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BİLDİRİ ÖZETLERİ
ABSTRACTS

OP3
EVALUATION OF HONEY CELL-INDUCED EFFECTS ON BACTERIA

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Introduction
Although there exist numerous studies to establish antimicrobial activity of different types of honey, few studies describe the mechanisms of their antimicrobial action. Flow cytometry is an analytical method which allows to characterize cell populations at single cell level. Cells are suspended in a liquid media and illuminated by a laser beam, under these conditions cells produce signals that can be scattering signals and/or fluorescence signals when they are dyed. In this way and using specific dyes it is possible to study cell viability and cell functionality at different physiological target sites. The main aim of this work is to establish the mechanism of antibacterial action of various types of honey by flow cytometry. To our knowledge this is the first attempt directed at determining the antibacterial action mechanisms of honey using this technique.

Honey and microorganisms tested
Three Spanish types of honey from different floral origins, avocado honey (*Persea americana*), chestnut honey (*Castanea sativa*) and a multiflower honey were selected from a previous study as the samples that showed best antibacterial activity. These honey samples were compared with manuka honey MGO 550 + (Manuka Health, New Zealand), as a reference for a honey with a well described antimicrobial effect.
The effectiveness of these types of honey were tested against the Gram-positive *Staphylococcus aureus* 86 and the Gram-negative *Escherichia coli* 515 from the Spanish Type Culture Collection (CECT).

Cytometry assay
A cell suspension containing 106cel/mL in exponential growth phase was used for each microorganism. 1mL aliquots of this suspension were exposed to the different types of honey at previously established minimal lethal concentration (MLC) and twice of this, during different incubation times at 30 °C in a shaker incubator. After this treatment, cells were centrifuged and the pellet was washed twice in PBS (phosphate buffered saline) and then finally stained with different fluorochromes. Membrane integrity was studied using propidium iodide, membrane depolarization with Bis-(1,3-dibutylbarbituric acid) trimethineoxonol [DiBAC4(3)], cell metabolism using Calcein-AM, alterations in nucleic acids with SYTO 9 and ethidium bromide in tests to determine the action into efflux pumps and possible alterations in nucleic acids. The flow cytometric analysis was performed using a BD FACSCalibur TM flow cytometer and results were analysed by Cell Quest Pro software (version 4.0.2, BD Biosciences).

Results
No significant differences related to mechanisms of antibacterial action of honey were observed between Gram-positive and Gram-negative bacteria and no significant differences were observed between the different honey samples including manuka honey. Early depolarization was observed with all honey samples in both kinds of microorganisms. Metabolic inactivation in *S. aureus* was seen with all honey samples even at lower concentrations used, this study was not carried out in *E. coli* (this species is not able to metabolize Calcein-AM). Propidium iodide was incapable of entering in bacterial cells at the assayed honey concentrations and the incubation times used. Changes in nucleic acids were observed both using ethidium bromide and SYTO 9. Slightly modifications related to efflux pump were observed. Taking the observed effects into account is necessary to approach new strategies to confirm these results.

Keywords
honey, flow cytometry, antibacterial mechanisms