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Original article

Properties of beeswax antifungal coatings obtained by high-pressure homogenisation and their application for preserving bananas during storageBridget Agyemang,¹ Joel Grabulos,^{1*} Olivier Hubert,¹ Claire Bourlieu,² Michael Nigen,^{2,3} Marc Lebrun,¹ Fanny Coffigniez,² Valérie Guillard² & Pierre Brat^{1*} ¹ CIRAD - UMR- Qualisud, Dpt Persyst Qualisud, Univ Montpellier, Avignon Université, CIRAD, Institut Agro, IRD, Université de La Réunion, Montpellier, France² Département Génie Biologique Alimentaire - Équipe I2M, Polytech Montpellier, UMR IATE, Montpellier, France³ IATE, Univ Montpellier, INRAE, Institut Agro, Montpellier, France

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Summary Bananas are tropical fruits that are perishable and susceptible to fungal diseases, mainly caused by *Colletotrichum musae*. Antimicrobial emulsion-based coating materials have been used extensively to inhibit the growth of these fungi and extend the shelf life of bananas. Targeted emulsion-based film functionalities offer excellent mechanical, physicochemical and barrier properties and limit fungal growth kinetics. Antifungal compounds, such as essential oils or phenolic compounds, are added to emulsion-based (wax/biopolymer) coatings to enhance their antimicrobial properties. This study set out to formulate a beeswax-based emulsion for banana coatings using high-pressure homogenisation (HPH) and hydrophilic food-grade/organic coating materials. The particle size distribution, stability and mechanical properties of the film-forming emulsions, water vapour and the oxygen permeabilities of the resulting coating were investigated. The most promising sucrose emulsions were tested as coatings on banana fruits to assess their efficacy against moisture and firmness loss during storage. Adding thymol (0.5% w/w) to the sucrose emulsions further enhanced the inhibition of *Colletotrichum musae* growth to its barest minimum of <20%. Emulsion stability was clearly enhanced using HPH while the best inhibition was obtained with a 20% diluted sucrose emulsion containing 0.5% thymol, with or without HPH.

Keywords Banana, beeswax, coating, *Colletotrichum musae*, crown rot, high-pressure Homogenisation, storage, thymol.

Introduction

As a climacteric fruit, banana is harvested at the green stage and undergoes ripening upon maturity when subjected to an ethylene gas atmosphere (Liu *et al.*, 1999). However, during the shipping phase lasting from 10 to 30 days, banana is highly susceptible to several fungal diseases, resulting in massive and extensive post-harvest losses during transportation and storage (Bhuthia *et al.*, 2016). Crown rot disease is the most critical post-harvest disease of bananas, resulting in 2% to 10% losses of marketable fruit (Ranasinghe *et al.*, 2002). In most banana-growing areas, crown rot is mostly controlled by synthetic post-harvest fungicide treatments resulting in potentially health-hazardous

residues of chemical fungicides (Waltner-Toews and McEwen 1994). There has, therefore, been increasing pressure on the banana industry to minimise synthetic fungicides and develop sustainable non-chemical alternative fungicides for controlling post-harvest diseases (Maqbool *et al.*, 2010).

Of the numerous natural antimicrobial components that have been tested for food preservation, several plant essential oils are effective and possess GRAS status. These essential oils include several phenolic compounds, such as thymol with high antimicrobial activity possibly mediated by hydrophobic interaction and destabilisation of micro-organism membranes (Sivropoulou *et al.*, 1996, Guarda *et al.*, 2011). In recent years, thymol has been tested in various bio-active coatings for fruit preservation (Wang *et al.*, 2016, Robledo *et al.*, 2018, Saki *et al.*, 2019).

