BIO-BASED ANTIMICROBIAL PACKAGING PAPER COATINGS CONTAINING COMPLEX COACERVATION MICROCAPSULES

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Abstract: *The main purpose of the research was to develop a bio-based environmentally-friendly antimicrobial packaging paper coating, containing microcapsules with a natural antimicrobial core, and a natural polymer wall. The essential oil of Cymbopogon citratus (citronella oil, lemongrass oil), known for its broad-spectrum antimicrobial activity and uses in food, pharmaceutical, cosmetic and repellent products, was chosen as the active compound. Microcapsules were produced by the complex coacervation process. Two pairs of macromolecular colloids of opposite charges were used for the formation of microcapsule walls: gelatin-carboxymethyl cellulose, and gelatin-gumi arabic. Sodium dodecyl sulphate was optionally used as an emulsifier. Microcapsule walls were cross-linked with glutaraldehyde or hardened with a natural tannin (extracted from chestnut wood) also known for its antimicrobial activity. Microcapsules were characterised by light-transmission and scanning electron microscopies. A container type microcapsules with a distinct liquid core and elastic natural polymer wall were obtained, with diameters 10 to 200 µm. Antimicrobial effects of microencapsulated citronella oil were determined on Bacillus subtilis and Escherichia coli. Freshly prepared aqueous microcapsule suspensions were formulated with or without binders and coated on packaging paper on a laboratory coating device. Mechanical activation of pressure-sensitive microcapsules was achieved by a 3 kg or 5 kg metal weight pulling*

test. Antimicrobial activity of coated papers with citronella oil coacervation microcapsules was tested by a modified agar plate method in sealed petri dishes, where mechanically activated microcapsules released vapors of citronella oil into the atmosphere of the petri dish, thus affecting the growth of microorganisms. The conclusion is that complex coacervate citronella oil microcapsules can be used for environmentally-friendly antimicrobial coated packaging paper products that require a combined prolonged antimicrobial activity and a quick release of the active substance by mechanical pressure.

Keywords: antimicrobial paper coatings; bio-based; microcapsules; complex coacervation; citronella; antimicrobial testing

1 INTRODUCTION

Interest in the microencapsulation of active compounds has rapidly increased in the last three decades in a wide range of industries and applications. The primary purpose of microencapsulation is to make a protective envelope around active compounds in a way that protects them from the surrounding environment and releases them at a desired place or time. The products are small microcapsules, typically with a core-shell structure (Figure 1).

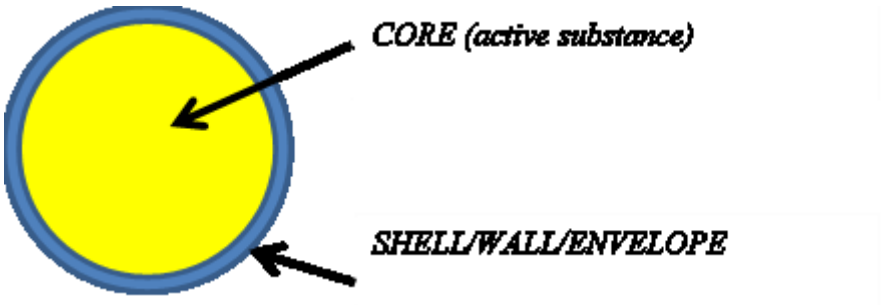


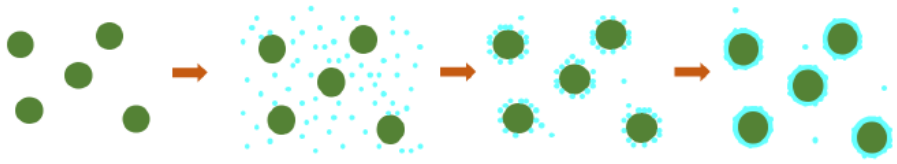
Figure 1: Typical container-type microcapsule structure

Examples of microcapsules used in paper industries include pressure-sensitive copying papers (Kukovič, 1995; Hirasawa et al, 2002), thermochromic and photosensitive papers (Li et al, 2007), electronic paper (Kim et al, 2013), fragranced papers (Watanabe et al, 2014), and antimicrobial paper products (Li et al, 2018). Some examples of products are presented in Figure 2.

(a) *(b)* *(c)* *(d)*

Figure 2: Examples of paper products with incorporated microcapsules: (a) copying paper (koehlerpaper.com), (b) scratch and sniff (newtonprint.co.uk), (c) scented ink (rtdodge.com), (d) electronic paper (electronicproducts.com)

For the preparation of microcapsules, different technologies have been developed, depending on the intended use/desired characteristics, such as final application, size and, the shape of microcapsules, mechanical properties of microcapsules, aggregate state of active compound, type of active compound, etc. Microencapsulation methods are generally classified into physical, chemical and physicochemical techniques. Complex coacervation belongs to physicochemical techniques and exploits macromolecular colloids phenomena of coacervate formation under specific conditions to create microcapsules shell. The process is shown in Figure 3.



