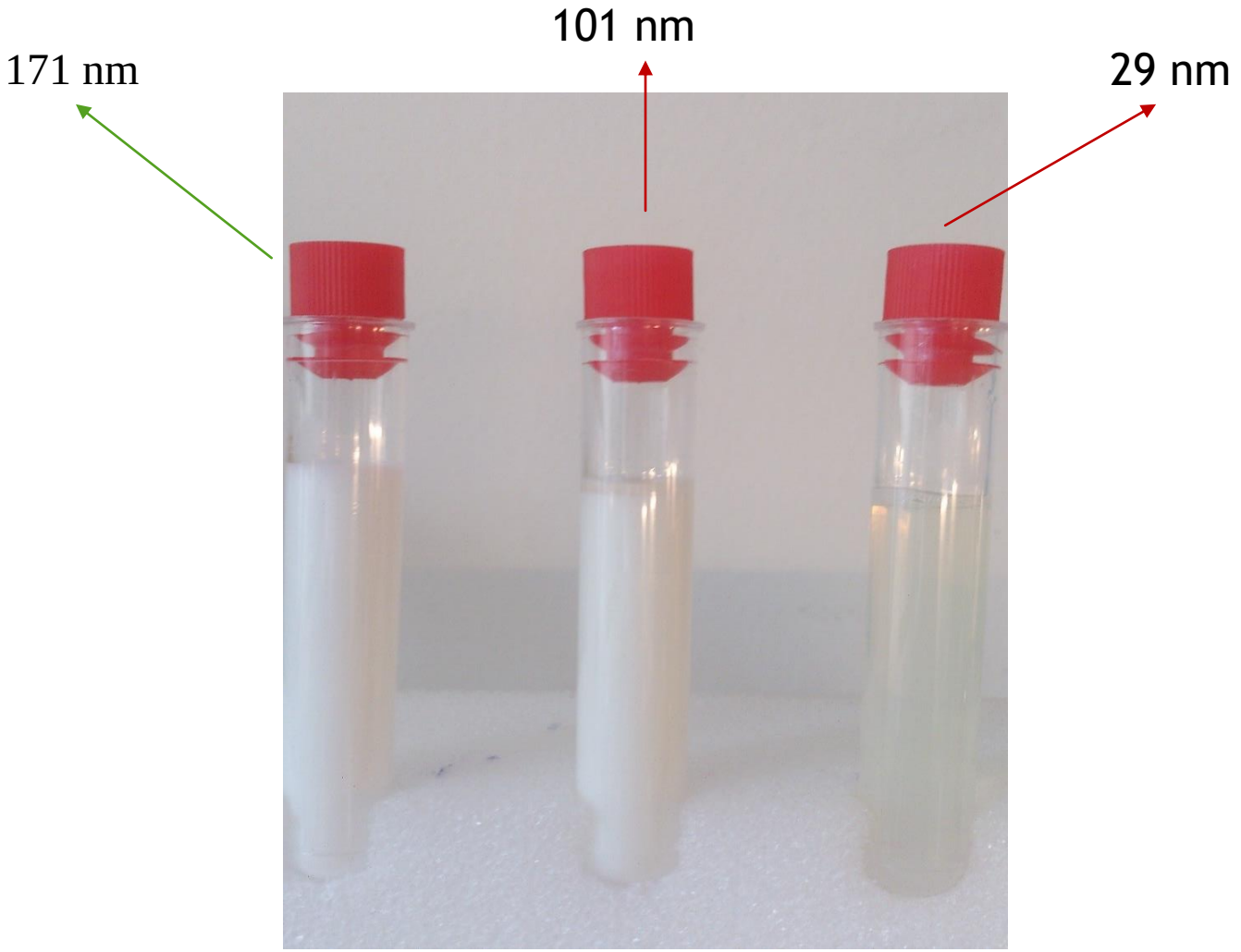
Aqueous phase

Distillate Water

Nanoemulsion

- **Carrier oil**
  - LCTs (Long chain triglycerides) and MCTs (Medium Chain Triglycerides)
  - Coconut oil - Medium Chain Fatty Acids > 50 wt. % of fatty acids

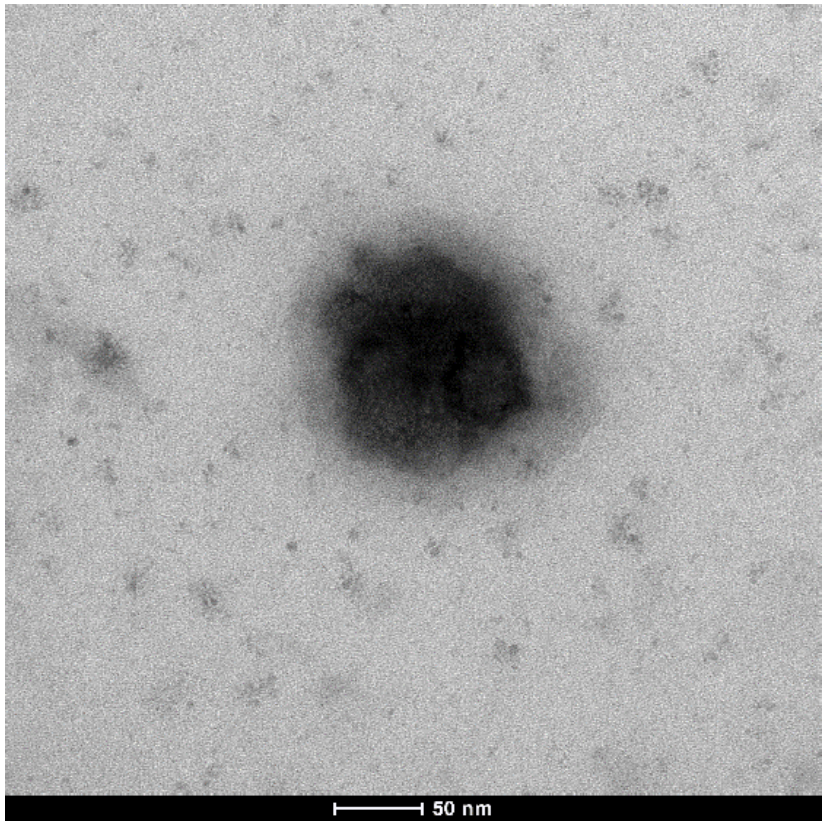
- **Effect of carrier oils on Ostwald Ripening**
  - Ripening Inhibitor
  - Entropic Stabilization

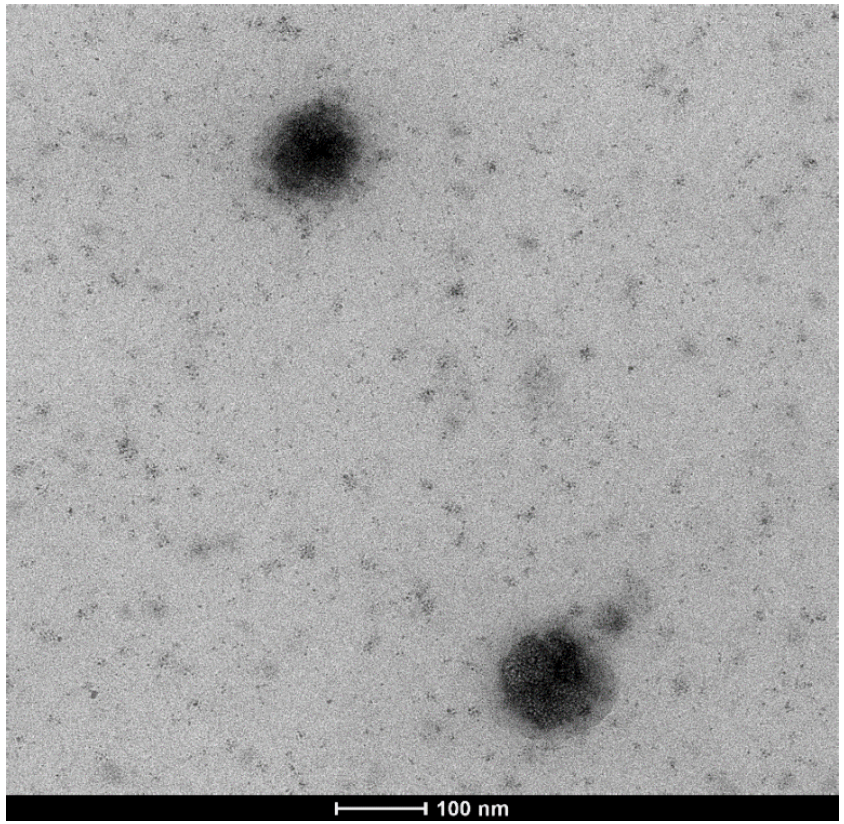


Effect of *Surfactant to Oil Ratio* on Nanoemulsions.

From left to right the photograph shows SOR in the system of 0.5, 1, 2 respectively.

