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Incorporation of the Next Generation Probiotic Akkermansia muciniphila in a chocolate matrix

Rita VEDOR[1], Daniela MACHADO[1], Joana Cristina BARBOSA[1], Ana Maria GOMES [1], José Carlos ANDRADE[2]

[1]*Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biot, Porto, Portugal*

[2]*TOXRUN—Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, Gandra, Porto, Portugal*

Chocolate is one of the most attractive food products worldwide and has been proposed as a suitable carrier for probiotic delivery. Probiotics are recognized as live microorganisms that, when administered adequately, confer health benefits. Among those, *Akkermansia muciniphila* has emerged as a promising next generation probiotic candidate, given its key bioactivities in diet-related disorders. Herein, *A. muciniphila* DSM 22959 strain was incorporated into chocolates with 3 different cocoa contents (33.6%; 54.5%, and 70.5%), and its viability was assessed throughout aerobic storage at 25ºC for 28 days. Moreover, each probiotic chocolate was characterized in terms of pH, phenolic compound content, antioxidant, antihypertensive, and antidiabetic activities, using control chocolates (without bacteria) for comparative purposes. Our results showed that higher *A. muciniphila* levels were obtained in chocolates with 33.6% and 54.5% of the cocoa content (≥ 106 CFU/g). Furthermore, similar pH values for control and probiotic chocolates were detected without considerable oscillations during storage. This suggests that the probiotic bacteria are metabolically inactive, which may be related to their viability maintenance over time. Additionally, the phenolic compounds content analysis revealed an increase in total compounds with increasing cocoa content in the food matrix. However, probiotic incorporation did not interfere with the phenolic content. Also, antioxidant, antihypertensive, and antidiabetic analyses showed a growing trend with the increase of cocoa content in the chocolate matrix. In conclusion, chocolate may be considered a potential and valuable carrier for the next generation probiotic *A. muciniphila* and is concurrently emerging as a novel functional food.

Keywords: Next Generation Probiotic, Akkermansia muciniphila, Bioactivity, Probiotic delivery

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