Condensed Summary: Bio-az Synbiotic Survivability & Stability White Paper

Background & Challenge

Probiotics in FMCG face four major challenges: survivability, stability, enumeration, and cleaning. Most probiotics die during pasteurisation, can't survive the GI tract, or degrade on shelves—even under refrigeration. Existing probiotic spore products like DE111, BC30, and BSN287 cannot withstand the 90°C+ pasteurisation processes common in FMCG, nor maintain viability long-term in high-water, low-pH, preservative-rich conditions.

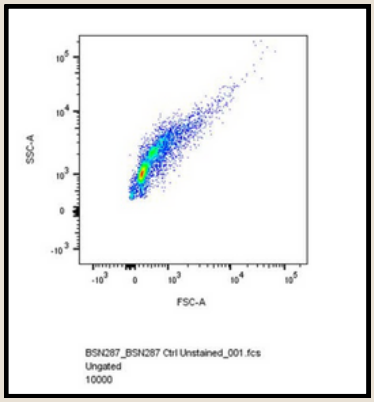
Bio-az's World-First Synbiotic Methodology

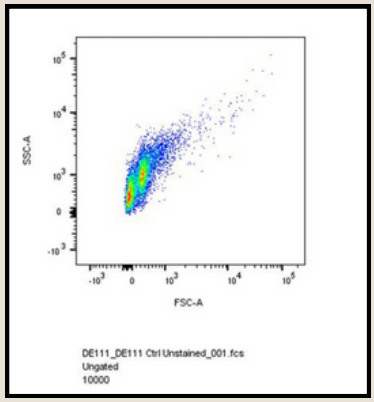
Bio-az developed a unique patented synbiotic methodologies (Method A & B) using commercially available spore forming probiotics and the proprietary BA142 Bacillus subtilis strain and specially formulated synbiotic compositions . Unlike commercial alternatives, Bio-az synbiotics are proven to survive pasteurisation up to 97°C, remain shelf-stable for 24+ months at ambient temperatures, and resist formulation breakdown under high acidity and carbohydrate stress. Patent Reference: PCT/AU2022/050657 – A method for creating an ambient temperature shelf-stable synbiotic for FMCG use.

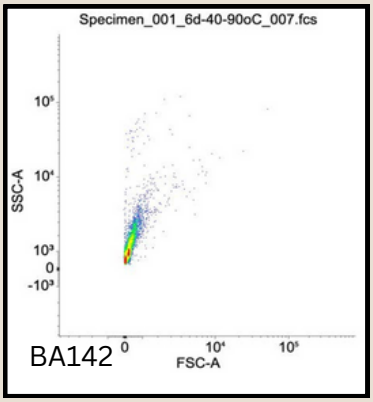
Research Highlights

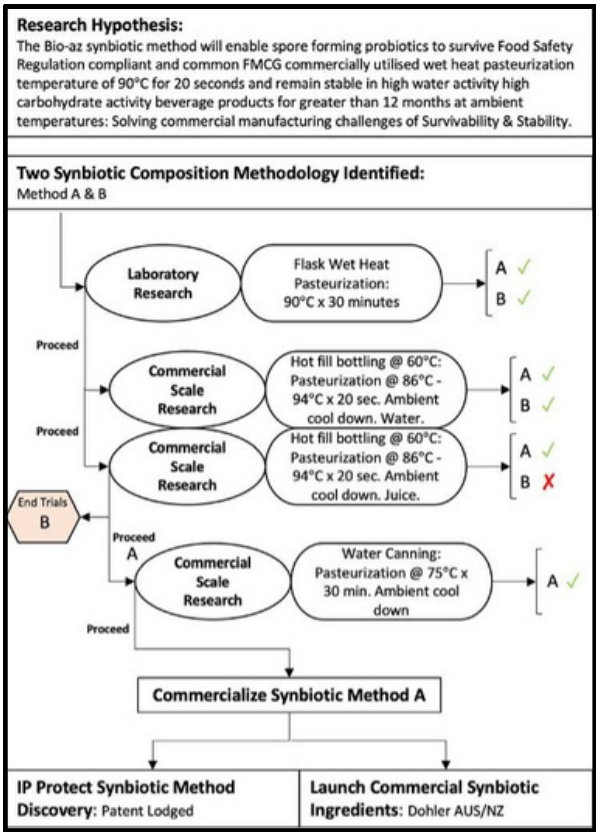
Spore Coat Formation & Stability

Bio-az research confirmed that spore quality (maturity, coat structure) directly determines survivability during heat processing. Flow cytometry showed commercial probiotics are up to 93% poorly coated spores and debris, while BA142 samples were 60% viable spores with tighter uniformity. Bio-az developed a synbiotic methodology to improve spore coat quality of all spore subjects in the research project









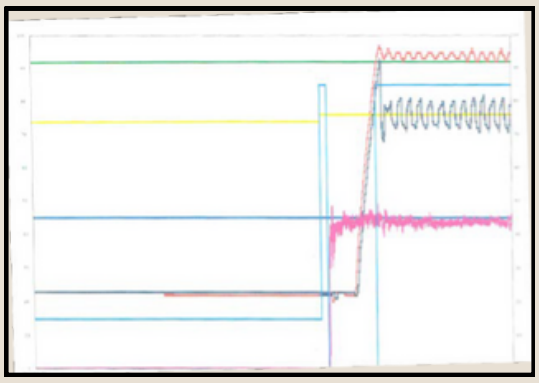
Laboratory-Scale Validation

Synbiotic Methods A & B were tested on DE111, BSN287, BC30, and BA142 at 90°C for 30 minutes. All showed 100% survivability with both methods under lab conditions.

Commercial Bottling Trials (Water & Juice)

Using Method A:
Water products retained >90% CFU stability over 12 months at room temp.

Juices (pineapple/lemon, apple, strawberry blends) showed CFU retention despite acidic pH, thanks to spore protection—even under bromelain stress.

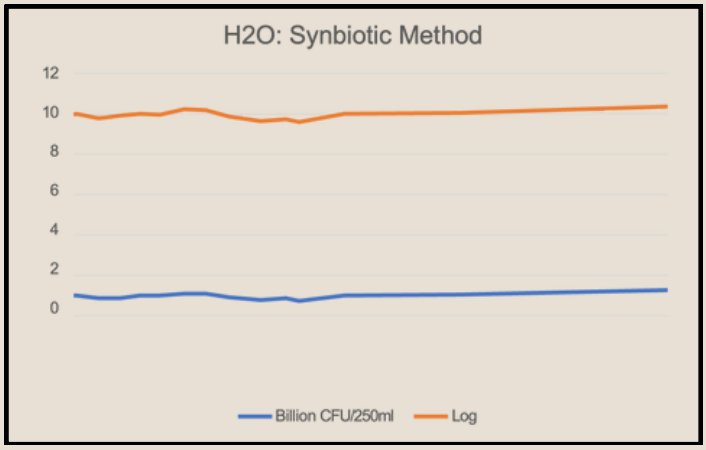


Green Line = Pasturization set point 92°
Orange Line = Actual Pasturization temp 92°-97°
Yellow Line = Bottling set point 77°
Blue = Actual bottling temp 72°-80°
Blue Line = Flow (wash off water) set point 45°
Pink Line = Actual Flow Temp 40-48°

