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Effect of the nano- and microencapsulation of vanillin and cinnamaldehyde on their antimicrobial activity in a whey protein beverage

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The food industry generally uses synthetic antimicrobials for food preservation as an effective tool to inhibit the growth of microorganisms. However, the use of these chemicals generates rejection by consumers, leading to interest in natural antimicrobials. However, many natural antimicrobials, such as vanillin (VAN) and cinnamaldehyde (CIN) are hydrophobic and direct incorporation into high-moisture foods is challenging. One way to increase miscibility in the food matrix is by encapsulating the antimicrobials through oil-in-water emulsions. In this sense, the objective of this study was to evaluate the antimicrobial activity of microencapsulated vanillin and cinnamaldehyde (ME-VAN and ME-CIN) in comparison with the respective nanoencapsulated systems (NE-VAN and NE-CIN) against *Escherichia coli* and *Listeria innocua* inoculated in a pasteurized whey protein beverage, and on the microbiota of the same unpasteurized beverage. For this, the whey protein beverage flavored with apple juice (70:30 v/v) was pasteurized (80°C, 10 min) in a Thermomix. Then, the beverage was fractioned (50 mL) and the bottles were added with 0.75 or 1 g/L of ME-CIN and NE-CIN, or 1 and 1.5 g/L of ME-VAN and NE-VAN. The bottles were inoculated with *E. coli* or *L. innocua* (final concentrations ≈ $5x10^5$ CFU/mL) and stored at 4°C for 14 days. The microbiological counts were conducted every 2 days. To study the effect of the antimicrobials on the microbiota of the beverage, the unpasteurized beverage was used, the same concentrations of antimicrobials were added, and was stored in the dark at 4°C. Microbial enumeration (total aerobic count (TAC), fungi, and yeasts) was conducted every 2 days. Regarding the nanoemulsions, NE-CIN had a notably greater effect than NE-VAN, exceeding 5 log cycles of inactivation of both *L. innocua* and *E. coli*. While NE-VAN (1.5 g/L) achieved only 2.0 and 1.7 log CFU/mL inactivation, respectively. Comparing NE-CIN and ME-CIN, the nanoemulsion was significantly more effective than the microemulsion ($p < 0.05$); however, vanillin showed no significant difference between de NE-VAN and ME-VAN. Regarding the effect of the emulsions on the beverage microbiota, NE-CIN and ME-CIN (0.75 g/L) reduced the TAC count from the initial $1.4x10^3$ to ≈ $1x10^1$ CFU/mL in 21 days of storage; while NE-VAN and ME-VAN reduced TAC only up to 3.8 $x10^2$ and $1.2x10^2$ CFU/mL, respectively. However, ME-VAN was ineffective in inhibiting yeast growth ($3.0x10^4$ CFU/mL after 21 days), while NE-VAN and both encapsulated cinnamaldehyde systems reduced yeast and molds counts in the stored beverage. In conclusion, for cinnamaldehyde the nanoemulsion was significantly more effective than the microemulsion. However, for vanillin the type of encapsulation did not affect the antimicrobial activity. *L. innocua* was more sensitive to encapsulated natural antimicrobials than *E. coli*. Furthermore, cinnamaldehyde nano- and microemulsions were also the most effective in controlling the growth of total aerobic

counts, molds, and yeast in the protein beverage during 21 days of storage.

Agradecimentos: