FRUTAROM SAVORY SOLUTIONS

Gewürzmüller® GEWÜRZMÜHLE NESSE

Meat starter cultures

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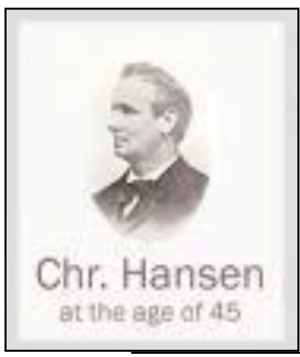
PASSION FOR TASTE AND HEALTH

History of starter cultures

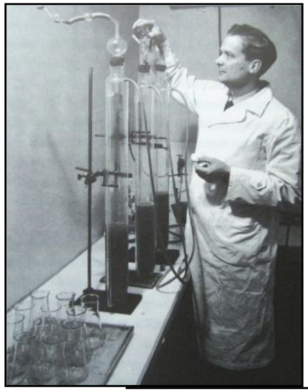
1866 - PASTEUR discovered micro-organisms as source for fermentation processes



1892 - Chr. Hansen starts selling the first commercial starter cultures for the dairy industry

1935/1940 - JENSEN and PADDOCK established the usage of lactic acid bacteria for the ripening of fermented sausage in the USA

History of starter cultures



1955 - Dissertation of NIINIVAARA „Über den Einfluss von Bakterienkulturen auf die Reifung und Umrötung der Rohwurst“ is commonly accepted as birth of defined starter cultures for meat fermentation

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1966 - NURMI develops the first mixture of lactic acid bacteria and micrococci as a starter preparation

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1972 - First International Symposium of Starter Cultures in Helsinki helps to get starters accepted by the butchers and the meat industry

Definition of starter culture

Starter cultures are preparations of live microorganisms or their resting forms, whose metabolic activity has desired effects in the fermentation substrate, the food.

The preparations may contain unavoidable residues from the culture substrate and additives that support the vitality and technological functionality of the microorganisms (such as antifreeze or antioxidant compounds).

- **Single-strain cultures: contain one strain of a species;**
- **Multi-strain cultures: contain more than one strain of a single species;**
- **Multi-strain mixed cultures: contain different strains from different species.**

Source: Senate Commission on Food Safety of the DFG, 2010.

Microbial ecology of meat fermentation

Endogenous factors

Meat
- type / quality (*e.g.* beef, pork)
- fat content
- reduction rate

Casing

Size

Curing agent
(salt, nitrate, nitrite)

Spices

Other ingredients
- ascorbic acid
- sodium glutamate
- glucono δ-lactone
- sugar

Meat, spice and environmental biota

Exogenous factors

Temperature
Relative humidity
Oxygen (airflow)
Smoke
Fermentation time
Starter culture

Starter organisms

Implicit factors, *e.g.* bacteriocins

Contaminating biota

Product quality

Color
Flavor
Texture
Shelf life

Advantages of use of starter cultures

- **Reduction of hygienic risks**
- **Ensuring constant high product quality**
- **Control of development of color and flavor**
- **Control of fermentation time**
- **Prevention of fault fermentation**

→ Reduction of costs by shortening fermentation times and assures production of products of high safety and sensory quality

Key components of R&D activities in the starter culture business

- **Screening for strains with desired properties**
  - microbiology (metabolism, performance, etc.)
  - meat technology (acidification, development of flavor and color, etc.)
  - safety (bacteriocins, antagonistic principles, etc.)
- **Safety assessment of strains**
  - identity
  - possible pathogenicity
  - acquired antibiotic resistances
- **Check for producibility of strains**
  - yield (fermentation, freeze-drying)
  - stability
  - functionality

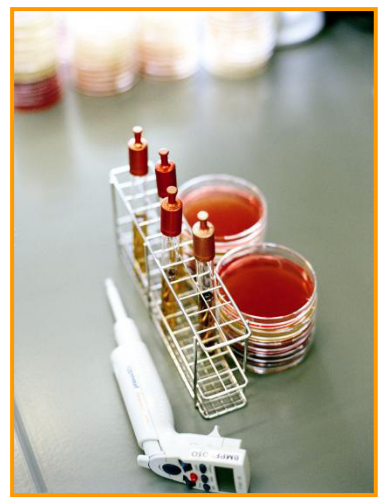
Antagonistically acting metabolites and substances of lactic acid bacteria