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The ability of edible coatings to improve the shelf life of fresh fruits and vegetables by reducing the migration of moisture and solids, oxidative reaction rates and modulating gas exchange respiration has led to interest in their research and applications (Shehata *et al.*, 2020, Falguera *et al.*, 2011). Edible coatings can be used to encapsulate anti-browning and antimicrobial agents, as well as other bioactive compounds that can improve shelf life and inhibit pathogen growth on food surfaces (Zanetti *et al.*, 2018, Pinzon *et al.*, 2020). Lipid-based constituents, such as natural waxes, for example, beeswax or carnauba wax, display excellent water barrier properties given their very high hydrophobicity and very low water vapour sorption (Bourlieu, Guillard, *et al.*, 2009), but they have poor mechanical properties. Such lipids are, therefore, often combined with hydrocolloids to take advantage of the distinct functional properties of each class of film-forming materials (Bourlieu, Ferreira, *et al.*, 2009, Bourlieu, Guillard, *et al.*, 2009). Pérez-Vergara *et al.* (2020) confirmed that high concentrations of beeswax in cassava starch-based films produced films with good mechanical properties, lower water vapour permeability (WVP) and less water sorption than the controls without beeswax.

Over the two last decades, high-pressure homogenisation (HPH) has significantly reduced emulsion particle size, leading to more stable emulsions (Amiri Samani and Naji 2019). Indeed, HPH can reduce droplet size to <1 µm and improves the shelf life of emulsions by reducing the emulsion creaming rate (Salem and Ezzat 2019).

Several beeswax-hydrocolloid fruit coatings have been proposed in the recent literature (Amin *et al.*, 2021, Velickova *et al.*, 2013, Perez-Gallardo *et al.*, 2015, Xie *et al.*, 2020, Trinh *et al.*, 2022), but to our knowledge the combined use of HPH to obtain a stable beeswax nano-emulsion and thymol addition, as a natural antifungal compound, has never been tested for fruit preservation. In view of that, the objective of this work was to formulate a beeswax-based antifungal emulsion for banana coatings using the HPH process and food-grade/organic coating materials, for example, beeswax-based materials loaded with thymol as an antimicrobial agent. Our strategy consisted in developing several emulsion-based films with excellent mechanical, physicochemical and barrier properties and assessing their potential for controlling *Colletotrichum musae* growth.

Materials and methods

Materials

Polyvinyl alcohol (Mowiol® 4-88, Mw ≈ 31 000 Da) and sucrose esters of fatty acids (E473) were obtained

from Sigma-Aldrich (Kuraray Europe) and Louis Francois (France), respectively. Beeswax (melting point 64–66.5 °C) was purchased from Droguerie Ecologique® (France). All other chemical agents were of analytical quality and used without further purification.

Formulation of beeswax/water emulsions

Emulsions were prepared as follows: the continuous phase of the emulsion consisted of distilled water pre-heated to 75 °C supplemented with either Mowiol® 4-88 (50 g L⁻¹) or sucrose ester (E473, 50 g L⁻¹) used as the emulsifier. The discontinuous oily phase consisted of 100 g L⁻¹ of beeswax and was also melted at 75 °C using a hot stirrer plate before being added to the aqueous phase. The mixture, kept at 75 °C, was pre-dispersed at 20 500 rpm on a T25 Ultra-Turrax® (IKA) for 10 min with a 5-min rest interval, and the same cycle was repeated twice more and resulted in oil-in-water nano-emulsions (Haq *et al.*, 2016). Lastly, the emulsions were allowed to cool at room temperature (Amin *et al.*, 2019). One portion of the emulsion was used directly for further characterisation, and another portion was processed by high-pressure homogenisation (HPH). The emulsions obtained were coded as sucrose and Mowiol® without HPH (SNH and MNH, respectively) and sucrose and Mowiol® emulsions with HPH (SH and MH, respectively).

High-pressure homogenisation of emulsions

Sucrose or Mowiol® emulsions (SH and MH) were further homogenised using a two-valve high-pressure homogeniser, model APV-1000 bar (SPX, Denmark). The homogeniser containing the emulsion was pre-heated at a pressure of 300 bar for 5 min to achieve the specified temperature of 77 °C. Nine cycles were repeated for the emulsions treated by HPH to narrow down the particle size distribution. At the end of the nine cycles, samples were collected for particle size analysis.