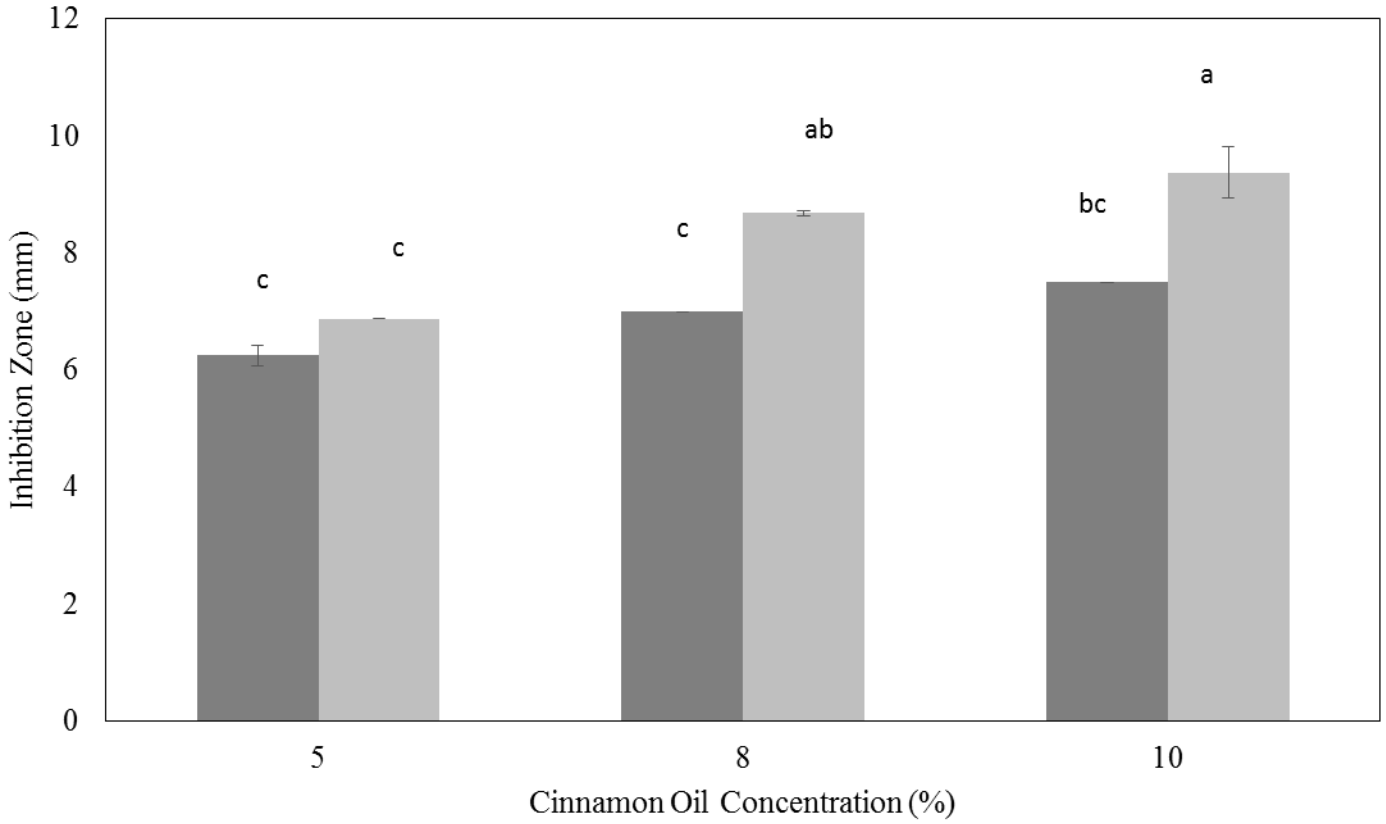
Transmission Electron Microscopy





Transmission Electron Microscopy Bright Field Images of Nanoemulsion

Comparison of Antimicrobial Activity of Cinnamon Oil Solutions and Cinnamon Oil Nanoemulsions obtained by SE



Effect of CO Concentration on Antimicrobial Activity.

Solution: (■) and Nano-emulsion:(■) Different letters represent significant difference ($p \leq 0.05$)

Active Paper Packages With Plant Extracts: Advantages & Uses

- Exerting antimicrobial activity;
- Providing antioxidant activity;
- Enrichment in total phenolic content;
- Natural antimicrobials, food grade;
- Renewable and biodegradable sources.

→ Prolong shelf life of various foods

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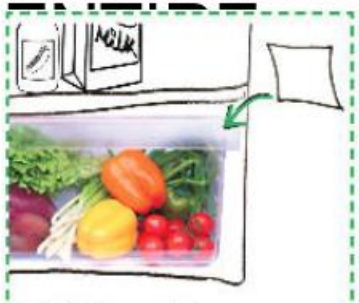
FreshPaper sheets are infused with organic spices that keep fruits & veggies fresh for 2-4x longer, naturally.

Just keep a sheet anywhere you store produce - no wrapping needed!

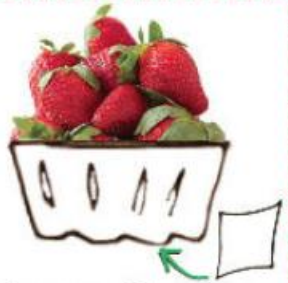
- ✓ Saves Money & Reduces Waste
- ✓ Award-Winning & Patented
- ✓ Made in USA with Organic Spices
- ✓ Recyclable & Biodegradable

Get Fresh >

Just use ONE FreshPaper sheet for an



Fridge Drawer



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Fruit Bowl

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-Chris. Boston. MA

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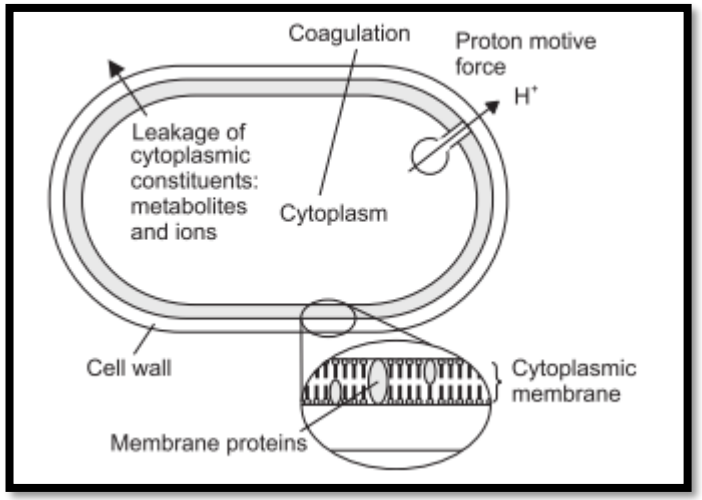
Alternatives to Fenugreek : Essential Oils

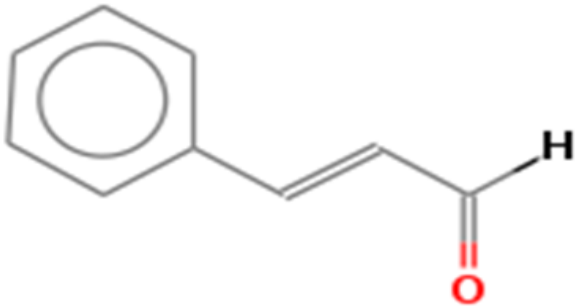
- Secondary metabolites of aromatic plants.
- Hydrophobic subtances
- Antimicrobial properties
  - *Penetration*
  - *Disruption of cell membranes*
  - *Leakage of ions or vital components*



Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods--a review. *International Journal of Food Microbiology, 94(3), 223-53.*

- ✓ Cinnamaldehyde is the major component of CO
  - ✓ **antifungal** (Burt, 2004).
  - ✓ **antiaflatoxigenic** (Manso, Pezo, Gómez-Lus, & Nerín, 2014)
- ✓ **antityrosinase activiy** (Marongiu et al., 2007; Rao & Gan, 2014).
- ✓ The mechanism of action of antimicrobial activity on E. Coli 0157:H7 is destruction of plasma membrane.

- ✓ One of the most studied EOs in the literature with their various and significant antimicrobial activity.
- ✓ Although EOs are generally more effective on G(+) rather than G(-), CO is effective on both.
- ✓ **gram positive bacteria** *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus feacalis*, *Listeria monocytogenes*;,
- ✓ **gram negative bacteria** *Escherichia coli*, *Yersinia enterocolitica*, *Salmonella cholereaesuis*;
- ✓ **fungi** *Candida albicans*;
- ✓ **mold** *Penicillium islandicum*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger* (Chang et al., 2001; López, Sánchez, Batlle, & Nerín, 2005; Wong, Ahmad-Mudzaqqir, & Wan-Nurdiyana, 2014).

