Advances in Spore-Forming Pathogen Treatment and Predictive Modelling: pasteurised, chilled products

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Pasteurised, chilled food products: micro-organisms of importance

1. *Listeria monocytogenes*: most heat resistant vegetative pathogen. Especially a risk due to post-contamination (after pasteurisation), psychrotrohic

2. *Clostridium botulinum*: heat resistant sporeformer, botulinum toxin, non-proteolytic psychrotrophic variant

3. *Bacillus cereus*: very heat resistant pathogenic sporeformer, emetic and diahrea toxin, psychrotrophic variant

‘Classroom’ knowledge: pasteurisation

Pasteurisation baremas for prepared meals:

-P_{70} = 2 min → 6D of *L. monocytogenes*

-P_{70}=40 min → 12D of fecal streptococci

-P_{90}=10 min → 6D of psychrotolerant *C. botulinum*

What about *Bacillus cereus*?

? **Does psychrotolerant *B. cereus* pose a problem/is it a risk in industrially produced, chilled, pasteurised meals?**

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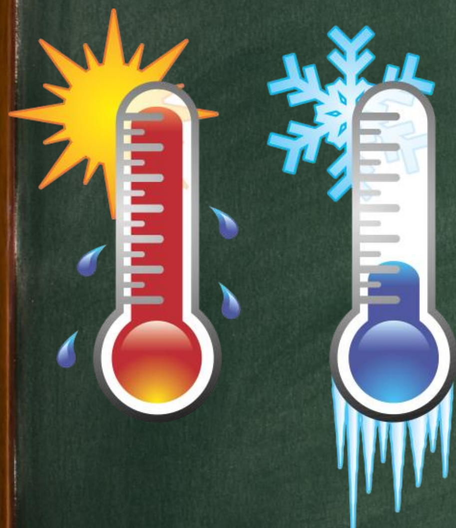
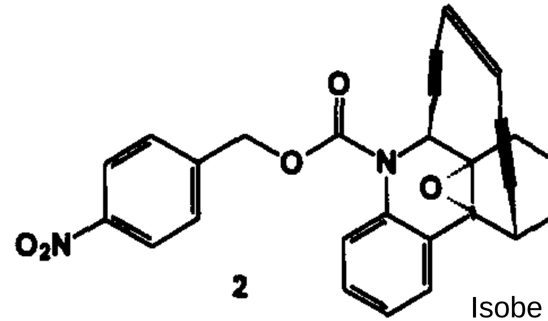
B. cereus, what do we 'kno



Mesophillic
Psychrotrophic

Facultative
anaerobic

Heat resistant
spores



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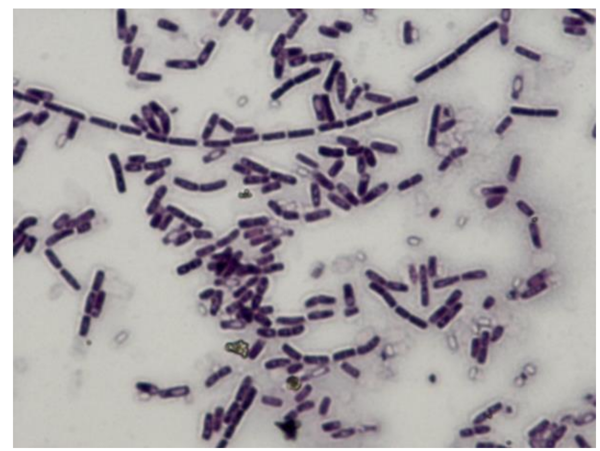
Isobe et al. 1995

Emetic



Diarrheal

Prevalence of *B. cereus* spp. food products marketed in Belgium

575 samples from REFPED compounds collected

parameters analysed

- prevalence of isolates *cereus* group
- psychrotolerance
- toxin production potential

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Prevalence

Product	No. samples	No. positive	% positive	% with spores
lasagna	83	58	69.9	37.3
béchamel	86	70	81.4	0
bolognaise	86	66	76.7	0
rice	80	80	100	6.3
carrots	20	8	40	15
pasta	80	20	25	0
paprika	20	1	5	0
celery	20	7	35	0
Chinese cabbage	20	6	30	0
minced meat	80	12	15	0
TOTAL	575	328	**57**	**6.8**

Samapundo *et al.* (2011)

Psychrotolerant nature of *B. cereus*

575 strains tested

Temperature	4°C	7°C	8°C	9°C	10°C
N# of isolates	1	22	43	174	416
% of total	0.22%	4.8%	9.5%	38%	91%

Samapundo *et al.* (2011), De Vriese *et al.* (2005), Deerens *et al*. (2006)

Can *B. cereus* spores survive pasteurization?

	Isolate				
	FF49	**FF67**	**FF137**	**FF140**	**FF360**
$D_{85°C}$ (min)	7.4	37.3	310	293	41.5
$D_{90°C}$ (min)	3.3	24.4	32.9	90.9	14.4
$D_{95°C}$ (min)	0.5	4.1	14	26.6	4.7
Z-value (°C)	8.8	10.4	7.4	9.6	10.5

All D-values were determined in TSB (a_w 0.99 & pH 7)

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The industrial demand

How to prove that *B. cereus* is unable to grow?

Product properties for REPFED (n=29)

	Min	Mean	Max
pH	5.2	6.0	6.5
a_w	0.977	0.990	0.997

Daelman et al.