Metabolite / substance	Remarks
Organic acids	
Lactic acid, acetic acid, propionic acid, formic acid, bencoic acid	Partly in use as additive
Phenyllactate	Antifungal
Other metabolites	
Reuterin (3-Hydroxypropionaldehyde)	Bacteria, yeasts, moulds, protozoe
Diacetyle	In use as flavoring agent
3-Hydroxy fatty acids	Antifungal
Other antagonistic substances	
Reutericyclin	Tetramic acid
Cyclic dipeptide	Antifungal
Bacteriocin	
Nisin	In use as additive
Others bacteriocins	Lantibiotics, Class II and cyclic bacteriocins

Origin of starter organism for food fermentation

Indigenous flora of traditional fermented food

Own strain collection



Public strain collections

NCTC
National Collection of Type Cultures
A Health Protection Agency Culture Collection

DSMZ

Screening of hundreds of strains is required !

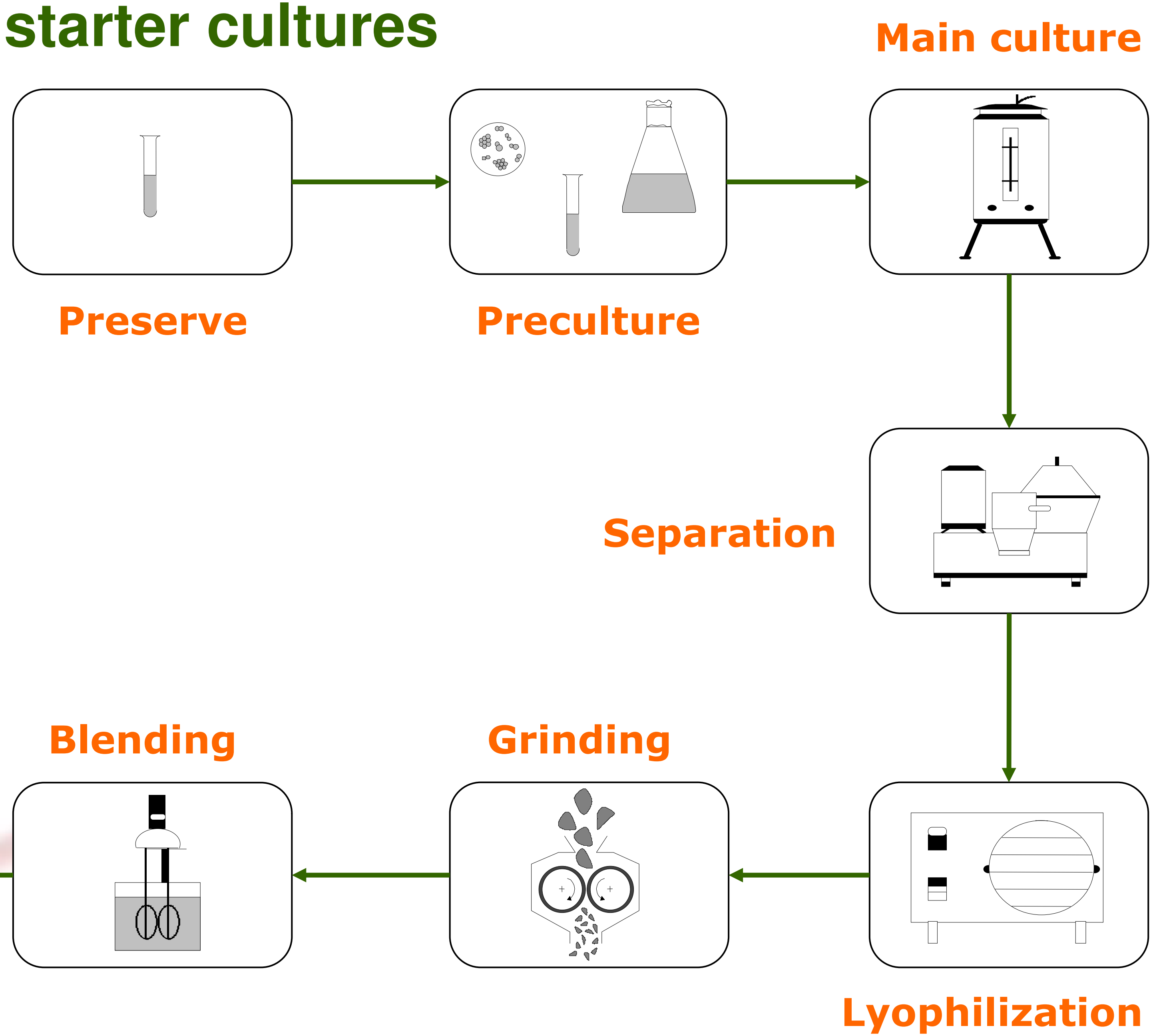
Requirements to starter organisms

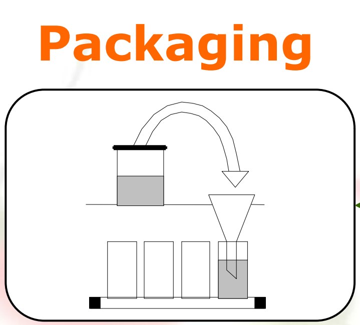
Production of the culture

- Optimal performance in an inexpensive artificial medium
- High cell densities (10^{10} - 10^{11} CFU/ml)
- High survival rate during lyophilization
- High storage stability

Fermenter at BITEC

Production of starter cultures





Requirements to starter organisms

Production of the culture

- Optimal performance in an inexpensive artificial medium
- High cell densities (10^{10} - 10^{11} CFU/ml)
- High survival rate during lyophilization
- High storage stability

Food fermentation

- Optimal performance (high competitiveness) in a complex food matrix, where ecological conditions are constantly changing
- Short lag phase (fast pH drop)
- Tolerance against prevailing ecological conditions (low pH)
- Expression of properties contributing to product quality

Prevailing ecological conditions

Factor	Fermenter	Sausage meat	Raw fermented sausage