(a) *(b)* *(c)* *(d)*

Figure 3: Microencapsulation with complex coacervation. (a) formation of oi in water emulsion, (b) initiation of coacervation, (c) shell formation, (d) crosslinking hardening the wall

The interest in the bio-efficacy of essential oils and their uses as natural antimicrobials in environmentally friendly products has during recent years considerably increased. One of them is Citronella oil, obtained from lemongrass plants of genus Cymbopogon, particularly of C. citratus (Figure 4), and predominantly consists of monoterpenes, such as citral (39-48%), neral (32-35%), mircen (11-15%) and geraniol (3-5%). Citronella oil has a broad-spectrum antimicrobial activity and is used in food (Alarono-Moyano et al, 2017) pharmaceutical and cosmetic products, natural pesticides and mosquito repellents (Songro et al, 2018).

Figure 4: Citronella plant and oil extracts (pureplantessentials.com)

Due to its reported antimicrobial activities in the scientific literature (Leimann et al, 2009; Bustos et al, 2016; Wang et al, 2018) and identified need of antimicrobial protection of paper products, it is a good candidate for wide paper applications. As the citronella oil components are volatile and subject to degradation under environmental influences, it cannot be directly used on paper products. To solve this problem, microencapsulation technologies seem to be the first choice. So, our work aimed to successfully microencapsulate citronella oil with the use of environmentally friendly components and develop and evaluate antimicrobial effectivity of packaging paper coatings.

2 MATERIALS AND METHODS

2. 1 Microcapsules preparation and their paper coatings

Complex coacervation process was carried out in a laboratory reactor equipped with an overhead stirrer turbine impeller according to our modified procedure using acid-treated gelatin (Sigma-Aldrich), low viscosity carboxymethylcellulose (CMC, Hercules) or gum arabic (Sigma-Aldrich) as shell materials. The temperature was maintained at T = 50 °C. The emulsification took place at 600–800 rpm, 10–30 min. To form microcapsules, the coacervation was initiated with dilution with water and by lowering pH to 3.9–4.3 (1–2 h, T = 50 °C, 600–800 rpm). After cooling the system to room temperature (1 °C/min, 600–800 rpm), the coacervate walls were crosslinked by glutaraldehyde (Acros Organics) or by tannin (Tanin Sevnica). The obtained suspensions of citronella microcapsules were applied to the paper surface (paper for flexible packaging, 53 g/m^2) using a lab-scale rod coater (rod No. 8)

(RK Printcoat Instruments). The coated sheets were dried in a lab dryer for 2 minutes at 110 °C.

2. 2 Mechanical activation and determination of antimicrobial activity

The mechanical activation of coated papers containing citronella oil microcapsules was performed with weight (3 kg and 5 kg) pulling tests. Weights were pulled three times along coated paper test specimens just before antimicrobial evaluation. The antimicrobial activity was tested on agar plates 140 mm Petri dishes with E. coli and B. subtilis. Both bacteria were cultivated in the medium Standard count agar, 37 °C, 24 h. After 24 h, 2 mL of Ringer solution was added. The inoculum ~1×10^6 CFU/mL was diluted to the range of concentrations from 10^5 to 10^0. The prepared microorganism suspensions were put in a Petri dish. The paper coated with microcapsules was attached to the inner part of Petri dish, mechanically activated and immediately sealed with parafilm. The results were evaluated after 24h and 48h of incubation.

3 RESULTS AND DISCUSSION

Non-diluted citronella oil was successfully microencapsulated with the complex coacervation method. Two pairs of macromolecular colloids of opposite charges were used for the formation of microcapsule walls: gelatin-carboxymethyl cellulose (CMC) and gelatin-gum arabic. Figure 5 shows both types of produced microcapsules under the transmission light microscope (TLM). A different shape of microcapsules can be seen; gelatin-CMC microcapsules are round with a standard core-shell form, while gelatin-gum arabic microcapsules have a characteristic rugby-ball shape. The microcapsule size ranged from 10-200 μm and was influenced by the use of emulsifiers and by process conditions