➔within growth range of *B. cereus*

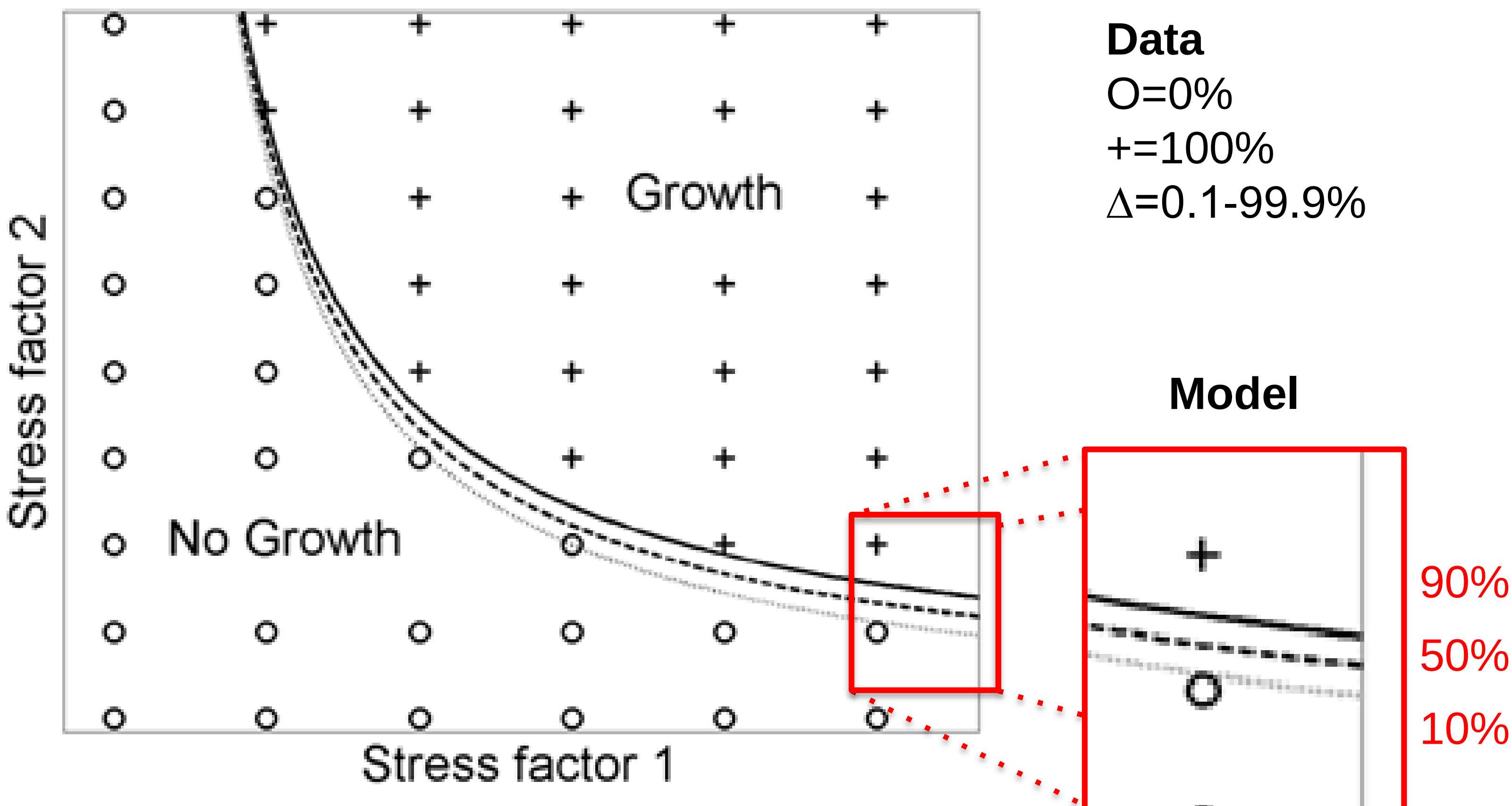
The industrial demand

- 56.3%-80% prevalence in various food products
- Intermediate psychrotolerant: ≤8°C – 9.5%
- pH and a_w within growth range
- It survives pasteurisation

When analyses are performed in final products at the end of the shelf life:

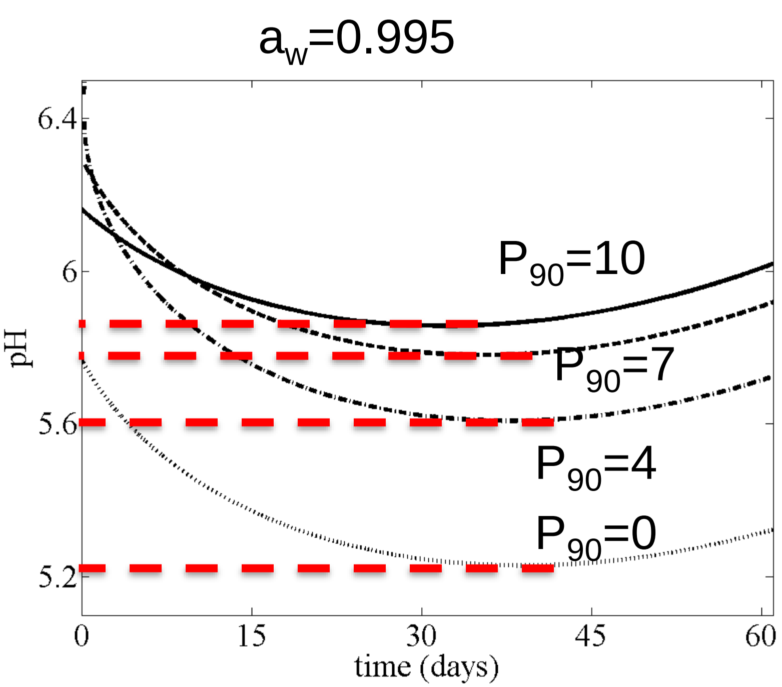
B. cereus << 10^4cfu/g

Is there an effect of pasteurisation on the germination and outgrowth of *B. cereus* spores?



Model 1: aw, pH and P_{90}

lines represent 10% growth probability



- Moderate a_w-P_{90} effect
- Strong pH-P_{90} effect
- Stabilization of GNG boundary after ±30 days

How does heat treatment in a broader T range affects the growth?

Growth in **TSB** and **food product**

- Heat intensity:
  10min @ 70°C, 80°C and 90°C
  or no heat treatment (NHT)
- a_w: 0.99 & 0.96 (adjusted with NaCl)
- pH: 5.5, 6.2 & 7
- Incubation temperature: 7°C & 10°C
- Air and MAP (30%CO_2, 70%N_2)

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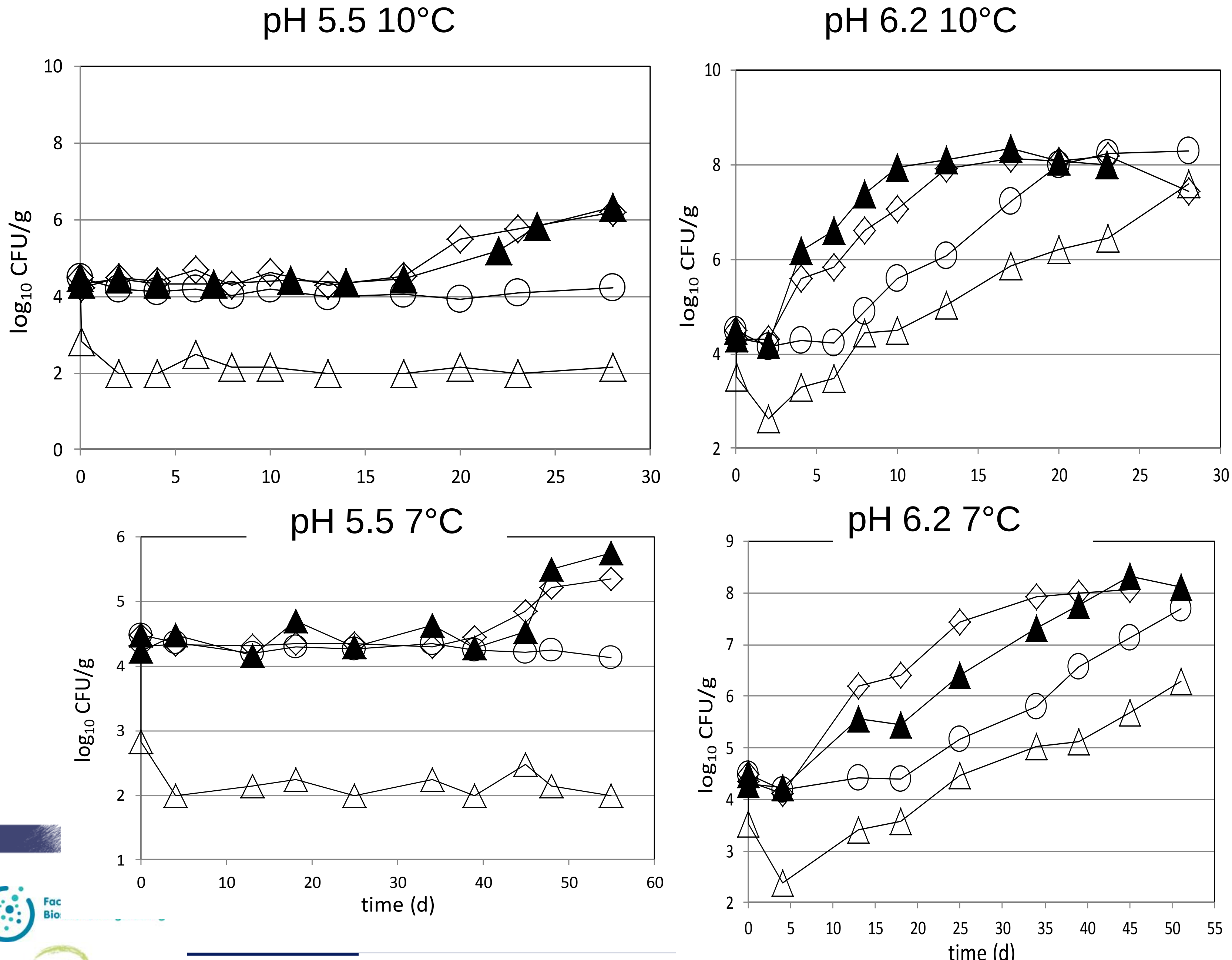
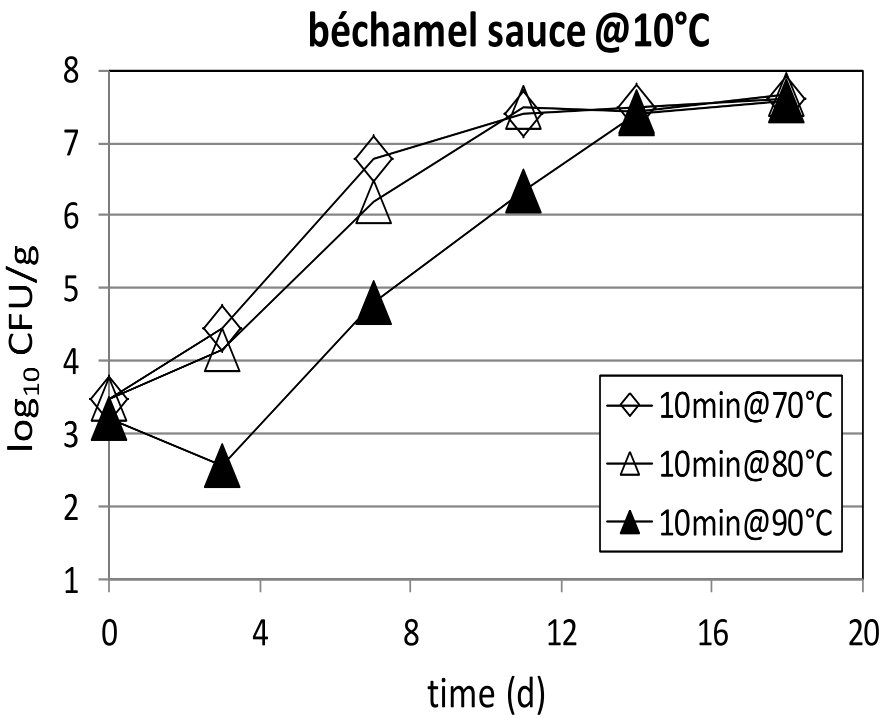
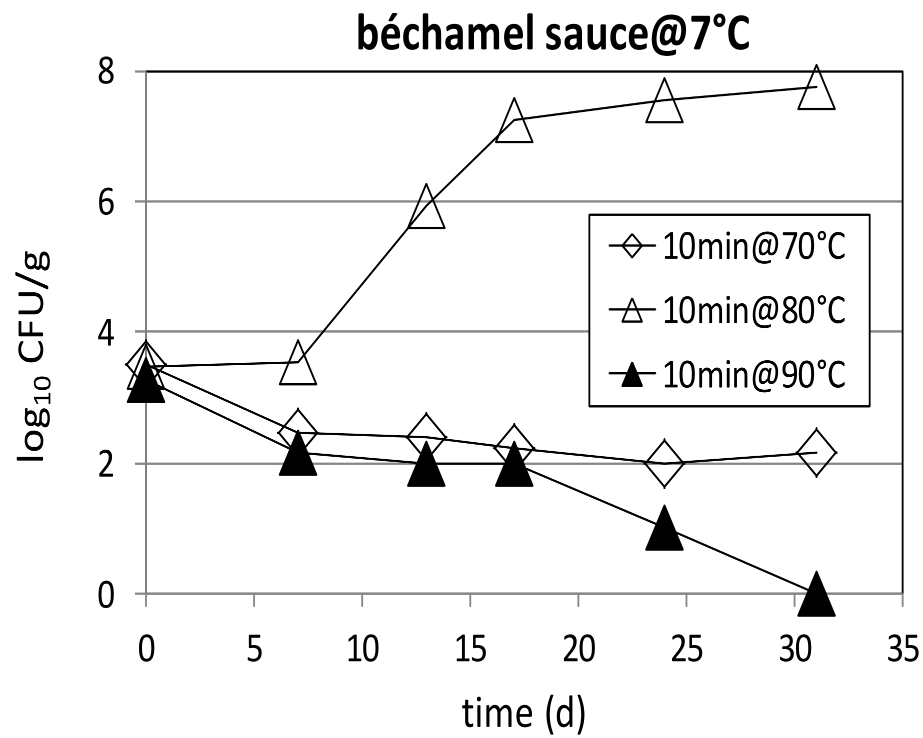


○ NHT ◇ 70°C ▲ 80°C △ 90°C





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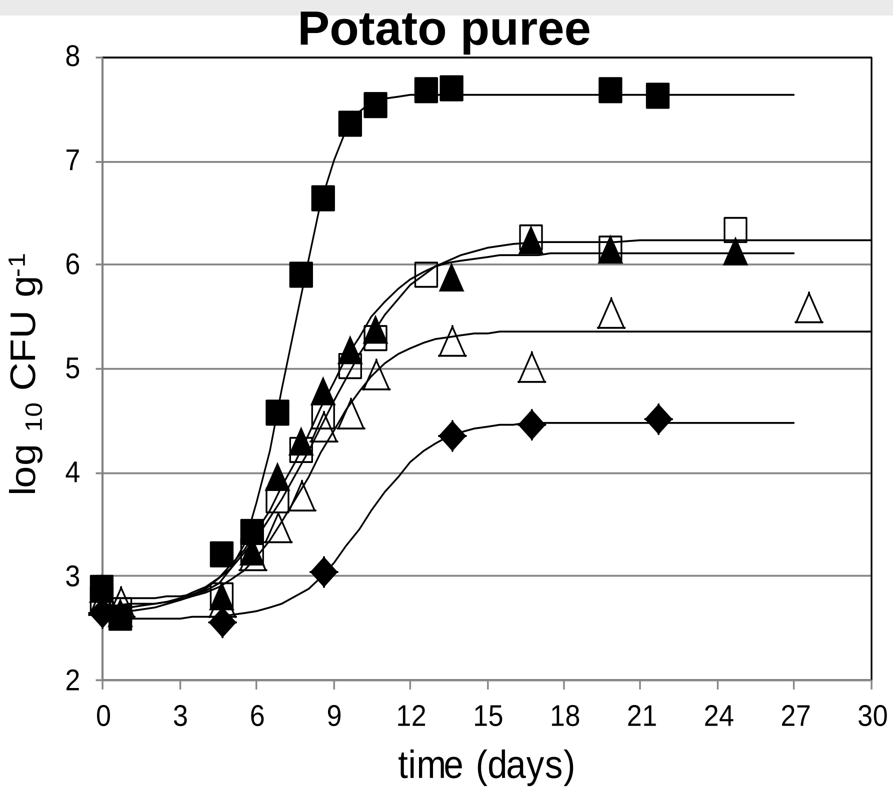
Effect atmosphere on growth after pasteurisation?

