Electronic Supplementary Material

Multifloral white honey outclasses Manuka honey in Methylglyoxal content: assessment of free and encapsulated of both Methylglyoxal and Anti-Microbial Peptides in liposomal formulation against toxigenic potential of *Bacillus subtilis* Subsp spizizenii strain

Journal: Food & Function Manuscript ID: FO-ART-02-2022-000566

Nadia Taïbi, *^{a, b} Rachid Ameraoui, ^a Amel Kaced ,^a Mohamed Abou-Mustapha, ^a Abdelghani Bouchama,^a Ahmad Djafri, ^a Amina Taïbi, ^c Kahina Mellahi, ^a Mohamed Hadjadj, ^a Souad Touati, ^a Zola Badria, ^a Souhila Djema, ^a Yasmine Masmoudi, ^a Sarah Belmiri, ^a Farida Khammar ^b

Corresponding author: Nadia Taibi Nadbio71.doc@gmail.com

^a Centre de Recherche Scientifique et Technique en Analyses Physico-chimiques CRAPC, BP 384, Bou-Ismail, 42004, Tipaza, Algérie

Supplementary Figures (s)

| S1: | The | linear | response | of | 2-methylquir | oxaline | at | different |
|--|--------------|--------|-------------|---------|--------------|---------|----------|-----------|
| concentrations | | | | | | | | |
| S2: Linear relationship between concentration found and concentration added by the metered | | | | | | | | |
| additions method2 | | | | | | | | |
| S3: | Chromatogram | of | protein and | peptide | standards | on P | oroshell | 300 Å |
| Column | | | | | | | | |
| S4: Reversed phase-high performance liquid chromatography (RP-HPLC) profiles of protein | | | | | | | | |
| fractions produced by solvent precipitation | | | | | | | | |
| S5: Chromatogram (a): sample # 5 eluted with 10% methanol and 90% water; (b): sample # 6 | | | | | | | | |
| received 100% ammonium hydroxide4 | | | | | | | | |

PP: Peptide-Loaded-liposome (green); **PT**: Protein-Loaded-Liposome (red); **EL**: Empty-Liposome (dark blue); **MIX**: PP-PT-MGO-Loaded-Liposome (sky blue); **MGO**: Methylglyoxal-Loaded-Liposome (black)

S: Sample; S0: purified protein; S1: protein precipitation with acetonitrile; S2: whitish mass obtained after centrifugation at the top of the tube; S3:_yellowish liquid obtained after centrifugation at the bottom of the tube; S3': translucent liquid after centrifugation; S4: 1^{st} centrifugation; S5: 1^{st} wash; S6: 2^{nd} wash; S7: 3^{rd} wash.



S1: The linear response of 2-methylquinoxaline at different concentrations



Concentration added

S2: Linear relationship between concentration found and concentration added by the metered additions method



S3: Chromatogram of protein and peptide standards on Poroshell 300 Å Column.



S4: Reversed phase-high performance liquid chromatography (RP-HPLC) profiles of protein fractions produced by solvent precipitation



S5: Chromatogram (a): sample # 5 eluted with 10% methanol and 90% water; (b): sample # 6 received 100% ammonium hydroxide



S6 Spectral signatures of drug-loaded-liposomes (full vs empty) by color code

PP: Peptide-Loaded-liposome (green); **PT**: Protein-Loaded-Liposome (red); **EL**: Empty-Liposome (dark blue); **MIX**: PP-PT-MGO-Loaded-Liposome (sky blue); **MGO**: Methylglyoxal-Loaded-Liposome (black)



S7: ATR-FTIR spectra of purified proteins after extraction with acetonitrile and several washes to remove sugars

S: Sample; S0: purified protein; S1: protein precipitation with acetonitrile; S2: whitish mass obtained after centrifugation at the top of the tube; S3:_yellowish liquid obtained after centrifugation at the bottom of the tube; S3': translucent liquid after centrifugation; S4: 1^{st} centrifugation; S5: 1^{st} wash; S6: 2^{nd} wash; S7: 3^{rd} wash.