The *in-vitro* bioactive properties of fermented brewer's spent grain using *Lactiplantibacillus plantarum*: aerobic vs anaerobic processes

Minh Quoc Ha[1,2], Victor Rimbault[1], Phuc Hanh Nguyen[2], Mathilde Berthevas[2], Maryam Ghamsary[2], Eileen Kitundu[2], Rothman Kam[1,2], Brent Seale[1,2], Claus Heiner Bang-Berthelsen[3], Thao T. Le[1,2]

[1]*AUT Centre for Future Foods, Auckland University of Technology, Auckland 1010, New Zealand*

[2]*School of Science, Auckland University of Technology, Auckland 1010, New Zealand*

[3]*DTU National Food Institute, Technical University of Denmark, Kongens Lyngby 2800, Denmark*

INTRODUCTION

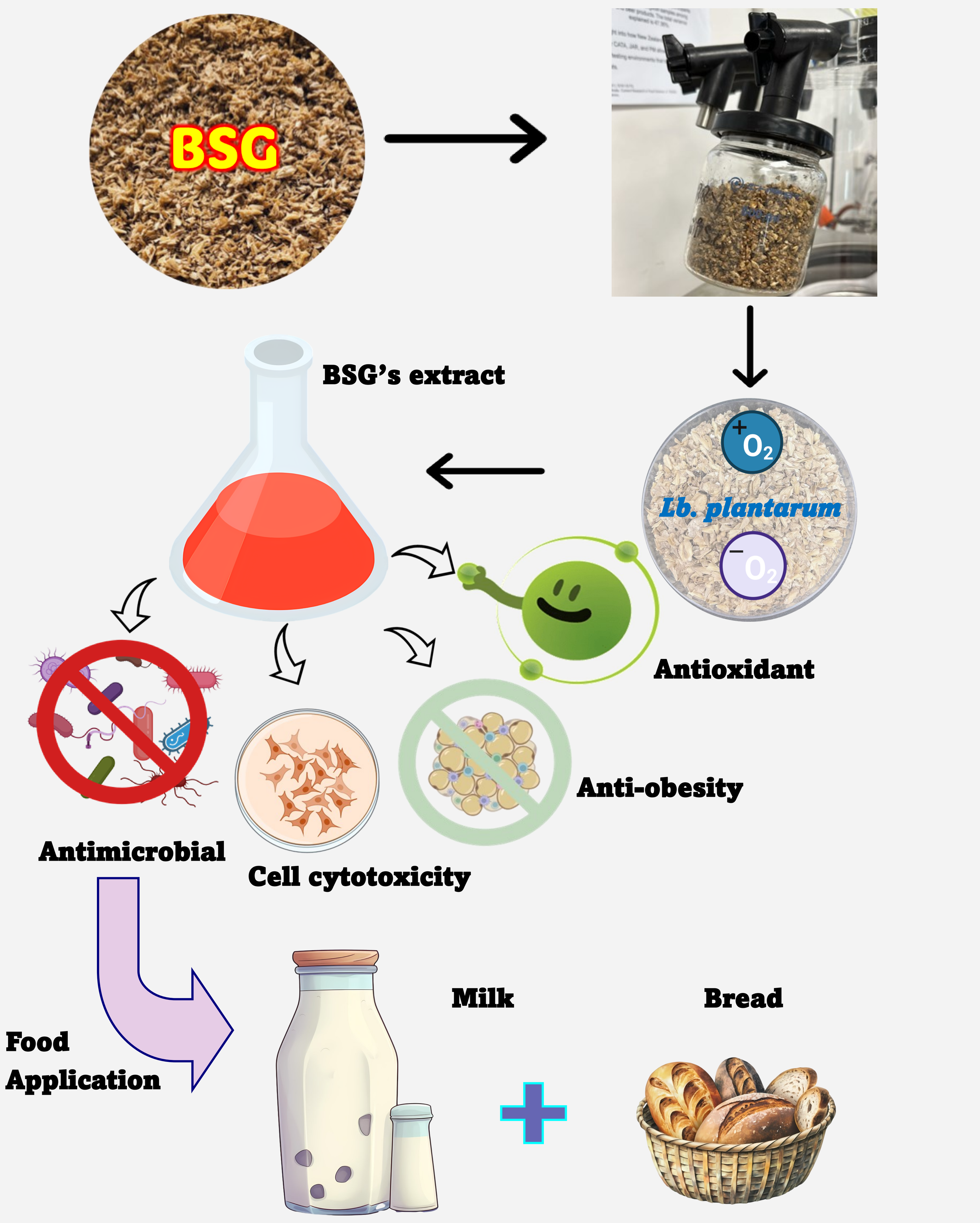
- Brewers' spent grain (BSG), a by-product of the brewing industry, is produced approximately 20 kg out of 100 beer liters, with around 36.5 million tons generated annually worldwide (Statista, 2021).
- BSG is mainly used for livestock feed or sent to landfill. However, transportation of this large amount of biomass is costly and increases carbon emissions (Mussatto, 2014).
- Enzymatic treatment has been considered a promising approach to valorise BSG for practical application, but limitations still exist, such as high enzyme costs and the use of non -renewable resources in the production and purification processes.
- Mindful of the environmental impact of this waste and limitations of current approaches, our study investigates the *in-vitro* bioactivities of fermented BSG using *Lactiplantibacillus plantarum* (*Lb. plantarum*), offering a sustainable solution to enhance the biological activity of this biomass.