Emulsion characterisation

Particle size distribution

The particle size measurements were reported as an area-based mean diameter: D[3,2] and volume-based mean diameter: D[4,3] and were calculated according to laser light scattering measurements performed on a Malvern Master-sizer 3000 system (Malvern Instruments, UK) (Qian and McClements, 2011).

Emulsion stability test

The colloidal stability of SNH and SH emulsions was analysed using a Turbiscan Tower (Fabra *et al.*, 2009)

equipped with a pulsed near-infrared light source ($\lambda = 880$ nm) and two synchronous detectors, a transmission (T) and a backscattering (BS) detectors (Formulation, France). The samples were loaded into cylindrical glass tubes and scanned throughout its entire height. The backscattering signal (BS, in %) was measured every hour for 6 days at 25 °C. The BS profiles were analysed using the TowerSoft software, version 1.1.0.36 (Formulation, France). The Turbiscan Stability Index (TSI) was determined as the following:

$$TSI = \sum_i \frac{h |scan_i(h) - scan_{i-1}(h)|}{H}$$

The TSI corresponded to the BS variation at all measured position (h) throughout the entire height (H) of the sample between the $scan_i$ and the $scan_{i-1}$. The plot of TSI according to time corresponds to the kinetic behaviour of the samples that takes into account all the physical phenomena (especially destabilisation mechanism) occurring during the measurement.

Self-standing film shaping & characterisation

The solution casting method was used to make the film using emulsions (FFE) with a volume of 10 mL dried at room temperature for 3 days until all water was removed. The drying setups included Teflon plates as an anchor, a 0.25-mm-thick metallic plate and clips to secure the Teflon plates to the metallic plates. The thickness of the resulting film was then measured using a digital micrometre on six different sections of the film to obtain an average thickness estimate (Wu *et al.*, 2020). The films were then analysed for their mechanical properties and barrier properties.

The mechanical properties were determined using a TA.XT2i Plus texture analyser (Texture Technologies Corp., Surrey, UK) equipped with Texture Expert software, at a crosshead speed of 10 mm s⁻¹ with a cell load of 5 kg. The samples followed the same standard and were evaluated with an initial probe length of 60 mm, film width of 5 mm, obeying the average maximum thickness of 0.25 mm in the total portion of the test. Film samples were tested in six replications. Tensile strength, maximum distance and maximum force were determined using a force (N) against time (s) test plot.

Water vapour permeability (WVP, (g cm⁻¹ day⁻¹ - kPa⁻¹)*10⁻³) was determined by the gravimetric method (Amin *et al.*, 2019).

The O₂P values (oxygen permeability) of the SN and SNH films were determined at 23 °C and 100% RH by an oxygen permeability tester (PreSens-GmbH, Fibox 4, Germany) according to Bourlieu, Ferreira, *et al.* (2009).

Banana fruit coatings

Whole banana fruits (4 fruits and 3 replications) were coated with emulsions at three different dilutions (10%, 20% and 50%, w/w) 1 day after formulation/HPH processing using a large paintbrush to ensure a uniform film on the fruit after drying for 24 h. Thymol was added at two different concentrations (0.25% or 0.5%, w/w) in the final diluted emulsion.

Banana samples coated with SNH and SH with different concentrations of thymol were tested in triplicate every two storage days up to the sixth day, after a 1-day ripening treatment. Uncoated control banana fruits ($n = 12$) were also introduced as standards.

In vivo physiological analyses of coated banana fruits vs uncoated control fruits

Fruit firmness was determined by tensile tests (maximum force, N) using a TA.XT2i Plus texture analyser. The test speed was set at 1 mm s⁻¹, with a compression depth of 2 mm, and the texture analyser was fitted with a 0.8 inch diameter spherical probe.