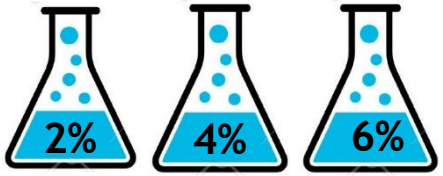


SAMPLE PREPARATION

Kraft Paper (120 g/m^2)

Cinnamon Oil

Ethanol

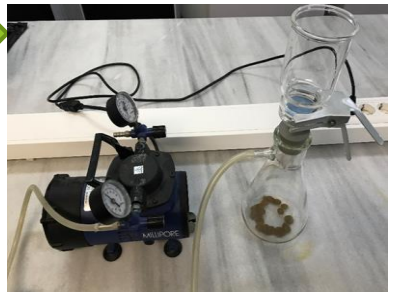


Utrasonic Coating

(80 kHz frequency and 100% power, for 5 min)

Dipping Coating

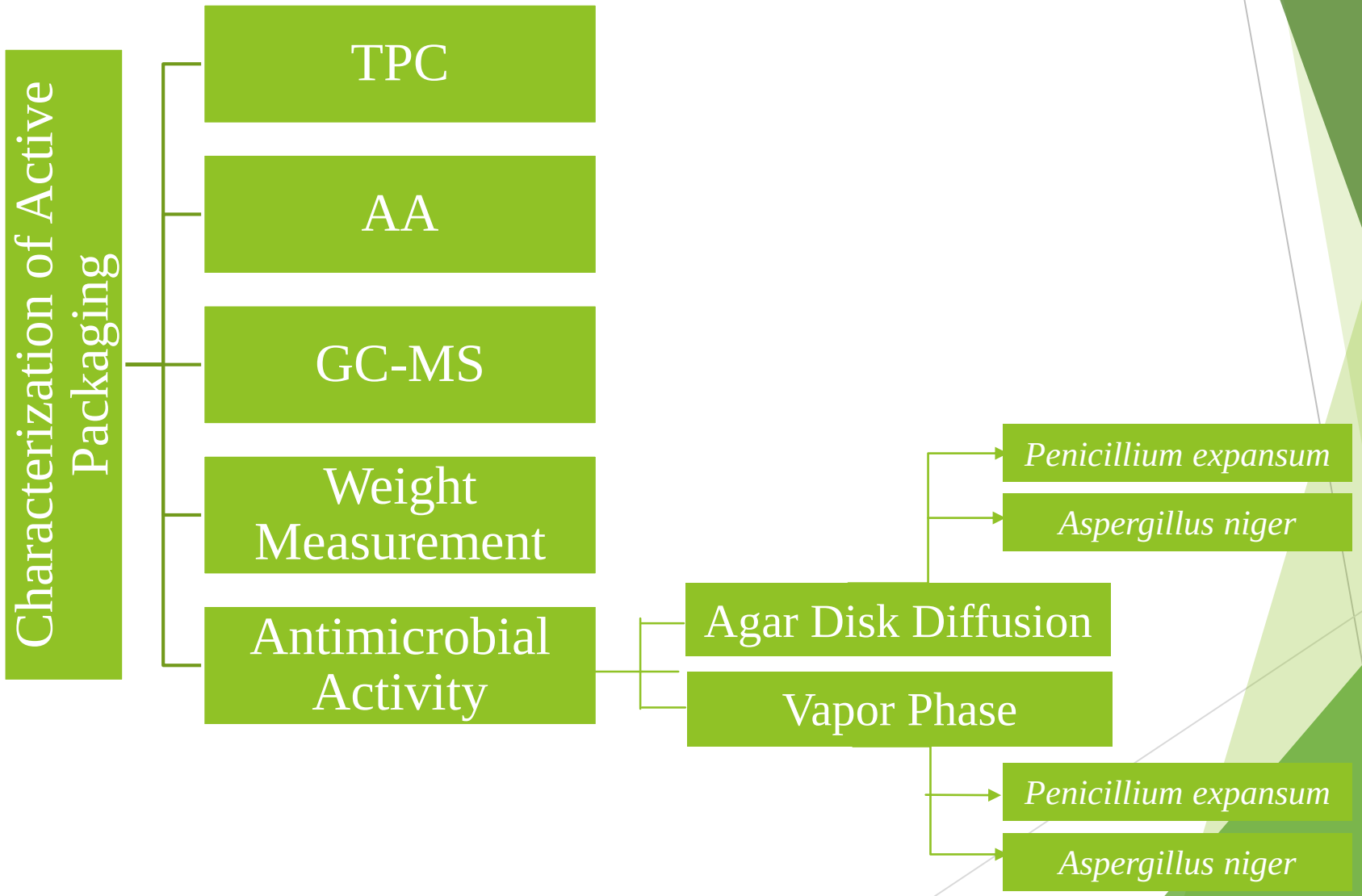
(22 ± 2°C for 5 min)



Vacuum Coating

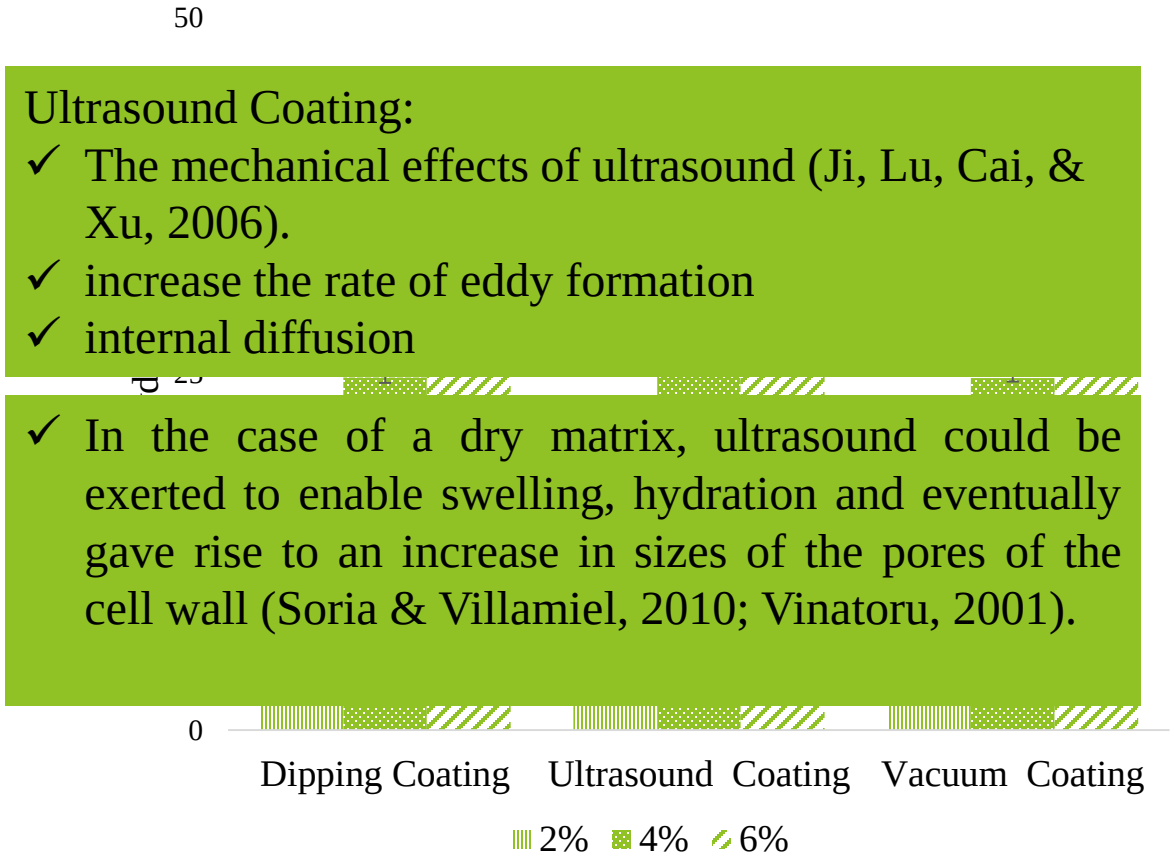
(-50 ± 2 kPa for 5 min)

Drying (22 ± 2°C for 5 min)


Characterization of Active Packaging
TPC
AA
GC-MS
Weight Measurement
Antimicrobial Activity
Agar Disk Diffusion
Vapor Phase
Penicillium expansum
Aspergillus niger
Penicillium expansum
Aspergillus niger

Effect of coating type and concentration of coating solution on cinnamaldehyde content:

‘’*vacuum = ultrasonic > dipping*‘



Ultrasound Coating:

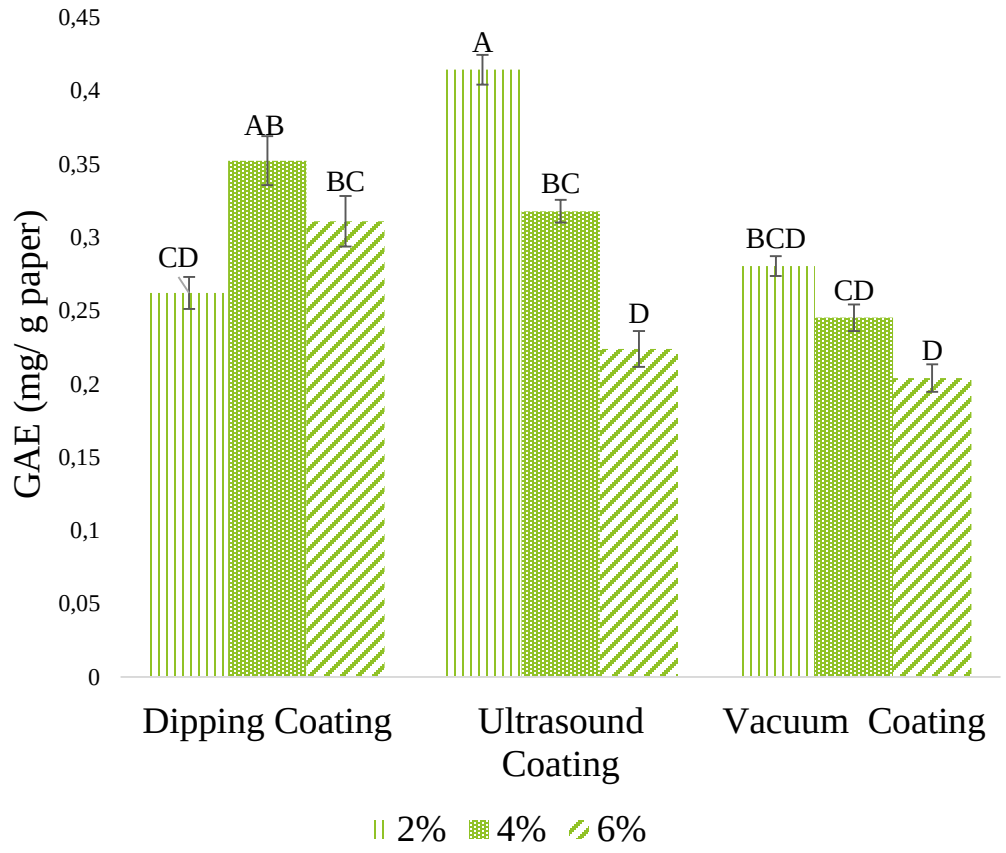
- ✓ The mechanical effects of ultrasound (Ji, Lu, Cai, & Xu, 2006).
- ✓ increase the rate of eddy formation
- ✓ internal diffusion
- ✓ In the case of a dry matrix, ultrasound could be exerted to enable swelling, hydration and eventually gave rise to an increase in sizes of the pores of the cell wall (Soria & Villamiel, 2010; Vinatoru, 2001).

Vacuum Coating:

- ✓ hydrodynamic mechanism (Anino, Salvatori, & Alzamora, (2006) removal of air from the pores

when atmospheric pressure is reached, the solution impregnates the intercellular spaces via capillary action and pressure gradients.

Effect of coating type and concentration of coating solution on TPC



'*ultrasonic = dipping > vacuum*'

Vacuum Coating:

✓ Volatilization of phenolic compounds

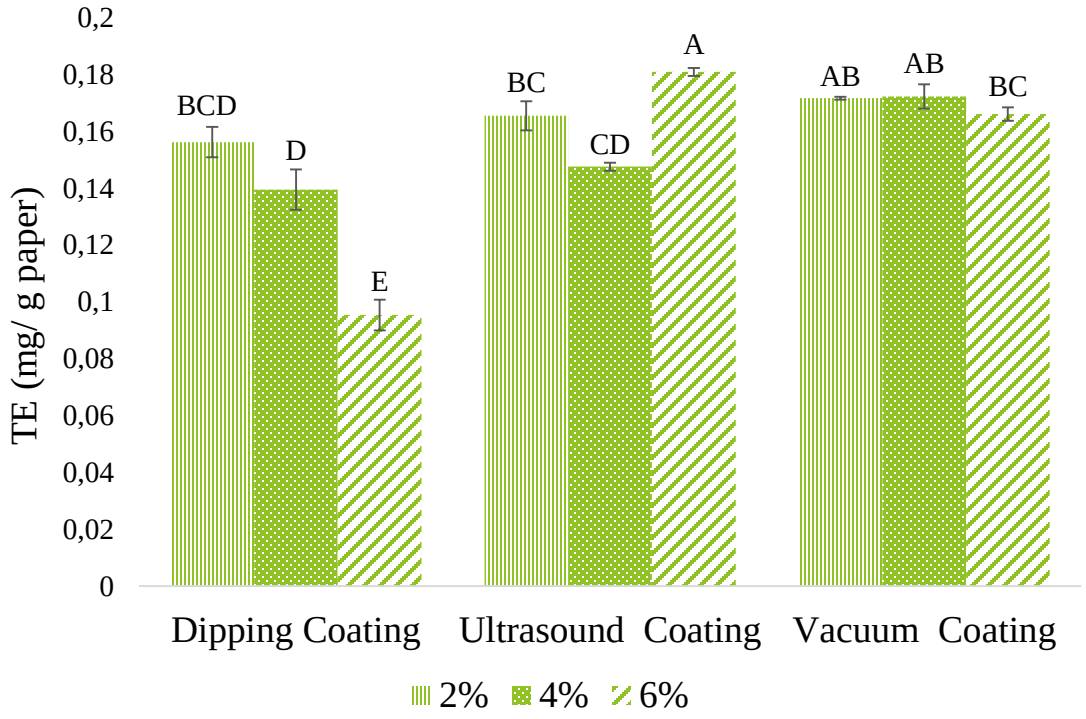
US Coating:

✓ Phenolic compound degradation by cavitation resulting formation of OH· radicals and pyrolysis (Pingret, Fabiano-Tixier, & Chemat, 2013; Rawson et al., 2011)

Effect of coating type and concentration of coating solution on AA:

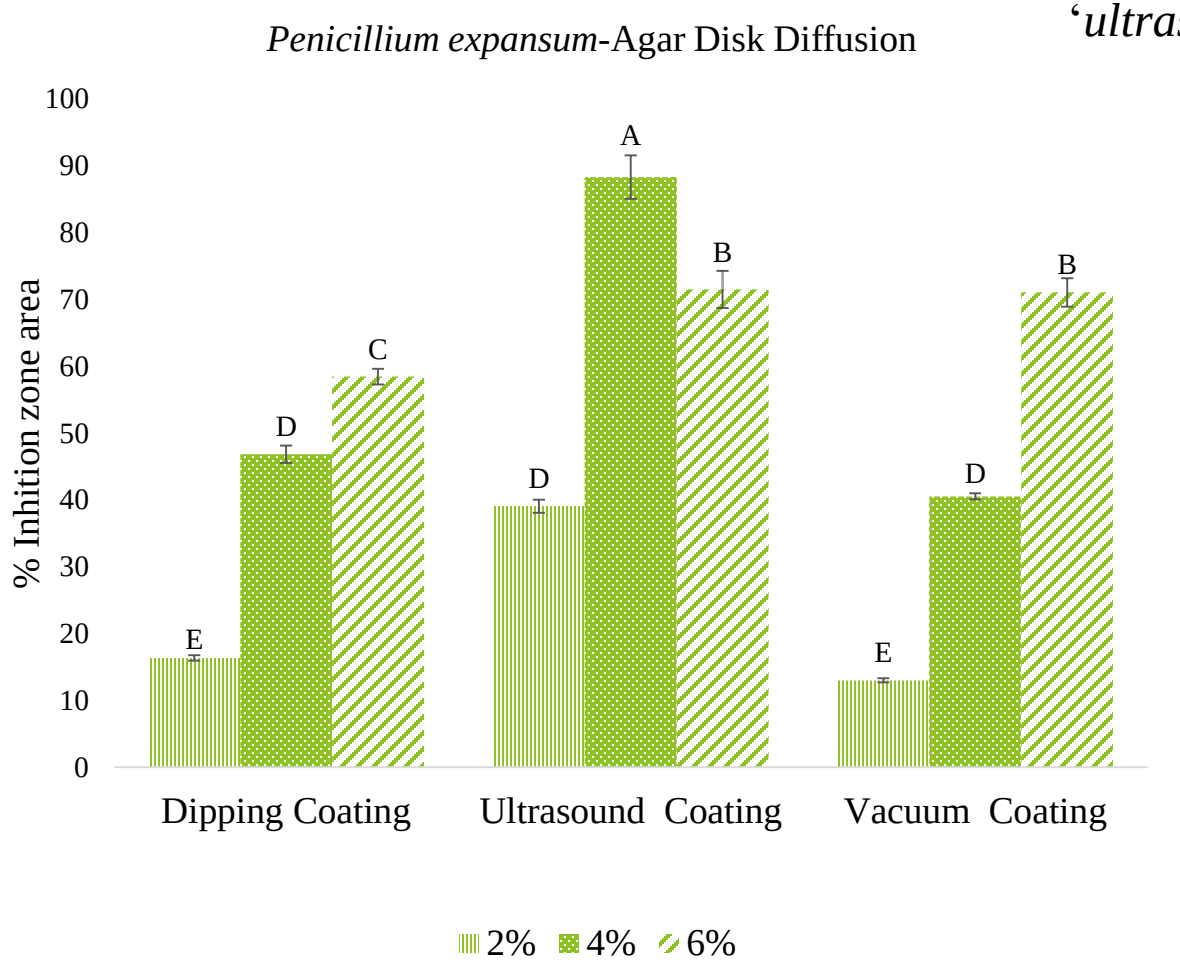
in mg trolox equivalent (TE) per gram paper

