Viability of protein microencapsulated *Lactobacillus plantarum* 299v during *in vitro* digestion process

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Introduction

Simulated gastrointestinal Test

Simulated gastrointestinal test is an experiment that carried *in vitro* for simulate the digestion process in the stomach of humans or the other living creatures. It can be used to investigate structural modifications, digestibility, and release of food constituents.

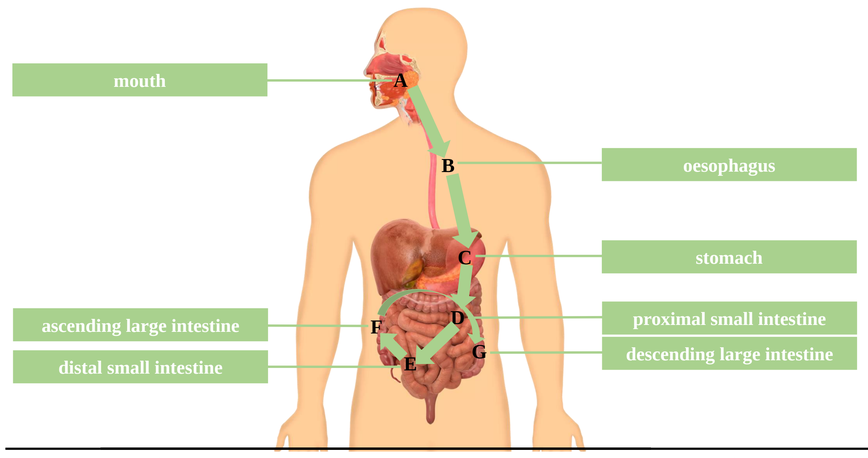
Microencapsulation

Microencapsulation is a talented technology that can protect the probiotics by coating them with wall materials to maintain their viability and functionality during the manufacturing, storage, and digestion process and let them apply their probiotic effect in the gut with an adequate dose level.

Aims

This study aimed to use *in vitro* simulated gastrointestinal test by exploring encapsulated probiotic samples into simulated gastric juice (SGJ) and simulated intestinal juice (SIJ) during the digestion process to check the survival ability of microcapsule samples with different core-to-wall ratios and wall materials formulation.

Materials and Methods



Letter	Region	pH	Retention Time
A	mouth	5.6-7.9	2-5 sec
B	oesophagus	~7	10-14 sec
C	stomach	1-2.5	161 mins
D	proximal small intestine	6.15-7.35	
E	distal small intestine	6.80-7.88	3.2 ± 1.6 h
F	ascending small intestine	5.26-6.72	
G	descending large intestine	5.20-7.02	variable

Fig.1. Characteristics of GI tract of human beings and the pH and retention time at the different part during the digestion process

Materials

- **Microencapsulated *Lactobacillus plantarum* 299v**

  Samples are produced by lyophilization of probiotics mixed with different core-to-wall ratios and wall material formulation.
- **Simulated Gastric Juice**

  To prepare SGJ, sodium chloride solution (5 g/L) was adjusted to pH 2 by using 6 M HCl and sterilized at 121℃ for 20 min followed by adding 0.3% pepsin (Sigma-Aldrich, USA).
- **Simulated Intestinal Juice**

  To prepare SIJ juice, 0.6% bile salt (Sigma-Aldrich, USA) was added into autoclaved 0.05 M KH_2PO_4 solution.

Methods

The sampling process was by adding 0.1g microcapsules of each sample into 9.9 mL above mentioned SGJ and SIJ. The living cell number was checked by using a plate-counting method by taking samples based on the incubation time 0 h, 0.5 h,1 h,2 h,3 h, and 0 h, 3 h, 6 h for SGJ and SIJ samples, respectively.