To determine physiological moisture loss, coated ($n = 6$) and uncoated (control, $n = 6$) banana samples were weighed: (i) just after ripening and (ii) on each storage day at (25 °C), over 6 days.

In vivo antifungal assay of coated banana fruits vs uncoated control fruits

The *in vivo* efficacy of sucrose SH emulsions (supplemented or not with thymol, 0.25 and 0.5% w/w emulsion) on the growth of *Colletotrichum musae* was evaluated on bananas. The method developed by Lassois *et al.* (2010) was used, and fruits were evaluated at colour stage 4 (yellow with green tips).

Statistical analysis

All tests were performed in triplicate unless otherwise stated. The means were compared with the IBM SPSS Statistics *T*-test at 5% error probability for the minimum difference in multiple comparisons. An analysis of variance (ANOVA) was used to distinguish between the treatments at $P < 0.05$.

Results and discussion

Particle size distribution

After HPH was applied on the formulated emulsions (600/60 bar, 77 °C, 9 cycles), whatever the stabiliser (sucrose or Mowiol®) and in agreement with previous studies (Chen *et al.*, 2010, Tan and Nakajima 2005), a large reduction (~5 to 10 times) in the size (D_{50}), the

average surface diameter D [3; 2] and the average volume diameter D [4; 3] of the particles was observed (Table 1).

The overall particle size distribution of emulsions is displayed in Fig. 1. MH and SH had a narrow uni-modal distribution (span of $0.8 \mu\text{m}$, between $0.1 \mu\text{m}$ and $1 \mu\text{m}$) with a mode at $0.5 \mu\text{m}$ (Fig. 1). In comparison, SNH and MNH displayed pluri-modal curves spreading widely from 0.1 to $1000 \mu\text{m}$. The decrease in droplet size with increasing pressure can be attributed to the magnitude of the disruptive forces generated within the homogenisation chamber (Jafari *et al.*, 2008), with turbulence leading to the breakup of the dispersed phase into small droplets (Floury *et al.*, 2000).

Stability of the emulsions

The backscattering (BS) profiles (%) of non-homogenised (SNH) and homogenised (SH) sucrose emulsions are shown in Fig. 2. After the SNH storage during 6 days at 25°C , the BS decreased at the bottom of the emulsions while it increased at the top (Fig. 2). This behaviour reflects the destabilisation of SNH emulsion with its clarification at the bottom and its creaming at the top. This SNH destabilisation was confirmed by the constant increase of the TSI during the 6 days of storage (Fig. 2). The BS measurements of HPH sucrose emulsion (SH) evidenced very little changes during the 6 storage days as shown both by the superimposition of the BS profiles from days 0 to 6 and the TSI evolution (Fig. 2). Therefore, the HPH treatment of sucrose emulsions allowed their stabilisation, especially by reducing and homogenising the droplet sizes (Fig. 1). The reduction and homogenisation of the droplet sizes are usually associated with an increase in the number of the droplets in the emulsion that was confirmed by a higher BS value (72%) at day 0 for SH emulsion in comparison with SNH one (52%).

As revealed by Grillet *et al.* (2004), the degree of backscattering explains also the levels of viscosity of

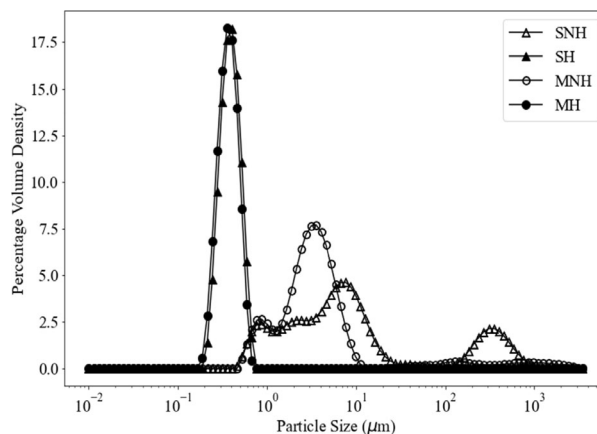


Figure 1 Representation of the particle size distribution of sucrose and Mowiol® direct (SNH and MNH) and HPH (SH and MH) emulsions.

the emulsion: with increased viscosity, the percentage of backscattering increases. Consequently, it unambiguously appeared that sucrose as an emulsifier combined with HPH process could be promoted as a potential optimum film-forming emulsion.