METHODOLOGY

Fermentation: Two BSG, wheat (FWB) and stout (FSB), underwent anaerobic and aerobic solid-state fermentation for 10 days at 35 °C, using *Lactiplantibacillus plantarum* starter cultures, with sample collection every 2 days.

Extraction: Fermented BSG was extracted with 60% acetonitrile (ACN) for 30 minutes.

***In-vitro* bioassays:** The supernatant extracts were analysed for antioxidants, antimicrobial, anti-obesity, cell cytotoxicity on RAW 264.7 macrophages cell lines, and *ex-vivo* antimicrobial on food products (dairy- and wheat- based products).



RESULTS

Antioxidant

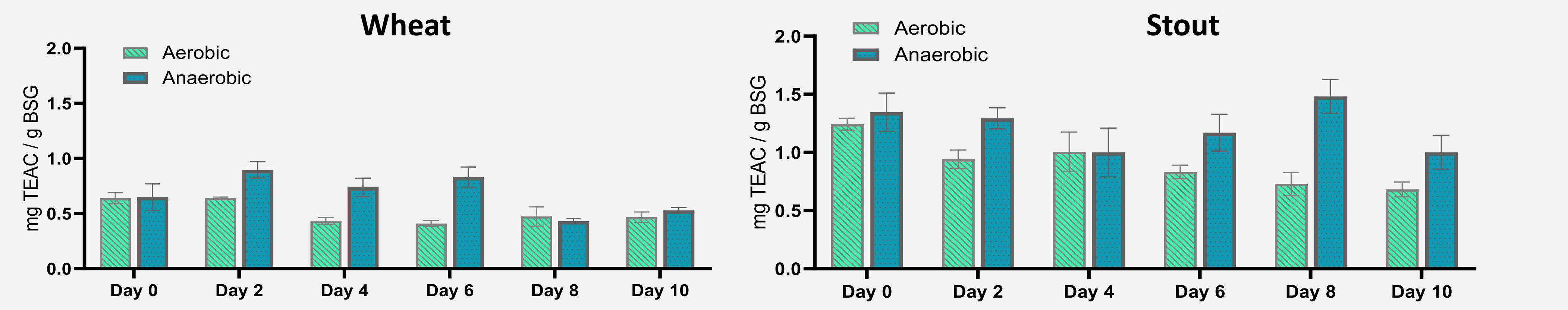


Figure 1. The Trolox equivalent antioxidant capacity (TEAC) values of DPPH assays on wheat and stout BSG under aerobic and anaerobic fermentation.

