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Incorporation of *Akkermansia muciniphila* DSM 22959 in chocolate matrices

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Introduction

Currently, consumers are seeking **healthier food options**, prioritizing goods with improved organoleptic and health properties. Consequently, the food industry has been focusing on developing **functional foods**, which are characterized by the incorporation of **bioactive ingredients**. Recently, ***Akkermansia muciniphila*** has risen as a potential candidate for the **Next Generation Probiotics**. *A. muciniphila* represents about 0.5 to 5% of the bacteria in the human gastrointestinal tract and is a crucial symbiont of intestinal microbiota by shaping host immune responses and participating in immune. Herewith, chocolate rises as a **potential vehicle to deliver probiotic bacteria**, as it's a highly popular food product among consumers.

Objective

Assess the **most adequate chocolate matrix** for the incorporation *Akkermansia muciniphila* in terms of **cell viability** during **aerobic storage** and **bioactivities.**

Methodology

Growth conditions

Strain: *Akkermansia muciniphila DSM 22959*
Medium: PYG médium + 0.05% mucin
Temperature: 37ºC
Atmosphere: 85% N_2, 5% H_2 and 10% CO_2
Incubation time: 24-hours with 3 culturing steps

Chocolate preparation

1) Centrifugation: 12000 x *g*, 4ºC, 30 minutes

2) Incorporation: Bacterial pellet added to melted chocolate (37ºC)

Aerobic storage

Conditions: ambient air at room temperature (20ºC) for 0, 7, 14, 21, 28 days

Viability assessment: Colony-Forming Units (CFU) enumeration technique

Bioactivity assays

1)pH measurement

2) Quantification of total phenolic compounds

3)Determination of **antioxidant, anti-diabetic and anti-hypertensive** activities

Results

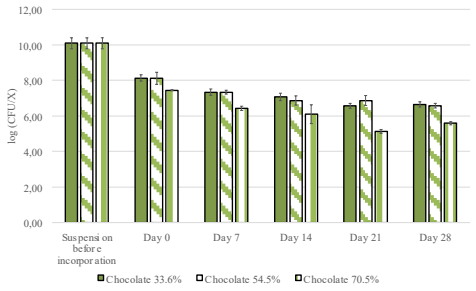

Figure 1. Viability of *A. muciniphila* DSM 22959 incorporated in different chocolate matrices: 33.6% cocoa content [solid bar]; 54.5% cocoa content [diagonal bar] and 70.5% cocoa content [vertical bar]

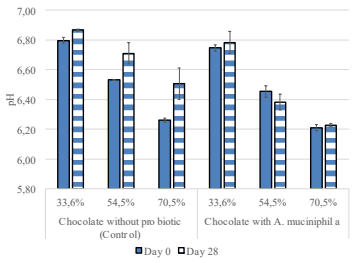

Figure 2. pH values of control and incorporated with *A. muciniphila* DSM 22959 chocolate at timepoint day 0 [solid bar] and day 28 [horizontal bar]

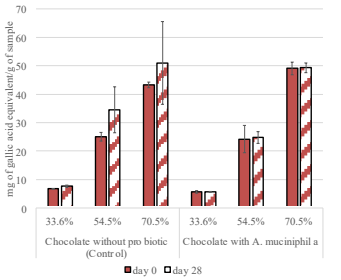

Figure 3. Total phenolic compounds at timepoint day 0 [solid bar] and day 28 [diagonal bar]

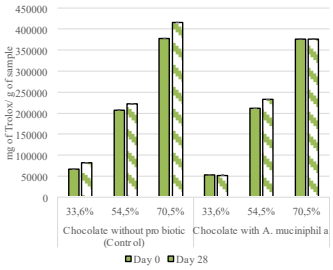

Figure 4. Antioxidant activity at timepoint day 0 [solid bar] and day 28 [diagonal bar]

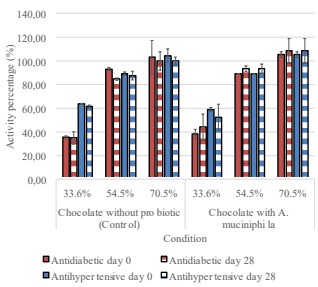

Figure 5. Anti-diabetic [red] and Anti-hypertensive [blue] activity at timepoint day 0 [solid bar] and day 28 [diagonal bar]

Main Findings

1) Chocolate matrices with **33.6% and 54.5% cocoa content** have similar protective effect on probiotic viability → survival around **10^6 CFU/g after 28 days of aerobic storage**. Matrix with **70.5% cocoa content** did **not** achieve the **minimum required levels.**
2) **Addition of probiotic** to chocolate **did not change the pH values** compared to the control values → probiotic was with a **slowed down metabolism** or **metabolically inactive** throughout storage
3) The **higher** the **cocoa content**, the **higher** the **total phenolic compounds** values. **Addition of probiotic** did **not** alter the values of total phenolic compounds. **Exception**: Chocolate matrix with **54.5%**, indicating possible **consumption**
4) The **higher** the **cocoa percentage**, the **higher** the **bioactivity**. This conclusion is valid for **antioxidant, anti-diabetic and anti-hypertensive** activity.
5) **Addition** of probiotics does **not** affect the **bioactivities**

Acknowledgements

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