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Microencapsulation improves the selective antibacterial activity of citrus essential oils

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The use of essential oils in animal breeding have been growing during the last 20 years due to their potential bioactivities to replace antibiotics. In pig production, antibiotic have contributed in improving the productivity by controlling enteric diseases caused by enterotoxigenic *Escherichia coli* (ETEC) colonization in the pig gut. Particularly, citrus EOs, a vital by-products of citrus processing industries, could turn an excellent and feasible alternative for this purpose due to the vast availability of these oils in the global market. Currently, Brazil is the main producer and exporter country of citrus oils worldwide (United Nations 2019). Thus, this study aimed to evaluate the antibacterial activity of commercial citrus EOs, raw and microencapsulated, on ETECs strains and on *Lactobacillus* species. Firstly, six citrus EOs were screened by disc diffusion method on four ETECs isolated from pig gut and on two *Lactobacillus* species. Results showed the citrus EOs had a selective antibacterial activity, that was, higher activity on ETECs (pathogenic bacteria) than on *Lactobacillus* sp. (beneficial bacteria). Furthermore, *E. coli* U21 was the most resistant ETEC and *L. rhamnosus* was the most sensitive beneficial bacteria to the activity of the citrus oils. Subsequently, polar and non-polar GC-MS analysis showed that limonene was the major compound present in them. However, a multiple factorial analysis (MFA) showed that four of the six citrus EOs had a very similar chemical composition profile and that nine minor common compounds were detected in these four oils. In addition, a principal component analysis (PCA) revealed that these four oils presented the most selective antibacterial performance, but Brazilian Orange Terpenes (BOT) oil highlighted as the most selective one ($p \leq 0.05$). MIC and MBC determinations by microdilution method showed that BOT presented lower MIC and MBC on *E. coli* U21 (1.85 mg/mL) than on *L. rhamnosus* (3.70 and 7.40 mg/mL), thus reaffirming its selective activity. SEM analysis also showed that higher alterations, damages and cell destruction were produced on *E. coli* U21 cells than on *L. rhamnosus* cells by BOT effect. In addition, BOT was microencapsulated by spray-drier and four different formulations of wall material was proved, being the best one the composed by chitosan and modified starch. The total oil content of microcapsules was 13.2% and the efficiency of encapsulation reached 61.2%. The antibacterial effect of the microencapsulated BOT (MBOT) was tested, and MIC and MBC values shown that microencapsulation enhanced the BOT antibacterial effectiveness since lower amount of the raw BOT oil in the capsules were needed to inhibit or kill *E. coli* U21 (3.5 mg of MBOT/mL ≈ 0.463mg of raw BOT/mL; 7.0 mg of MBOT/mL=0.93 mg of raw BOT/mL), than *L. rhamnosus* (14 mg of MBOT/mL ≈ 1.85 mg of raw BOT/mL).

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