SURVIVABILITY & STABILITY RESULTS

TRIAL: 1
PRODUCT: Water
SYNBIOTIC METHOD: METHOD A
Date of Production: 4/12/2020

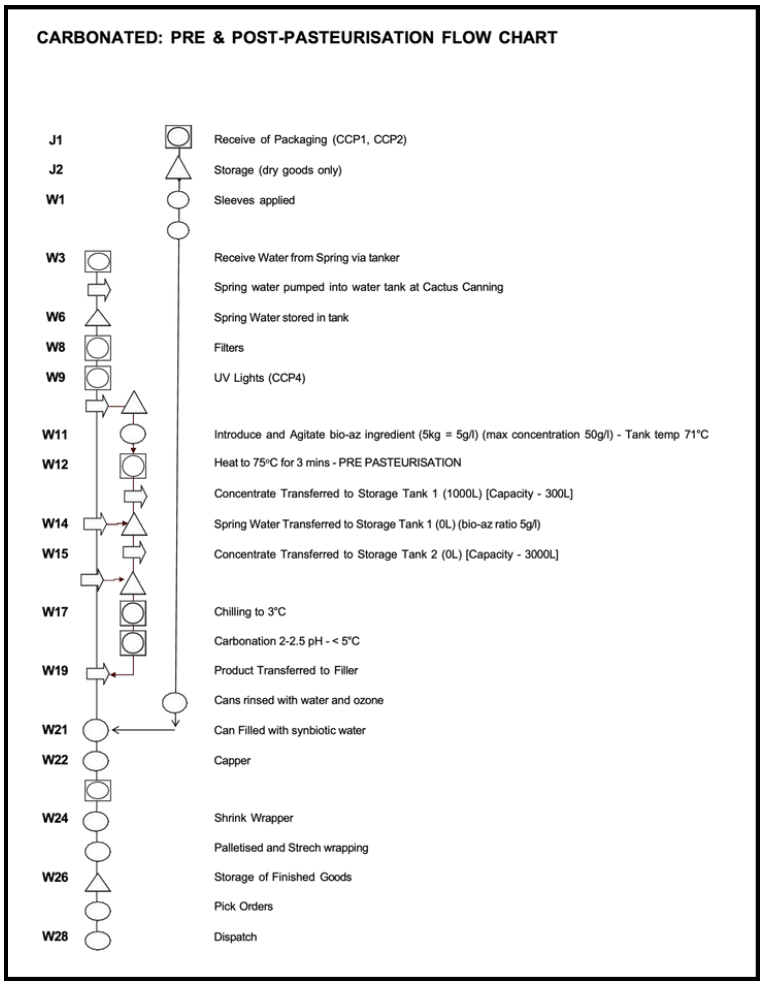
Date	CFU/ml	Billion CFU/250ml	Log	PH
4/12/2020	40X10^5	1	9	6.5
7/12/2020	41X10^5	1.025	9	6.5
7/1/2021	35X10^5	0.875	8.93	6.5
5/2/2021	36X10^5	0.9	9.05	6.5
5/3/2021	41X10^5	1.025	9	6.4
1/4/2021	40X10^5	1	9	6.4
5/5/2021	45X10^5	1.125	9.12	6.4
3/6/2021	44X10^5	1.1	9.1	6.3
5/7/2021	37X10^5	0.925	8.97	6.3
18/8/2021	31X10^5	0.775	8.89	6.3
21/9/2021	35X10^5	0.875	8.9	6.2
10/10/2021	30X10^5	0.75	8.87	6.2
10/12/2021	41X10^5	1.025	9	6.2
19/5/2022	42X10^5	1.05	9.02	6.1
1/3/2023	51x10^5	1.275	9.1	6.1



Canning Trials (Still & Sparkling)

Bio-az synbiotics survived combinations of:

- Pre-pasteurisation (74°C/3 min)
- Post-pasteurisation (69°C/13 min)
- Carbonation (p 2.5)
- BA142 demonstrated survivability even at UHT conditions (up to 121°C for 5 mins).



Carbonated Water manufacturing process with pre and post pasteurisation stages included.
NOTE: Prior to carbonation the water or beverage must be chilled to a temperature lower than 5°C to ensure the targeted levels of carbonation remain in the finished product. To achieve this a chilling process was included and represented as step W17 in the production flow diagram.

Still water canning was successful with the probiotic component of the bio-az synbiotic ingredient demonstrating survivability and stability. However, the quality control of the nitrogen dosing at the commercial manufacturing facility was not consistent or accurate. Random canned still water samples from the different canning trials presented undesirable organoleptic properties.

Laboratory analysis revealed highly variable nitrogen levels in randomly selected samples. Samples that had very high nitrogen levels had the undesirable organoleptic properties. Despite variable nitrogen levels the probiotic component of the bio-az synbiotic ingredient demonstrated survivability and stability.

Further research on still canned water will be progressed when an alternative process to nitrogen injection is identified or a commercial manufacturing facility with consistent and accurate nitrogen dosing equipment can be identified and validated.