Mechanical properties and water vapour permeability (WVP) of the film-forming emulsion

According to Haq *et al.* (2016), maximum force interprets the tensile strength of each film, while the elongation at break explains the elasticity and flexibility of each film. Coatings made from emulsified beeswax exhibit high hydrophobic properties and relatively good mechanical properties (Rullier-Birat *et al.*, 2015).

Films of uniform thickness were successfully achieved for the different modalities after the solvent casting method. The force needed for film rupture (tensile strength) and elasticity were roughly divided by 2–4 when HPH was not applied (SH/SHH and MH/MSH) (Table 2).

Emulsions with a lower droplet size after HPH, using both Mowiol® and sucrose as the emulsifier (MH and SH, respectively), displayed better mechanical properties compared to those without HPH. Indeed, when HPH was used both the Mowiol® and sucrose emulsions turned from dispersive to colloidal form, with the more cohesive films being obtained after HPH (MH and SH), presenting a maximum force 2–4× higher than those without HPH (MNH and SNH).

The absence of any significant O_2P difference between the sucrose emulsion with HPH (SH) or without HPH (SNH) ($0.14 \pm 0.2/ 0.13 \pm 0.10 \text{ mol m}^{-1} \cdot \text{s}^{-1} \text{ Pa}^{-1} \cdot 10^{-14}$) showed that there was no significant difference between the two films at $P_{\text{value}} < 0.05$ and

Table 1 Particle size distribution (D_{50}), area-based particle size diameter (D [3; 2]) and volume-based particle size diameter (D [4, 3]) of sucrose and Mowiol® emulsions without HPH (SNH and MNH) and with HPH (SH and MH)

Emulsions	$D_{50} (\mu\text{m})$	D [3; 2] (μm)	D [4; 3] (μm)
SNH	6.54 ± 0.17^a	3.13 ± 0.05^a	76.90 ± 0.67^a
SH	0.41 ± 0.01^b	0.40 ± 0.01^b	0.42 ± 0.01^b
MNH	3.43 ± 0.02^c	2.48 ± 0.02^c	57.31 ± 0.02^c
MH	0.39 ± 0.01^b	0.37 ± 0.01^d	0.40 ± 0.01^b

Different letters in the same column indicate significant differences ($P < 0.05$).