‘*vacuum = ultrasonic > dipping* ‘



✓ 6% = 4% > 2%

Penicillium expansum-Agar Disk Diffusion

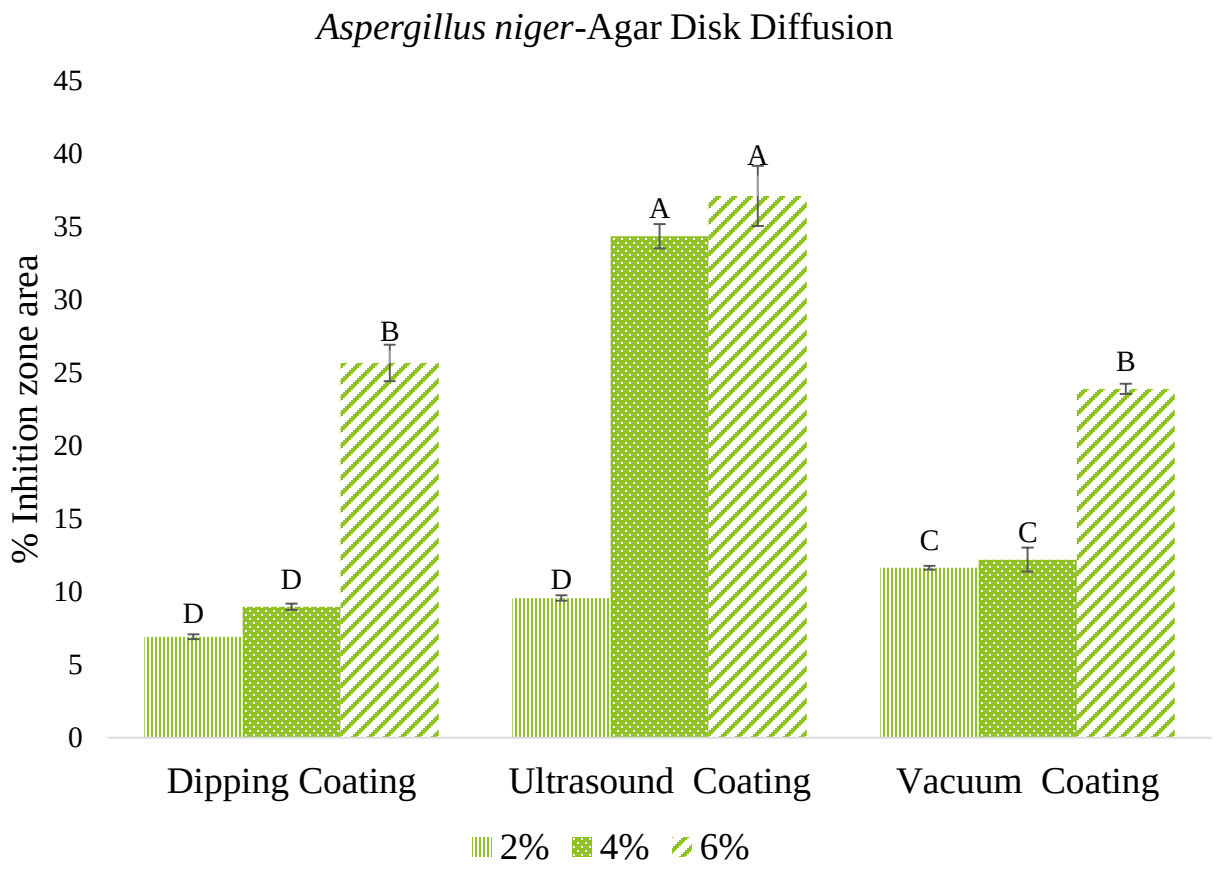


'*ultrasonic* > *dipping* = *vacuum*'

- ✓ Significant difference was found for different concentrations with regard to all coating types ($p \leq 0.05$).

Aspergillus niger-Agar Disk Diffusion

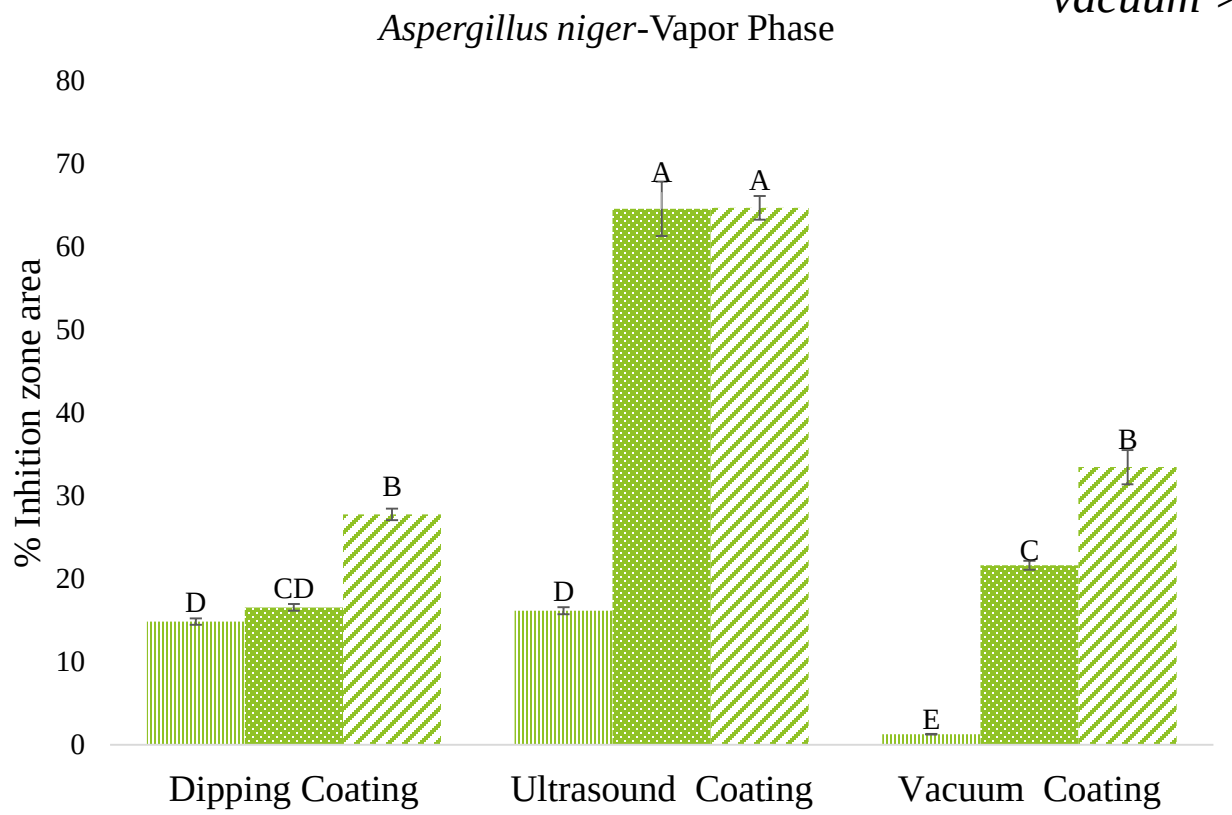
‘*ultrasonic > dipping > vacuum*’



- ✓ Significant difference was found for different concentrations with regard to all coating types ($p \leq 0.05$).

Aspergillus niger-Vapor Phase Test

‘*vacuum > dipping > ultrasonic*’



- ✓ Significant difference was found for different concentrations with regard to all coating types ($p \leq 0.05$).

- ✓ The vapor phase antimicrobial activity of the paper sheets were higher than agar disk diffusion antimicrobial activity. This was an important result, since its antimicrobial effect would reach more places in the refrigerator shelves or grocery baskets.

Olive Leaf Extract

- Important by-product => Olive tree culture and Olive oil industry (Molina Alcaide & Nefzaoui, 1996)

Pruning produces 25kg of by-products (twigs and leaves) per tree per year.

Leaves represent 5% of the weight of olives in oil extraction.