ACTUAL COMMERCIAL RESEARCH PARAMETERS

Run #	Product	Pasteurization Method	Pre-Pasteurization Temperature	Pre-Pasteurization Time	Filling Temperature	Post Pasteurization Temperature	Post Pasteurization Time	Cooling Time	Probiotic Spore CFU Dose	Probiotic Spore CFU Postproduction	Probiotic Spore CFU 12 Months
PRODUCTION 1	21/3/2022										
1	Sparkling (1000L)	PRE	74°C	3 min	<5°C	N/A	N/A	0 min	0.33 x 10⁹	0.33 x 10⁹	TBC
2	Still (1000L)	PRE	74°C	3 min	25°C	N/A	N/A	0 min	0.33 x 10⁹	0.33 x 10⁹	TBC
PRODUCTION 2	26/5/2022										
1	Still Water 1 (500L)	PRE + POST	74°C	3 min	25°C	69.2°C	13 min	24 min	0.225 x 10⁹	0.193 x 10⁹	TBC
2	Still Water 2 (500L)	POST	N/A	N/A	25°C	69.2°C	13 min	24 min	0.225 x 10⁹	0.225 x 10⁹	TBC
3	Sparkling 1 (500L)	PRE + POST	74°C	3 min	<5°C	69.2°C	13 min	20 min	0.225 x 10⁹	0.159 x 10⁹	TBC
PRODUCTION 3	4/7/2022										
1	Sparkling (1000L)	PRE + POST	74°C	3 min	<5°C	69.2°C	13 min	20 min	0.5 x 10⁹	0.6 x 10⁹	TBC
PRODUCTION 4	15/8/2022										
1	Sparkling (2500L)	POST	N/A	N/A	<5°C	69.2°C	13 min	20 min	0.5 x 10⁹	0.475 x 10⁹	0.309 x 10⁹

**TBC - Tob be completed

Bio-az's Synbiotic Method A is the only documented method to enable the inclusion of live, stable probiotics in ambient, shelf-stable FMCG products without refrigeration, artificial encapsulation, or post-manufacture blending. This fundamentally solves longstanding industry problems and positions Bio-az as a global category disruptor.

Bio-az Synbiotic Methodology Summary

The Problem

- Most probiotics die at pasteurisation (>80°C)
- Rapid shelf degradation (even refrigerated)
- Incompatible with high water activity, low pH, preservatives
- Label claims often misleading ("at time of manufacture")

The Breakthroughs

- Patent-Pending Synbiotic Method (Method A)
- Proprietary Spore Strain (BA142)
- Survives 97°C pasteurisation & 12+ months shelf life at ambient temp

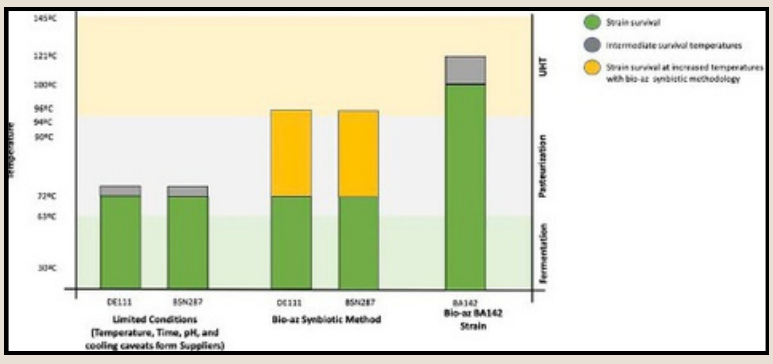
Proven Results

- Lab & Commercial Trials in juices, water, sparkling & canned formats
- 90–121°C survivability, including UHT conditions (BA142)
- Maintains colony-forming units (CFU) over 12+ months
- Compatible with high-acidity, carbonated, and preserved matrices

Competitive Advantage

Bio-az Synbiotic Methodology outperforms leading strains (DE111, BC30, BSN287)

- Validated in commercial FMCG bottling & canning manufacturing
- Eliminates need for cold chain or encapsulation
- The only synbiotic method proven to meet FMCG processing standards



Commercial probiotic spores do not solve commercial manufacturing challenges of survivability and stability.
The bio-az synbiotic method protects current commercially available probiotic spores up to pasteurization temperatures of 95°C.
BA142 currently known to survive up to 121°C.