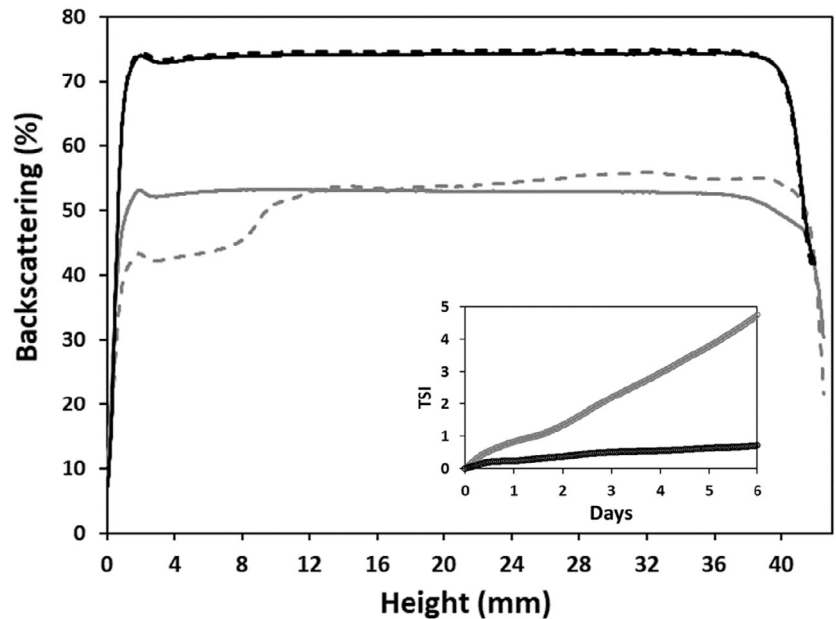


Figure 2 Backscattering profiles (%) of SH (dark) and SNH (grey) emulsions immediately after their preparation (continuous line) and 6 days of storage (dashed line) at 25 °C. The insert corresponded to the temporal evolution of the Turbiscan Stability Index (TSI) of SH (dark) and SNH (grey) emulsions.

Table 2 Mechanical properties and water vapour permeability (WVP) of the film-forming emulsion

Film/ Emulsion	Mechanical properties		WVP (g cm ⁻¹ day ⁻¹ kPa ⁻¹)* 10 ⁻³
	Tensile strength (N)	Elongation at break (mm)	
SNH	0.40 ± 0.19 ^a	3.52 ± 1.48 ^a	15.28 ± 0.05 ^a
SH	1.94 ± 0.20 ^b	4.56 ± 0.59 ^a	19.43 ± 0.04 ^b
MNH	1.95 ± 0.19 ^b	7.24 ± 2.04 ^b	67.54 ± 1.67 ^c
MH	5.59 ± 0.28 ^c	6.95 ± 0.94 ^b	31.94 ± 0.07 ^d

Different letters in the same column indicate significant differences ($P < 0.05$).

that both films were, therefore, suitable coating materials for reducing the rate of oxygen permeability. In all events, the controlled oxygen permeability of the films will facilitate slow ripening of bananas treated with the emulsions, by reducing the respiration rate that leads to rapid oxygenation from the tissues of banana peel (Taweechat *et al.*, 2021, Dwivany *et al.*, 2020).

As a consequence, at this stage, since the sucrose emulsions had less phase separation and lower tensile strength (flexibility), only these coatings (SNH and SH) were used for the next *in vivo* fruit coatings.

Fruit firmness and moisture loss

Fig. 3a shows that as the concentration of the coating emulsion increased (dilution ratio of the emulsion from 10 to 1), coated fruit firmness also increased.

These results are in agreement with those of Tanada-Palmu and Grosso (2005), Amal *et al.* (2010) establishing the beneficial impact of coating on overall quality. In addition, SNH coatings had increased firmness compared with SH coatings.

From Fig. 3b, it appears that as the concentration of the coating emulsion increased, fruit moisture loss also decreased. Moreover, fruits coated with the HPH film-forming emulsion (SH) exhibited higher water losses than those obtained without HPH. Indeed, homogenisation, when applied to sucrose, made the resulting film more permeable.

Reduction of crown rot using fruit coating

Lastly, the beeswax coating with thymol as the active component was evaluated as a potential trigger for better control of crown rot incidence. We, therefore, used two different dilution ratios (10% or 20%) supplemented, or not, with thymol (T, NT) at two different concentrations (0.5, 0.25% w/w dry emulsion). Whatever the dilution ratio or the applied concentrations, not any thymol odour traces were detectable on the coated fruits after 2 days storage or more.

Crown rot incidence was determined after 10 days of banana inoculation (Fig. 4) and the presence of the coating induced an effective inhibition of *C. musae* growth, hence less incidence of crown rot compared with the control. Adding thymol to the coatings further enhanced the inhibition of crown rot to its barest minimum of <20%. As the concentration of beeswax in the coating increased, *C. musae* incidence