- Turkey is one of the top five countries in terms of olive production.
- The bitter polar glycoside ***oleuropein***, the major constituent of the **secoiridoid** family in the olive trees, has been shown to have an antioxidant activity and its hydrolysis leads to anti-microbial compounds such as **hydroxytyrosol**

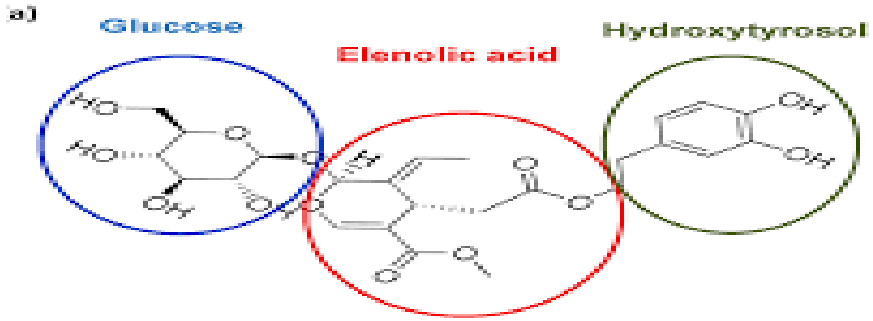


Figure 1. Oleuropein structure (Souilem et al., 2016)

Several biological activities of oleuropein

- Its surface activity is condemned for its biological activities (Di Mattia, Sacchetti, & Pittia, 2011).
- The antimicrobial activity of oleuropein can be attributed to its ability to damage the bacterial membrane (Bisignano et al., 1999).
- Oleuropein, as an antioxidant in a membrane of phospholipid bilayer, is associated with the membrane surface rather than penetration of oleuropein into the membrane (Souilem et al., 2016)
- Controlled release from emulsion system can be provided with the use of surfactant ability of olive leaf extract.
- There are studies about emulsified active agents showed better antimicrobial effect than nonemulsified ones. (Rodriguez, Nerin, & Batlle, 2008)

Objectives

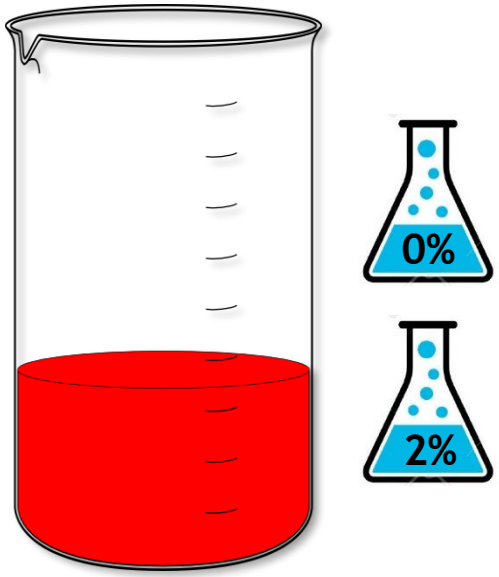
- **Production of the emulsions with olive leaf extract (OLE)**

1. Determining the suitable concentration of OLE in the emulsion
2. Emulsification method to produce the emulsions

- **Their application as active sheet:**

1. Emulsion infusion techniques to produce active sheets ?
2. Antifungal effect on *P. italicum* and *A. Niger* ?
3. Total phenolic content of the sheets ?
4. Antioxidant activity of the sheets ?

Preparation of Nanoemulsion



- Virgin Olive Oil
- Olive Leaf Extract
- Phosphate Buffer
- 1% Tween 80 @ (pH=7)

Blending using a high speed blender (**UltraTurrax**) At 20,000 rpm for 2 min

1400 bar, 5 passes

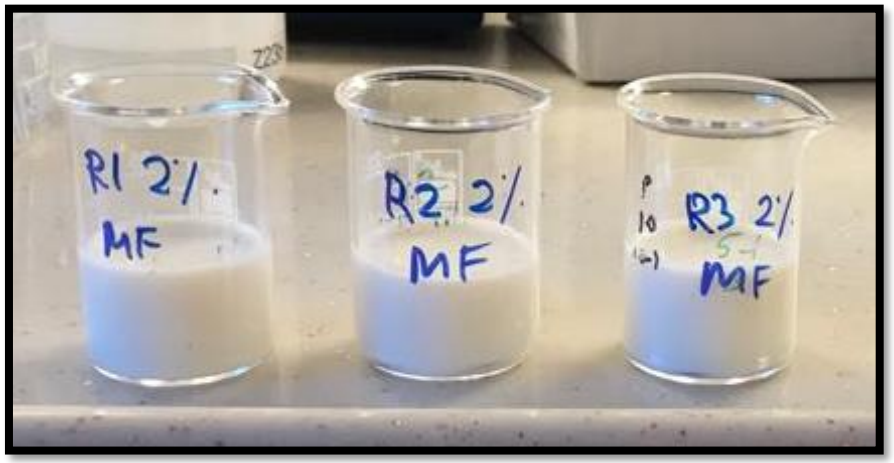
Homogenization by using microfluidization and ultrasonication

75% amp, 5 min

Preparation of the Active Sheets

Ultrasonic Coating

(80 kHz frequency and 100% power, for 5 min)



Dipping Coating

(At room T for 5 min)

Drying

(At room T for 10 min and at 40°C for 5 min)

Phenolics and Antioxidant Activity

5 paper disks were put into 15 ml methanol and waited for 30 min for extraction.

Antioxidant Activity

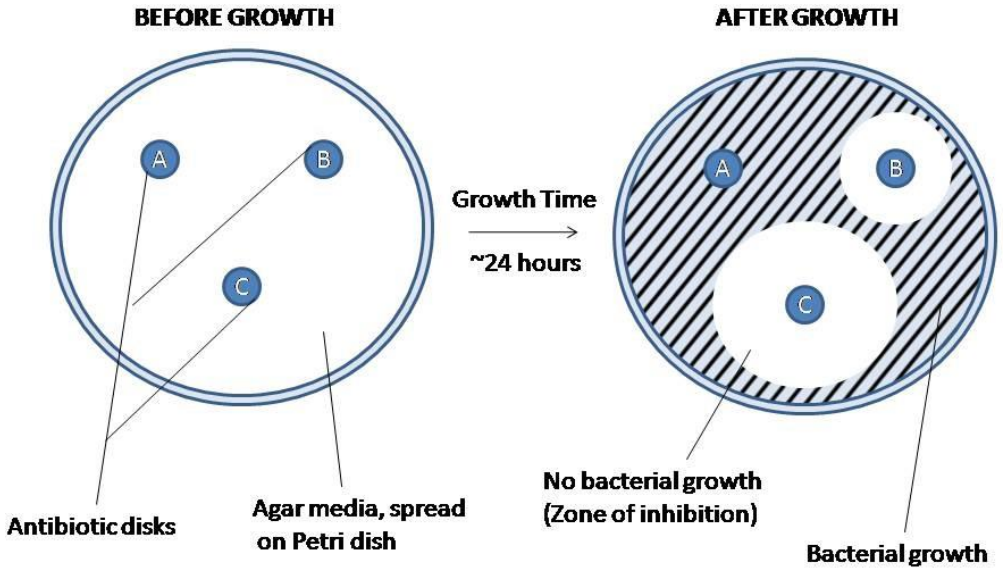
In order to measure antioxidant activity, DPPH free radical scavenging assay was used.

Total Phenolic Content

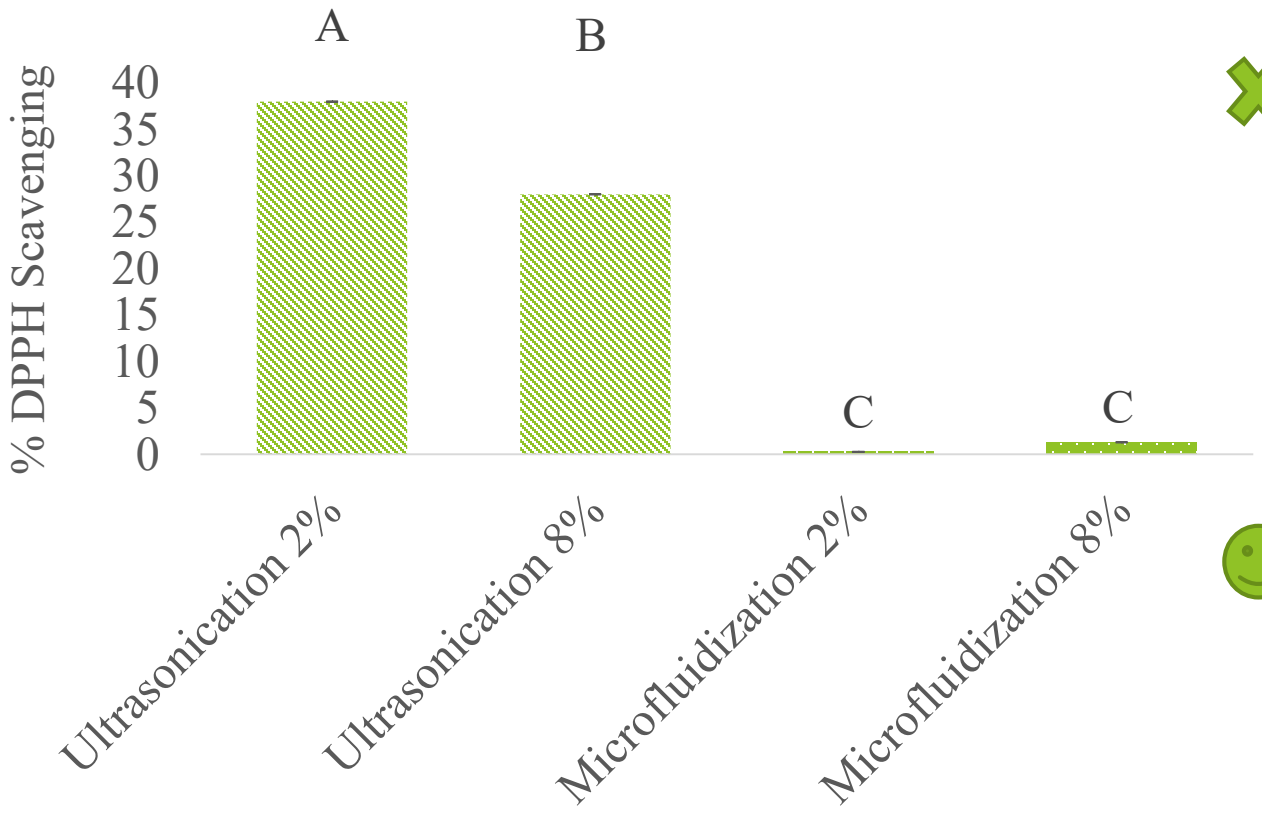
Total phenol content of extracts was determined by the method Folin-Ciocalteu assay.