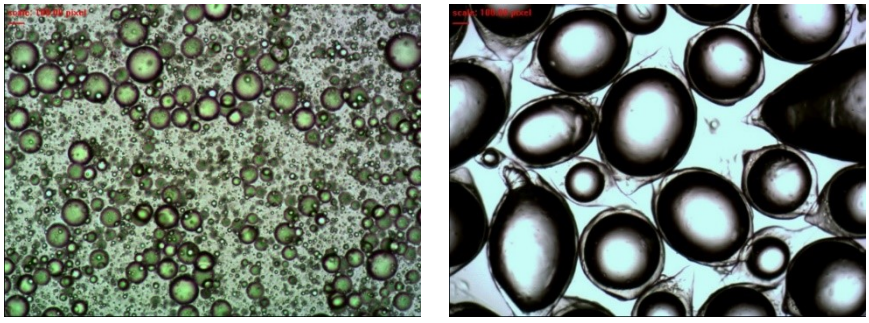


Figure 5: TLC of gelatin-CMC microcapsules (left, 40 ×) and gelatin-gum Arabic (right, 100 ×) microcapsules with citronella oil

Gelatin-gumi arabic microcapsules were successfully applied to the paper surface with a lab-scale rod coater. After mechanical activation, they broke and released microencapsulated citronella oil (Figure 6).

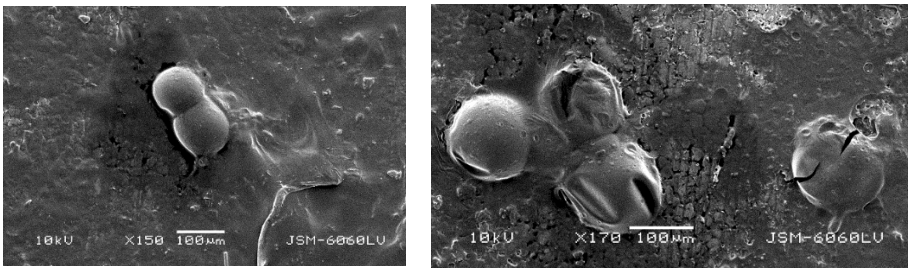


Figure 6: SEM of microcapsules (100 ×): left applied to paper and right releasing the citronella oil (broken)

The antimicrobial activity of coated papers with citronella oil coacervation gelatin-gumi arabic microcapsules was tested in sealed petri dishes. Inhibition of microorganisms for both tested bacteria E. coli and B. subtilis was evaluated after 24 and 48 hours (Table 1). For B. subtilis a total inhibition was observed after 48 hours for the activated and inactivated samples. This indicates a slow release of citronella oil from coacervation microcapsules due to their partially permeable walls without a need for their mechanical activation. With E. coli, a small growth in the inactivated sample was detected, so in this case, there was a need for mechanical activation of microcapsules to achieve the optimal antimicrobial activity.

Table1: Antimicrobial activity of coated paper with citronella oil coacervation microcapsules after 24 and 48 hours

	24h			***48h***		
	activated	***inactivated***	***blank***	***activated***	***inactivated***	***blank***
E. coli 10^1 CFU/plate	*0*	*18*	*63*	*1*	*23*	*74*
B. subtilis 10^1 CFU/plate	*0*	*0*	*68*	*0*	*0*	*72*

A comparison of antimicrobial activity between E. coli and B. subtilis indicates their different sensitivity to citronella oil components. E. coli seems to be more resistant to citronella oil than B. subtilis.

Examples of antimicrobial tests for E. coli are presented in Figure 7. Covers with added paper coatings with coacervation microcapsules are placed above the agar plates in petri dishes. In the blank sample, extensive growth of E. coli can be seen, while the test specimens remain intact. Similar growth can be

observed in the blank sample of B. subtilis (Figure 8), while in the activated and inactivated samples a total inhibition of growth can be observed.

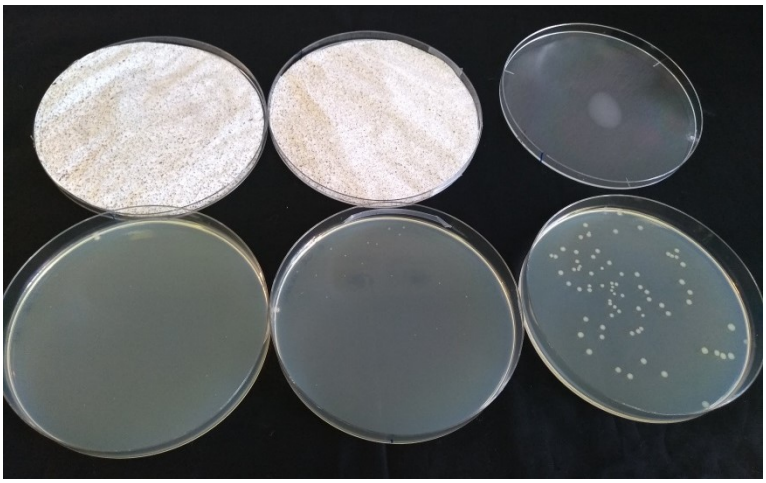


Figure 7: Antimicrobial activity to E. coli (left activated paper, middle inactivated paper, right blank sample) after 48 hours