- **No growth** (heat treated and native spores) in MA of 30% CO_2, rest N_2 @ 7 and 10°C
- Growth observed **only** in air

How does oxygen affect growth



◆ vacuum △1% O_2 ▲ 5% O_2 □ 10% O_2 ■ air

Estimation of the risk related with psychrotrophic sporeformers, major issue: variability

Storage time

Raw material contamination

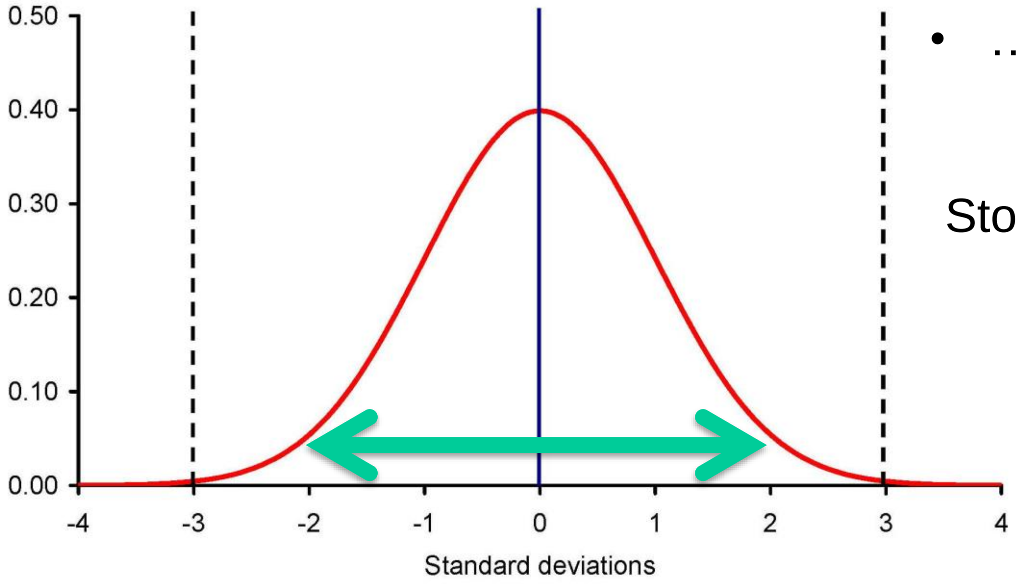
B. Cereus strains:

- Heat resistance
- Psychrotrophic?
- …

Contamination

Storage temperature

Waiting times



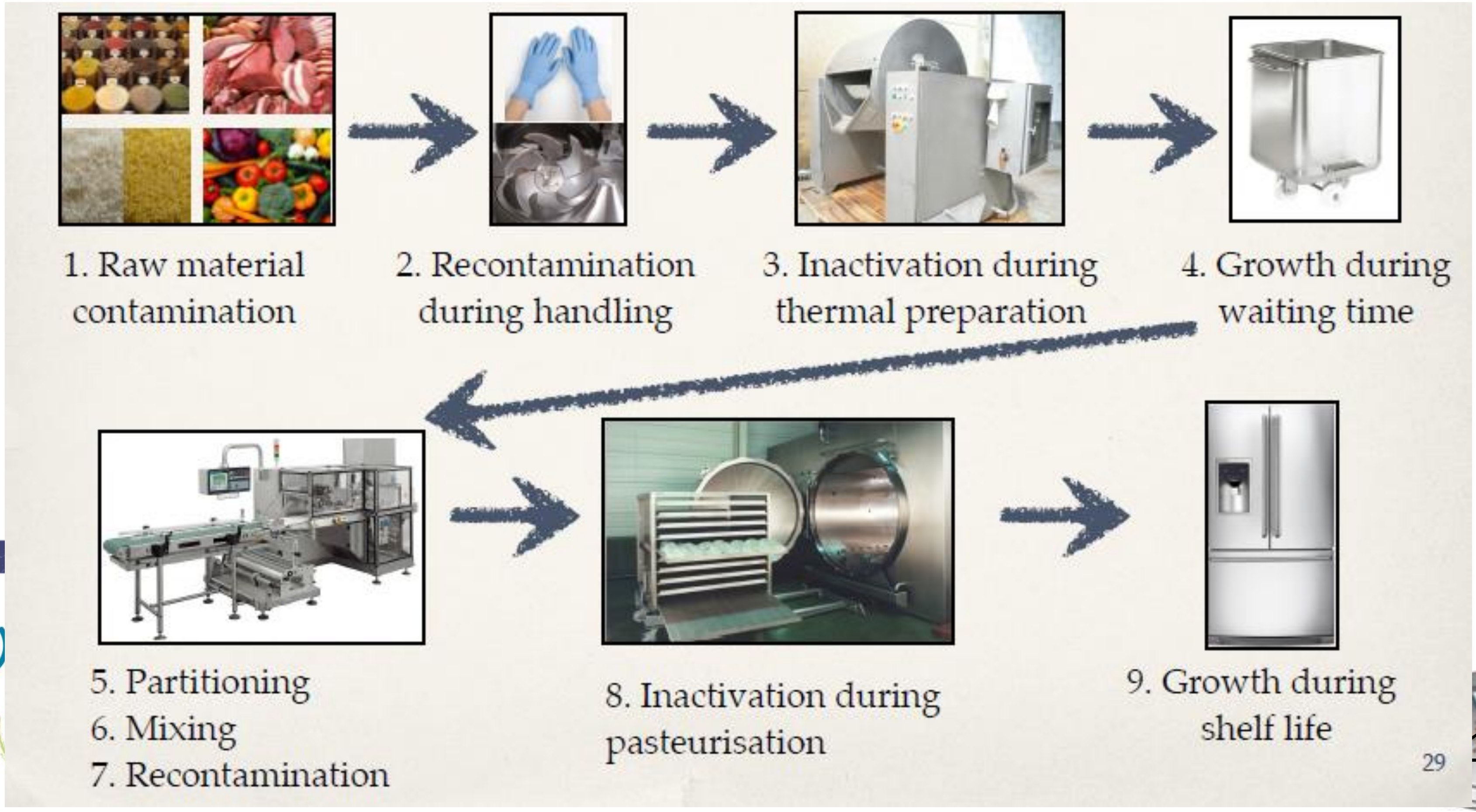
Consumerbehavior

A new approach: the iso-risk curves

An **iso-risk curve** is a graphical representation of combinations of risk determining factors (e.g. pH, heat treatment intensity, shelf life) that all lead to the same risk level.

Each point on these curves, i.e. each combination of variables, will lead to the same level of exposure e.g. the same fraction of 'risky' packages in a production (e.g. 0.48%).

Gathering **QUANTITATIVE information** regarding the different steps in the chain: Quantitative microbial exposure assessment



One of the key elements which was unknown

Sublethally damaged spores after pasteurisation: effect of pasteurisation intensity on the subsequent lag phase (germination and growth) of surviving *Bacillus cereus* spores during storage