Antifungal Activity by ‘Agar Disk Diffusion’

- Active paper disks loaded with 0% (control) and 2% OLE emulsion produced with dipping coating and ultrasound coating methods were impregnated into prepared PDA containing mold strains (10^6 spores/ml).
- After 80 hours’ incubation at 25 ± 2°C, the zones around the paper disks were expected to be measured.



Effect of Emulsification Method on AA

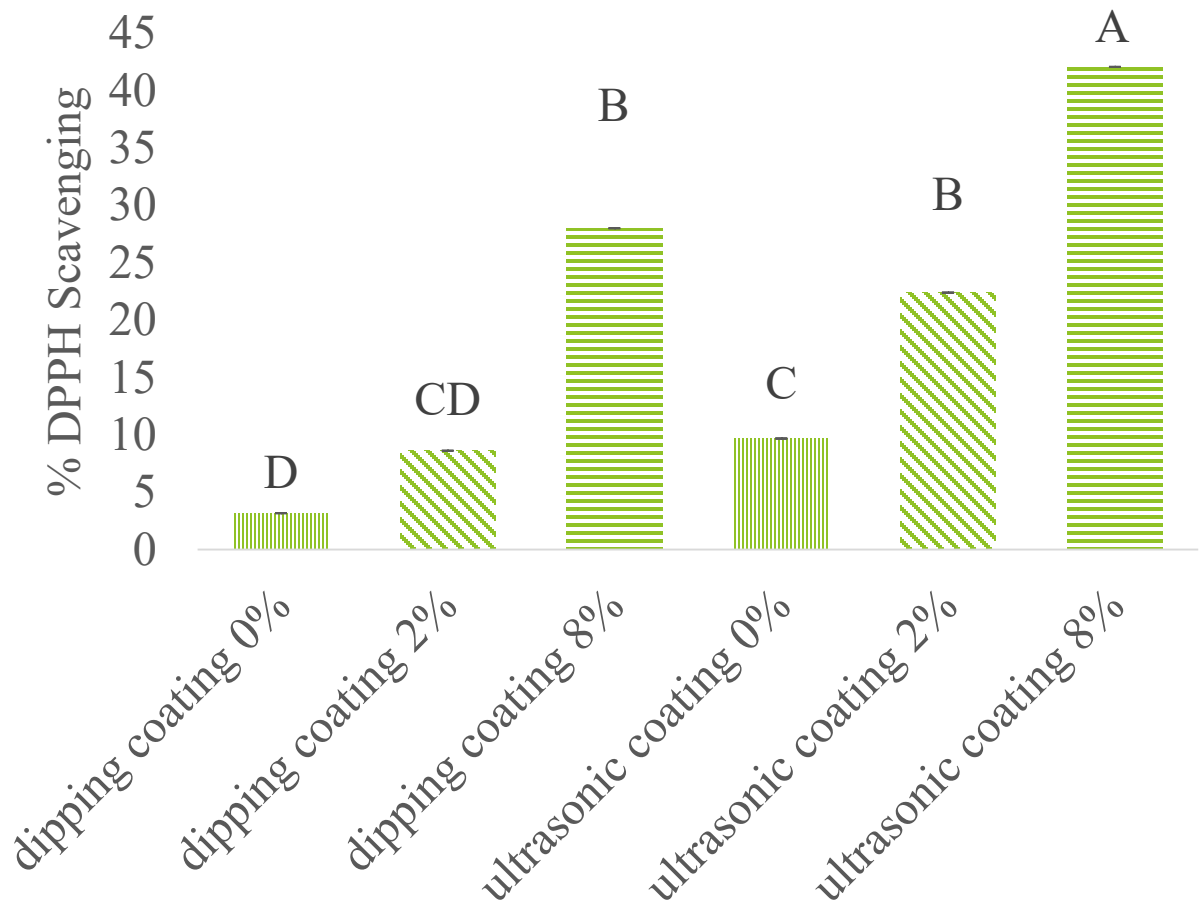


✓ Ultrasonication > Microfluidization

✖ High-pressure homogenizers apply energy input during emulsification is mostly converted to heat (i.e., temperature elevation) degrading heat-sensitive bioactives such as polyphenols and vitamins.

☺ Ultrasonication also produce acoustic streaming that causes microjets in fluids and forms eddies. ➡ Increased mass transfer

Effect of Coating method on AA

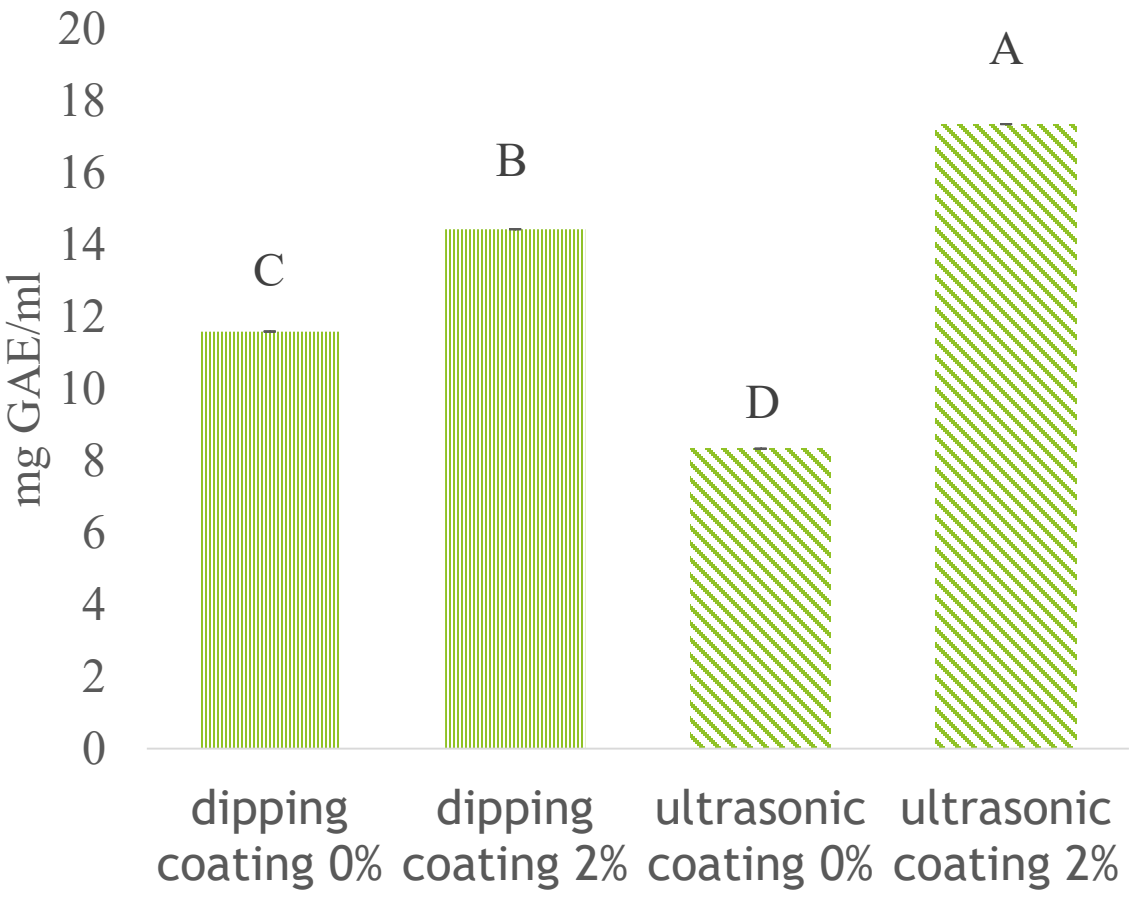


- ✓ Ultrasonic Coating > Dipping Coating
- ✓ 8% > 2% > 0%

US Coating:
- ✓ diffusion rate
- ✓ acoustic streaming

Ultrasonication also produce acoustic streaming that causes microjets in fluids and forms eddies. ➡ Increased mass transfer