- Anaerobic fermentation boosts antioxidant capacity in BSG compared to aerobic conditions in both BSG, in which wheat shows significance early to mid-fermentation (days 2, 4, 6; $p < 0.05$), while stout peaks late (days 6, 8, 10, $p < 0.05$).

Anti-obesity

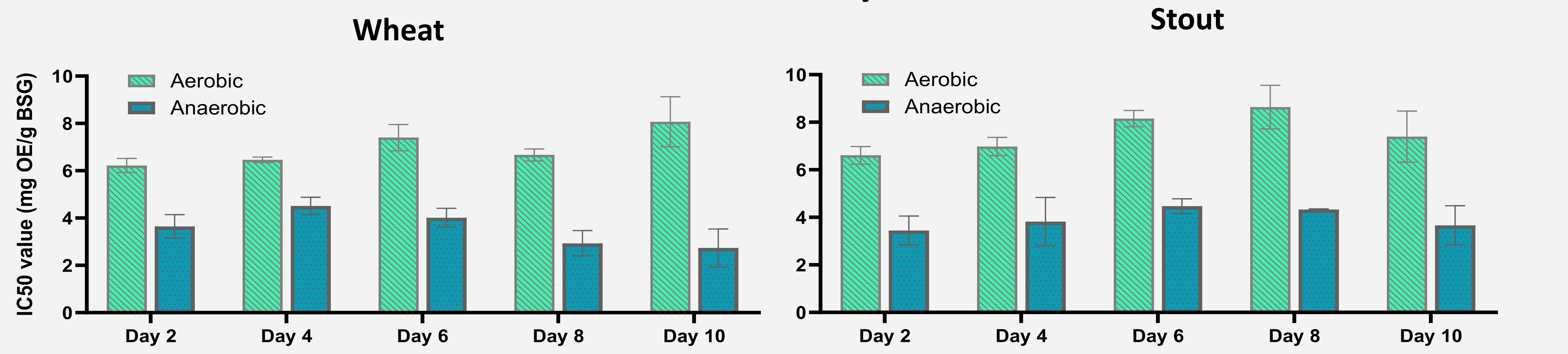


Figure 2. The lipase inhibitory activity of the BSG extracts expressed as mg of Orlistat Equivalent inhibitory capacity (OE)/ g BSG, both BSG groups under two fermentation conditions.

- Aerobic fermentation consistently outperforms anaerobic fermentation in anti-obesity capacity across all days ($p <0 .05$) , while stout shows superior anti-obesity capacity compared to wheat ($p < 0.05$) .

Cell cytotoxicity

- The BSG extracts are non-toxic and also promote cell proliferation.