Lag time model as a function of heat intensity (T,t), pH, aw, storage T

Heat treatment time as a function of product pH

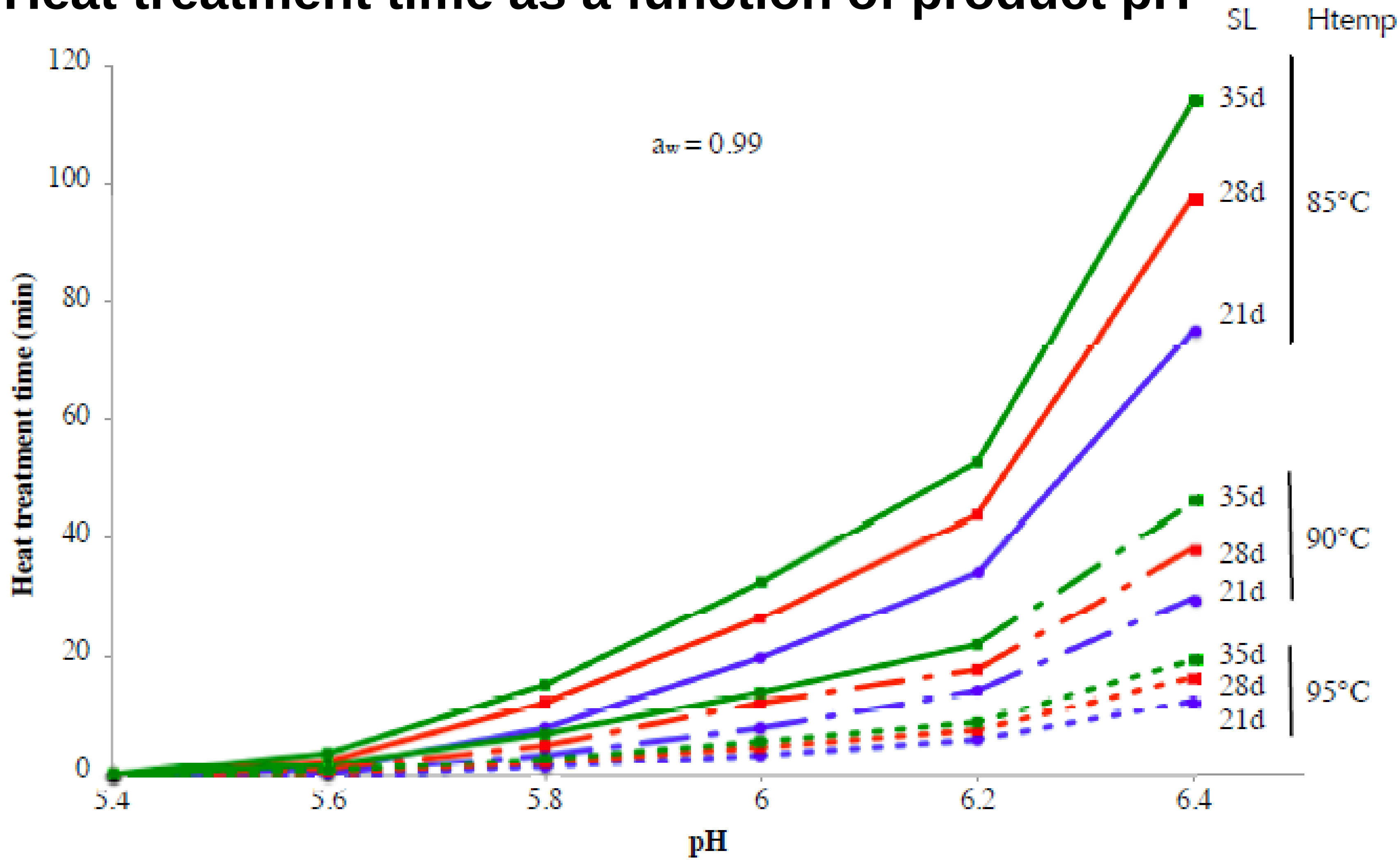


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Figure 9.2: Iso-risk curves for different shelf lives (SL) and heat treatment temperature (Htemp) combinations. Heat treatment time needed (y-axis) to obtain the same % of 'risky packs' as in the baseline scenario, for a product with a given pH (x-axis) (a_w: 0.99).