Results and Discussions

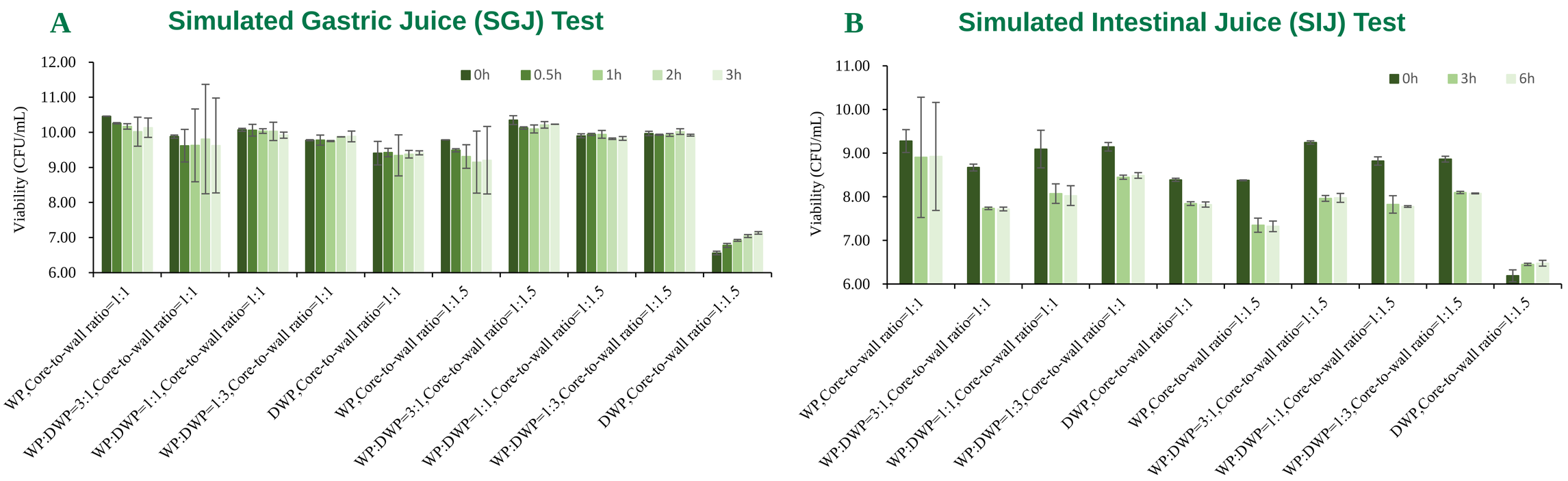


Fig. 2. Viability of lyophilized *Lactobacillus plantarum* 299v with different wall core-to-wall ratio and different wall material formulation after exposed to **SGJ for 3 h (Figure 2-A)** and **SIJ for 6 h (Figure 2-B)** at 37℃. Error bars represent standard deviation of means (n=2).
WP-whey protein, DWP-denatured whey protein.

- after 3 hours digestion, samples with core-to-wall ratio=1:1, with the increased content of DWP, the viability loss decreased, the same trend for samples with core-to-wall ratio=1:1.5.
- the highest viability of the sample with core-to-wall=1:1 and 1.5 is WP and WP:DWP=3:1, whose viable number is 10.13 CFU/g and 10.23 CFU/g, respectively.
- the viability of each sample has a significant difference between 0 h and 3 h incubation but does not have a significant ($p>0.05$) difference in the further incubation
- after 6 hours incubation, sample core-to-wall ratio=1:1, WP and core-to-wall ratio=1:1.5, WP:DWP=1:3 in each group has the lowest viability loss, which is 0.35 CFU/g. and 0.78 CFU/g, respectively.

Conclusions

- The core-to-wall ratios and wall material formulation have influence on the viability of protein microencapsulated probiotics during the *in vitro* simulated gastric juice and simulated intestinal juice test.
- The results of our research are very promising and may have some guidance on the simulated *in vitro* digestion process of probiotic microcapsules by lyophilization.

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Sun is a Ph.D. student at the Doctoral School of Food Science, Hungarian University of Agriculture and Life Sciences.