Effect of Coating Method on TPC

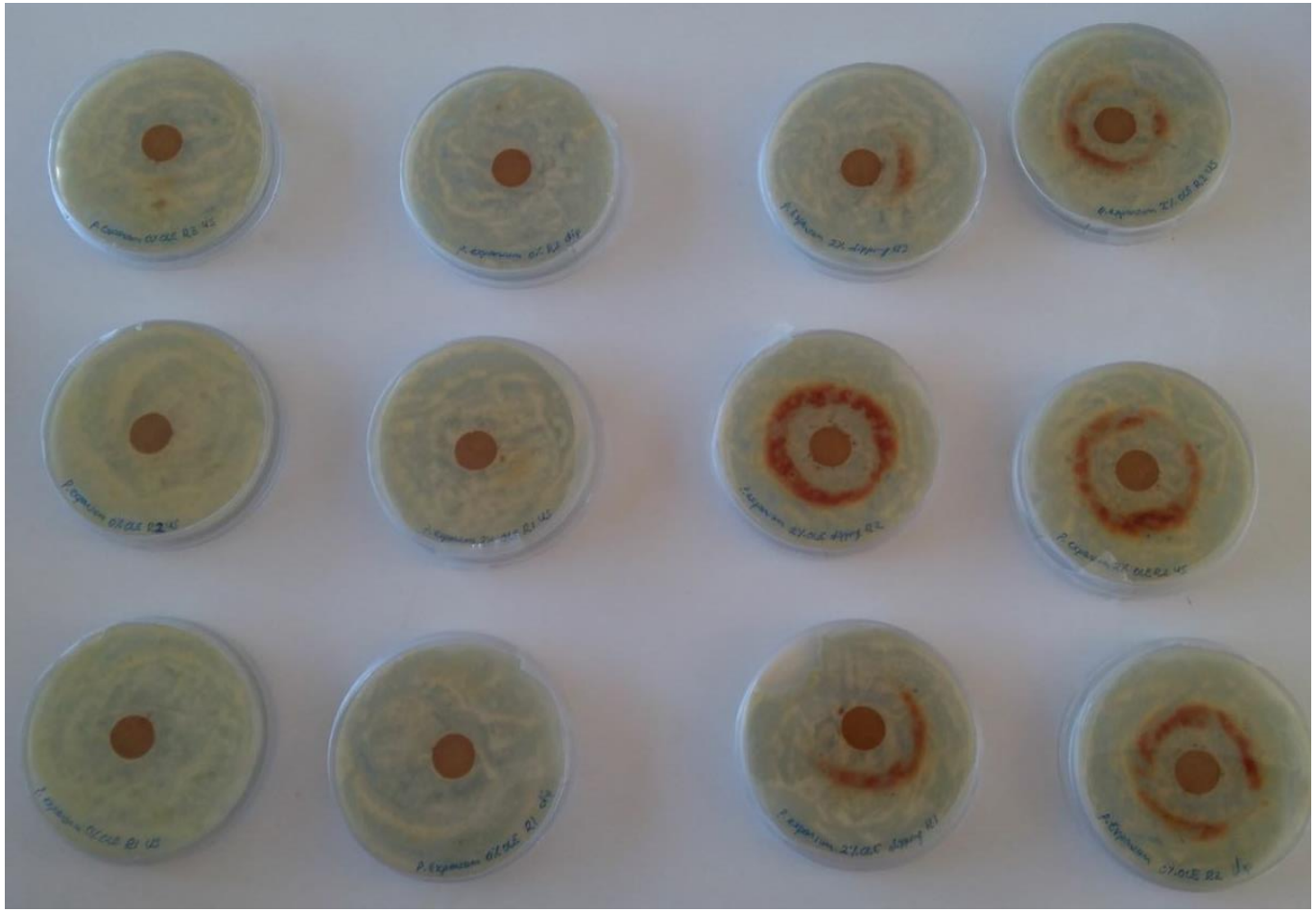


- ✓ Ultrasonic Coating = Dipping Coating
- ✓ 2% > 0%

US Coating:

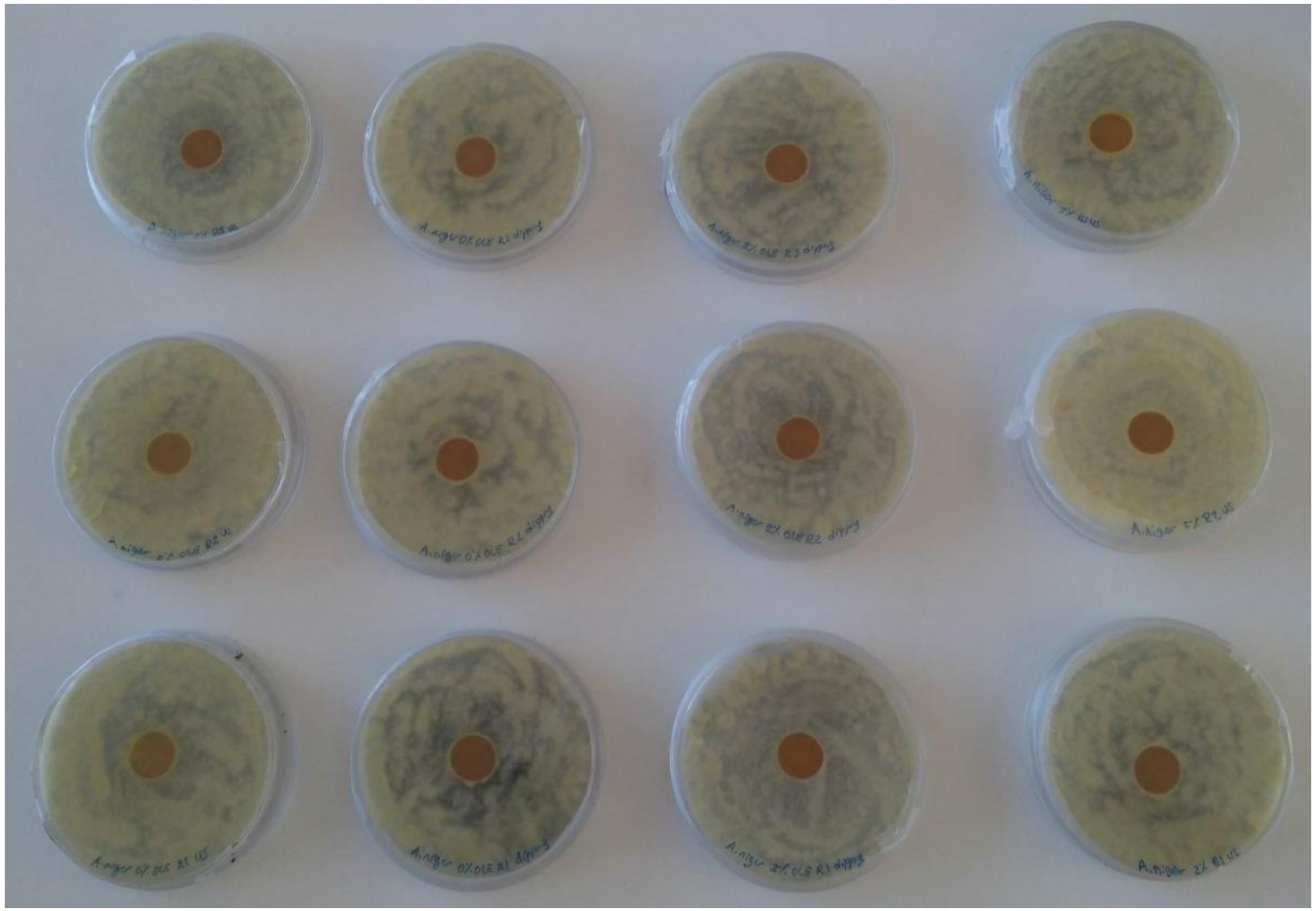
- ✓ Phenolic compound degradation by cavitation resulting formation of OH· radicals and pyrolysis (Pingret, Fabiano-Tixier, & Chemat, 2013; Rawson et al., 2011)

Antifungal Effect on *P. expansum*



0% US 0% Dipping 2% Dipping 2% US

Antifungal Effect on *A. niger*



0% US 0% Dipping 2% Dipping 2% US

Conclusion

- Prepared active sheets with ultrasonic coating showed antioxidant activity.
- In terms of total phenolic content, dipping coating and ultrasonic coating did not gave significantly different results.
- OLE is known to show appreciable activity on *Campylobacter jejuni*, *Helicobacter pylori* and *Staphylococcus aureus*. It was not effective on *P. italicum* and *A. niger*.