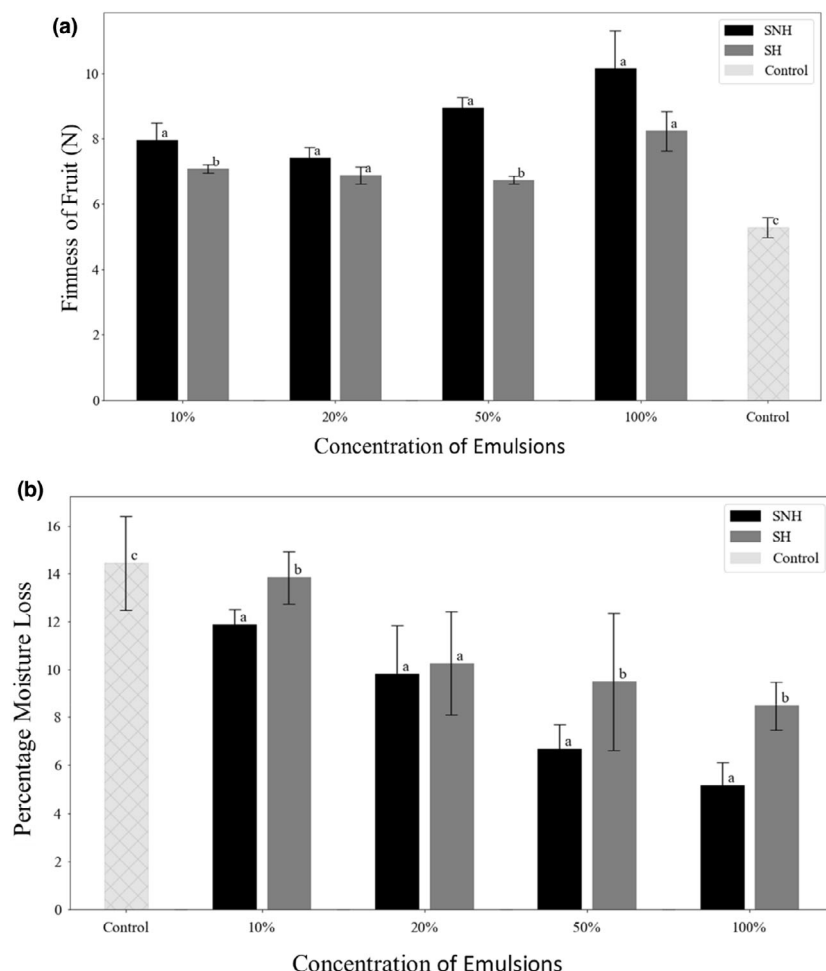


Figure 3 Firmness (a) and moisture loss percentage (b) of fruit coated with emulsions at different dilution percentages (10%, 20%, 50% and 100%, w/w). Different letters in the same poll indicate significant differences between the concentrations of the emulsions and the control ($P < 0.05$).

decreased. The best inhibition was achieved with a 20% dilution of the emulsion supplemented with 0.5% thymol. Although SNH and SH could differ in some properties, such as their particle sizes, their ability to release effective compounds like thymol was not jeopardised, suggesting that either of the emulsions will be effective for encapsulating thymol in a beeswax hydrophobic matrix for an antifungal assay (Amal *et al.*, 2010).

To conclude, while sucrose used as a beeswax emulsifier appears to be the best candidate, providing a more stable emulsion, the impact and merits of HPH need to be further assessed. By greatly reducing the average sucrose emulsion droplet size, this homogenisation process unambiguously increased emulsion stability. When a sucrose emulsifier was used, the resulting film on the fruit was thinner. Moreover, a sucrose film-forming emulsion without any emulsion treatment (SNH) maintained better fruit firmness and more effectively reduced water losses, again making the merits of this HPH process questionable.

Conflict of interest

The authors declared that there is no conflict of interest for any of the co-authors of this publication.