Maximum shelf life as a function of heat treatment temperature

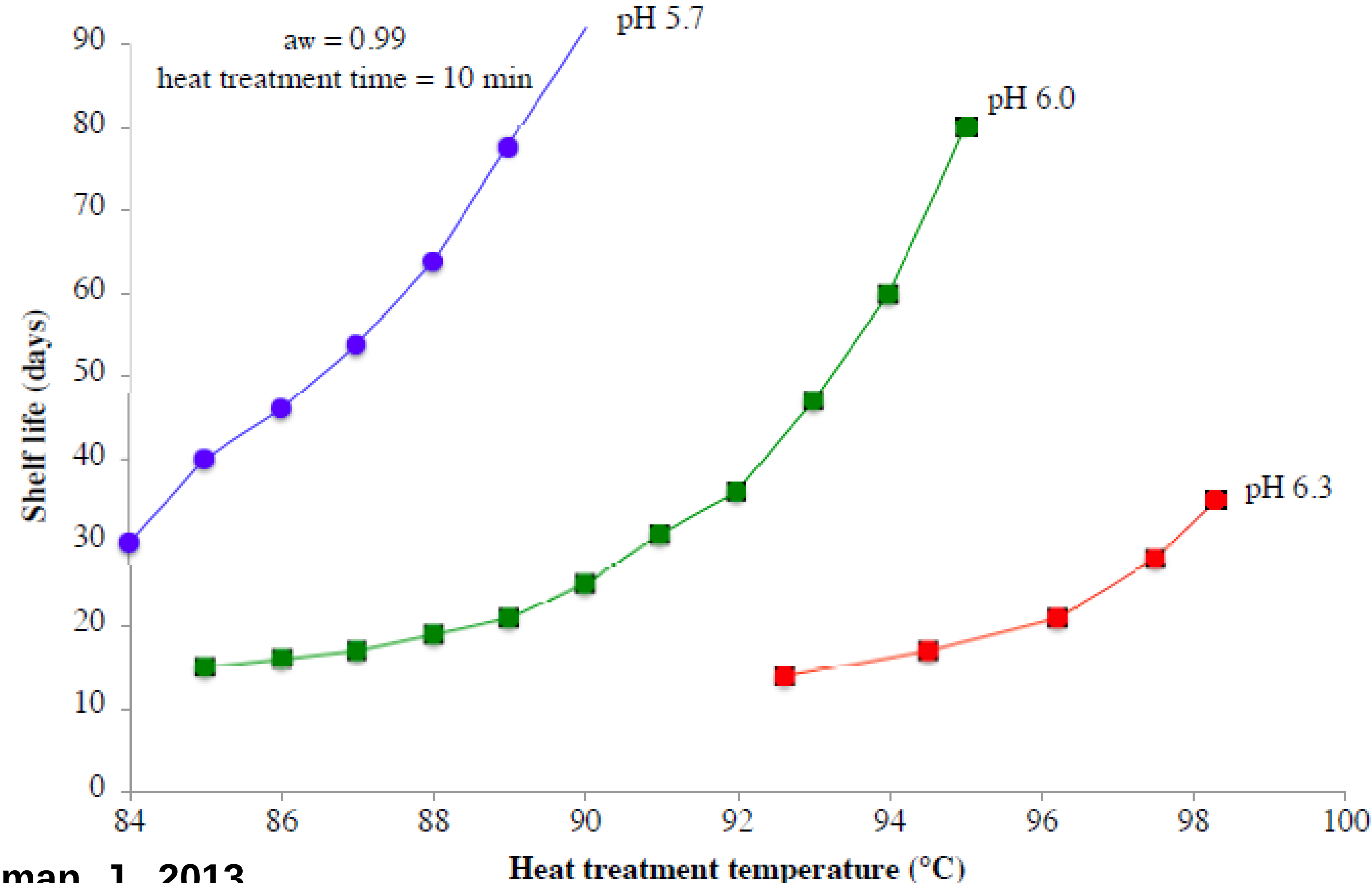

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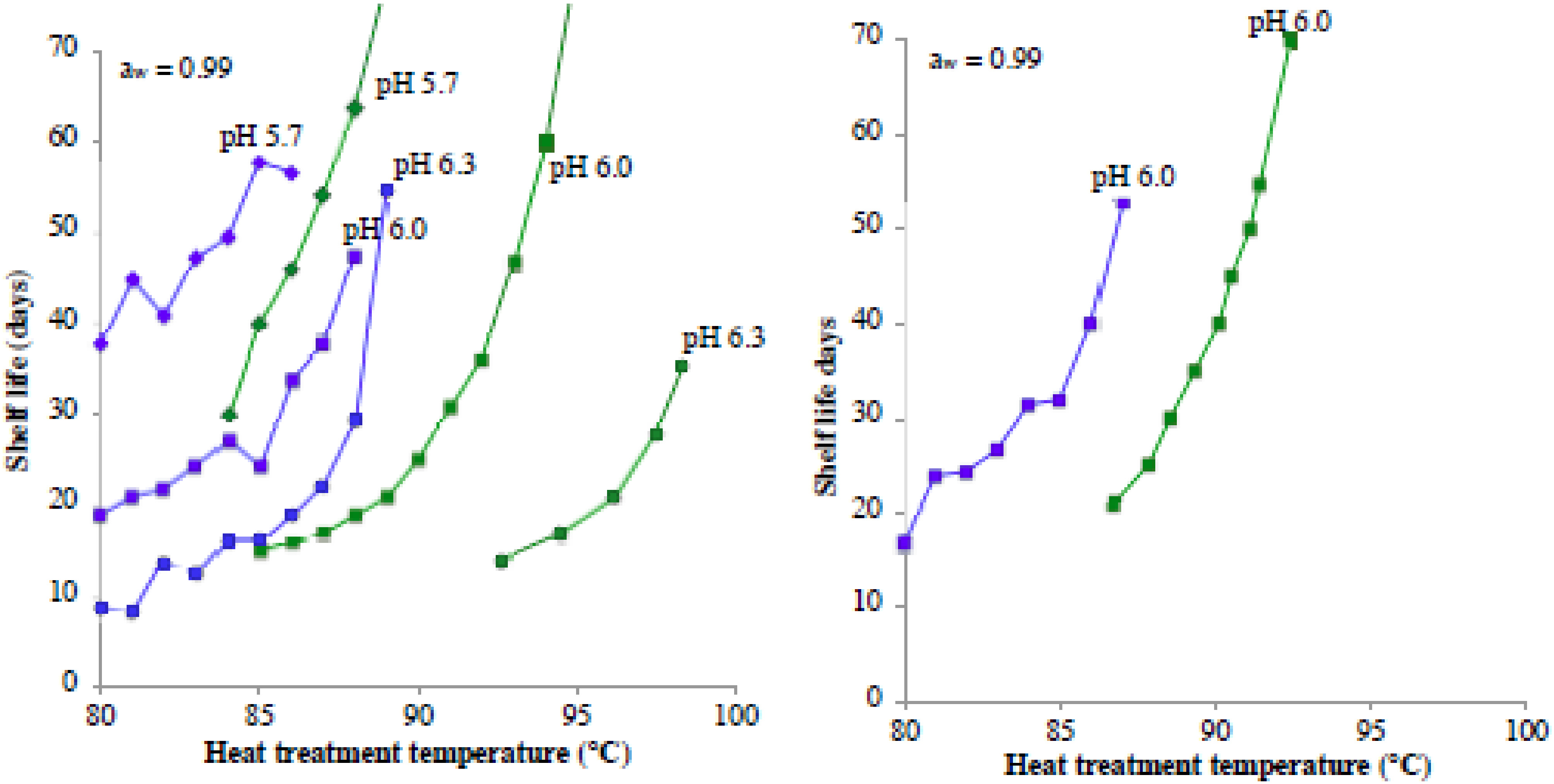
Figure 9.4: Iso-risk curves for different pH-values (blue: 5.7, green: 6.0 and red: 6.3), maximum shelf life as a function of heat treatment temperature to obtain the same % of 'risky packs' as in the baseline scenario (a_w:0.99, heat treatment time: 10min).

Psychrotrophic *C. botulinum*

Similar BUT:

- more sensitive for heat
- combined effect of heat and other parameters on germination and growth will be more pronounced
- However: no quantitative data available



(a) Heating time = 10 min

(b) Heating time = 15 min

Figure 9.6: Iso-risk curves for different pH-values (◇ 5.7, □ 6.0 and ○ 6.3) and two microorganisms: *B. cereus* (green) and *C. botulinum* (blue). Maximum shelf life as a function of heat treatment temperature to obtain the same % of 'risky packs' for *B. cereus* as in the baseline scenario (a_w: 0.99, heat treatment time: 10min).

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Conclusions

Does psychrotolerant *B. cereus* pose a problem/is it a risk in industrially produced, chilled, pasteurised meals?

Theoretically: all conditions are present for germination and growth of psychrotolerant spores of *B. cereus*

Conclusions

In reality: several factors are lowering the risk:

- limited growth at T<10°C at anaerobic conditions and no growth at 30% CO_2
- a clear combined effect of pasteurisation and other preservation factors (a_w, pH, T) especially at higher pasteurisation temperatures
- CO_2 inhibits growth
- low survival of preformed toxins and vegetative cells through gastro-intestinal tract

Conclusions

BUT:

- lower pasteurisation temperatures can stimulate growth
- low residual O_2 levels allow growth
- The consumer remains a bottleneck

➔Time (interpretation of use by date)

➔Temperature (refrigerators)

➔Reheating (micro-wave)

In cooperation with

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