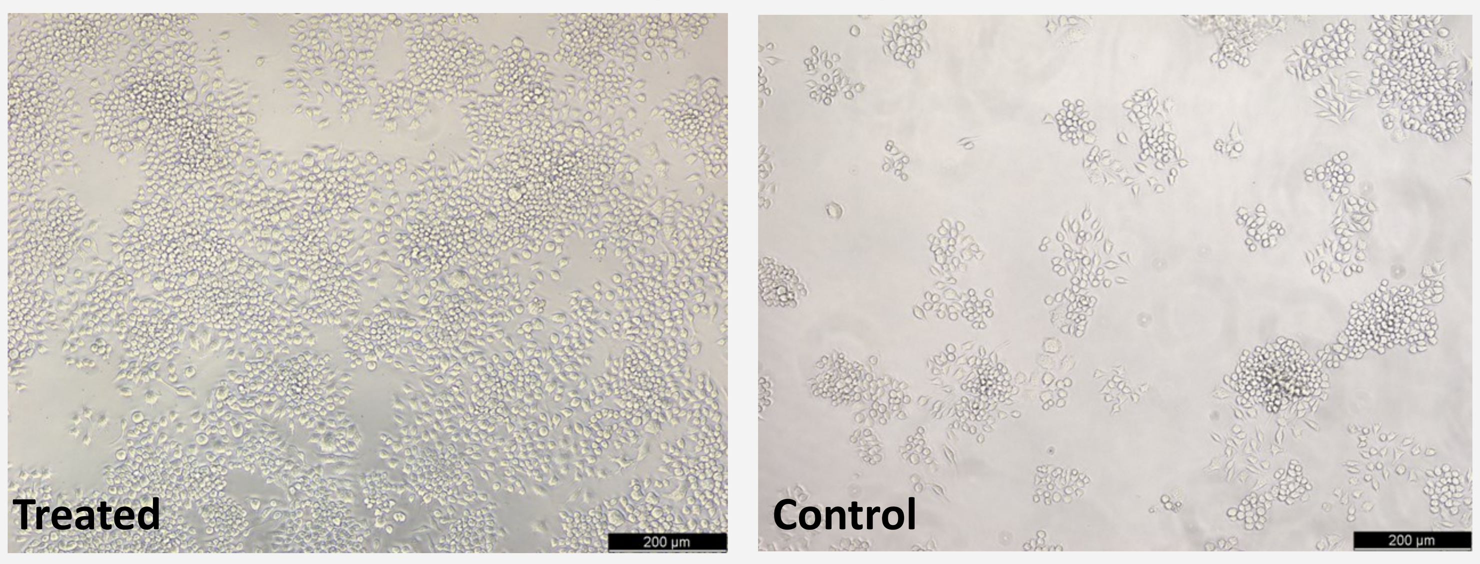


Figure 3. The cell confluence of RAW 264.7 macrophages under the BSG extract-treated vs the control (no treatment) after 48 hours.

Ex vivo antimicrobial on food products

Table 1. Antibacterial activity of aerobic fermented BSG extracts of day 4 on wheat and dairy-based media. For the control: "FG" is full growth and "LG" is low growth.

Food media	Bacteria	BSG types and BSG extract concentrations (mg BSG/mL)								
		FWB				FSB				
		0.333	0.167	0.083	0.042	0.333	0.167	0.083	0.042	Control
BREAD	*E. coli*	+	+	-	-	+	+	-	-	FG
	B. cereus	+	+	+	+	+	+	+	+	FG
	S. aureus	+	+	+	+	+	+	+	+	LG
MILK	*E. coli*	+	-	-	-	-	-	-	-	FG
	B. cereus	+	-	-	-	+	-	-	-	FG
	S. aureus	+	-	-	-	+	-	-	-	FG

- Both BSG extracts exhibit strong bactericidal activity on bread (0.167 mg BSG/mL), but a higher concentration (0.333 mg BSG/mL) is required to inhibit food-borne pathogens in milk.

NEXT STAGE

- What are the bioactive components in these extracts?

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CONCLUSION

- Anaerobic conditions enhanced antioxidant properties, while aerobic conditions improved antimicrobial and anti-obesity effects.
- Both fermentation conditions promote the growth of RAW 264.7 macrophages.
- Overall, fermentation with *Lactiplantibacillus plantarum* enhances BSG's bioactivity under both conditions, depending on the fermented conditions.
- Therefore, fermentation conditions can be adjusted to prioritise the desired bioactivity.

DISCUSSION

- In aerobic environments, oxygen catalyses the formation of reactive oxygen species (ROS)—such as hydrogen peroxide, superoxide, and hydroxyl radicals (Imlay, 2003; Serrazanetti et al., 2013) degrading antioxidant compounds in the BSG.
- Oxygen present in aerobic fermentation significantly increases bacterial biomass and metabolic activity (Tejedor et al., 2022), which likely leads to higher lipase inhibition activity.
- The nutrient-dense environment and more available minerals in milk require higher concentration to completely kill the food-borne pathogens, comparing to enzyme-assisted bread media (HorFSBurgh, Ingham, & Foster, 2001).

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