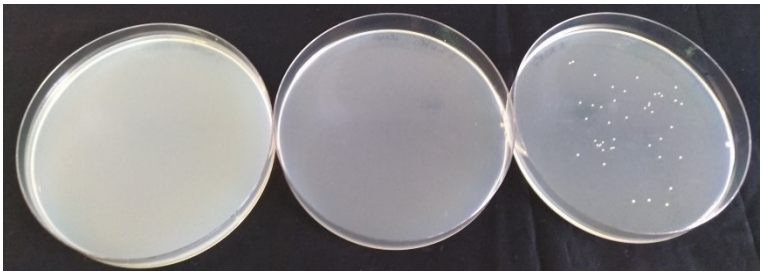


Figure 8: Antimicrobial activity to B. subtilis (left activated paper, middle inactivated paper, right blank sample) after 48 hours

The results indicated that citronella oil coacervation microcapsules can be used for environmentally-friendly antimicrobial coated paper packaging products, which require a combined prolonged antimicrobial activity and a quick release of the active substance by mechanical pressure.

4 CONCLUSIONS

Non-diluted citronella oil from *Cymbopogon citratus* was successfully microencapsulated with the complex coacervation of gelatin and carboxymethyl cellulose, or gelatin and gumi arabic coacervate pairs. The microcapsules from 10-200 µm in diameter were applied to the paper surfaces. Antimicrobial activity tests with standard bacteria indicated a slow release of citronella oil from

coacervation microcapsules, due to the partially permeable walls. To achieve a quick release and optimal antimicrobial activity, microcapsules need to be mechanically activated by pressure.

The citronella oil microcapsules can be applied in innovative environmentally-friendly packaging products with functional antimicrobial protection. Due to microcapsules structure and size, a combined prolonged antimicrobial activity and a quick targeted release of the active substance by a mechanical pressure can be achieved at multiple uses of the product.

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5 REFERENCES

Alarcón-Moyano, J.K.; Bustos, R.O.; Herrera, M.L.; Matiacevich, S.B. (2017) Alginate edible films containing microencapsulated lemongrass oil or citral: Effect of encapsulating agent and storage time on physical and antimicrobial properties. J. Food Sci. Technol., 54, pp. 2878–2889.

Bustos, R.O.; Alberti, F.V.; Matiacevich, S.B. (2016). Edible antimicrobial films based on microencapsulated lemongrass oil. J. Food Sci. Technol., 53, pp.832–839.

Hirasawa, A.; Naoi, S. (2002) Back Carbon Ink, and Tamperproof Pressure-Sensitive Copying Sheet Using the Same. Patent JP2002129077A, 9 May 2002.

Kim, C.A.; Oh, J. (2013) Color Electronic Paper Displays Using Black Matrices and Methods of Fabricating the Same. Patent US20130163067A1, 27 June 2013.

Kukovič, M. (1995) Tiskarska Brezsajna Kopirna Barva. Patent SI9300539A, 30 April 1995.

Li, X.; Zhang, N.; Sun, S.; Lai, W.; Fu, G. (2007) Imaging Method and Printer Applying Photo-Thermal Sensitive Material. Patent CN101020394A, 22 August 2007.

Li, Y.; Lei, Y.; Wei, L. (2018) Preparation Method of Color-Developing Antimicrobial Sucrose Wrapping Paper. Patent CN107604752A, 19 January 2018.

Leimann, F.V.; Gonçalves, O.H.; Machado, R.A.; Bolzan, (2009) A. Antimicrobial activity of microencapsulated lemongrass essential oil and the effect of experimental parameters on microcapsules size and morphology. Mater. Sci. Eng. C 2009, 29, pp. 430–436.

Songkro, S.; Yapong, P.; Puechpan, P.; Maneenuan, D.; Boonme, P. (2018) Microencapsulation of citronella oil for mosquito repellent: Preparation and evaluation of release characteristics. Songklanakarin J. Sci. Technol. 2018, 40, 767–765.

Wang, J.; Li, X.; Chen, M.; Chen, Z.; Wu, H.; Zhang, P.; Hu, Y. (2018) Fabrication of sustained-release and antibacterial citronella oil-loaded composite microcapsules based on Pickering emulsion templates. J. Appl. Polym. Sci. 2018, 135, 46386.

Watanabe, M.; Watanabe, Y.; Kawasaki, K.; Ogawa, A.; Yoshimatsu, T.; Kato, S.; Otohata, T. (2014) Printing Paper Containing Microcapsules of Oily Substances. Patent JP2014169512A, 18 September 2014.