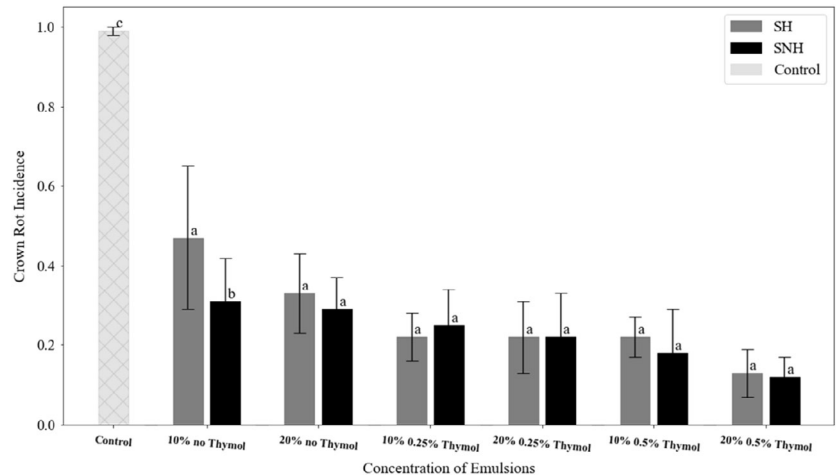
Ethical approval

Ethics approval was not required for this research.

Author contributions

Bridget Agyemang: Formal analysis (lead); investigation (equal); methodology (lead); validation (equal); writing – original draft (lead). **Joel Grabulos:** Formal analysis (equal); investigation (equal); methodology (equal); project administration (equal); resources (equal); supervision (equal); writing – review and editing (lead). **Olivier Hubert:** Validation (equal); writing – review and editing (equal). **Claire Bourlieu:** Formal analysis (supporting); supervision (supporting); validation (equal); visualization (equal); writing – review and

Figure 4 Crown rot incidence on banana determined at different sucrose dilution percentages (10% and 20%) and thymol addition (NT = No Thymol, T = Thymol) after 10 days of banana inoculation. The values are means \pm standard deviation. Different letters in the same poll indicate significant differences between the concentrations of the emulsions and the control ($P < 0.05$).



editing (equal). **Michael Nigen:** Methodology (equal); resources (equal); validation (equal); writing – review and editing (equal). **Marc Lebrun:** Investigation (equal); methodology (equal); software (lead); supervision (equal); writing – review and editing (equal). **Fanny Coffigniez:** Investigation (equal); resources (equal); supervision (equal); validation (equal); writing – review and editing (equal). **Valrie Guillard:** Supervision (equal); validation (equal); writing – review and editing (equal). **Pierre BRAT:** Conceptualization (equal); formal analysis (equal); funding acquisition (lead); methodology (equal); project administration (lead); validation (equal); writing – original draft (equal); writing – review and editing (lead).

Peer review

The peer review history for this article is available at <https://publons.com/publon/10.1111/ijfs.15865>.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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- These authors applied High Pressure Homogenization to realize very fine oil-in-water emulsions. The effect of homogenizing pressure (HPH from 20 to 300 MPa) was studied on model emulsions stabilized by whey proteins. As a major result, droplet size was reduced

with increasing pressure. We therefore decided to use HPH to reduce particle size of the beeswax film's forming emulsion and as a consequence, stabilize this resulting emulsion.

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- This team determined the antimicrobial (AM) properties of plastic flexible films with a coating of microcapsules containing carvacrol or thymol as natural AM agents. The results of this study confirm the suitability of using microencapsulated thymol incorporated in polymer films for antimicrobial food packaging. The authors definitely concluded that further studies on emulsified antimicrobials evaluated with real foods are required in order to confirm their assumption. We therefore used this paper first to confirm the antimicrobial properties of thymol but also to incorporate it in a specific fruit's coating.
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This team used the properties of beeswax to improve barrier properties of biodegradable materials. In order to study occlusive properties of these coatings, they measured water vapor permeability and contact angle measurements. They concluded that coatings made from emulsified beeswax exhibit high hydrophobic properties and relatively good mechanical properties compared to chitosan film without coatings. As a consequence, we decided to incorporate beeswax into our emulsions to improve their properties.

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