pH	5.8 – 6.5	5.6 – 5.9	4.8 – 5.3 (5.8)
Temperature	25 – 37°C	0 bis 2°C	RT
Water activity (a_W)	0,99	0.96 – 0.97	0.85 – 0.93 (0.95)
Salt content	< 0.1%	2.6 – 3.0%	> 2.6 – 3.0%
Sugar content	2.0 – 3.0%	0.2 – 0.7%	0%
Nitrite (NPS)	0	130 – 150 ppm	< 150 ppm
Redoxpotential	adjusted	high	low (high at surface)

Effect of starter cultures on the spontaneous flora

Day	Cell number of the lactic acid bacteria (cfu / g)				
	Culture 1	Culture 2	Culture 3	Culture 4	Culture 5
1	$1,0 \times 10^9$	$6,9 \times 10^8$	$7,6 \times 10^8$	$8,8 \times 10^8$	$4,2 \times 10^8$
7	$1,5 \times 10^9$	$8,3 \times 10^8$	$3,7 \times 10^8$	$6,0 \times 10^8$	$5,9 \times 10^8$
42	$9,1 \times 10^8$	$1,2 \times 10^9$	$5,1 \times 10^8$	$4,1 \times 10^8$	$8,7 \times 10^8$

Day	Cell count of the spontaneous flora of lactic acid bacteria (cfu / g)				
	Culture 1	Culture 2	Culture 3	Culture 4	Culture 5
1	n. d.	n. d.	$2,0 \times 10^6$	n. d.	$2,2 \times 10^6$
7	n. d.	n. d.	$6,3 \times 10^7$	$4,1 \times 10^6$	$2,0 \times 10^6$
42	n. d.	n. d.	$9,5 \times 10^7$	$2,1 \times 10^7$	n. d.

Technologically relevant properties of starter organisms

- Relationship to oxygen
- Type of fermentation
- Salt tolerance
- Nitrite tolerance
- pH tolerance
- Temperature range
- Spectrum of fermentable sugars
- Physiological enzyme activities
- Properties involved in the safety assessment

Potential of lactic acid bacteria from meat to form hydrogen peroxide and to exhibit catalase activity

Species	Formation of H_2O_2	Presence of the activity of catalase (heme-dependent)	Presence of the activity of pseudocatalase (Mn-dependent)
Lactobacillus curvatus	+	-	-
Lactobacillus sakei	+	+	-
Lactobacillus plantarum	+	+	+
Pediococcus pentosaceus	-	-	+
Pediococcus acidilactici	+	+	-

$$2\,H_2O_2 \rightleftharpoons O_2 + 2\,H_2O$$

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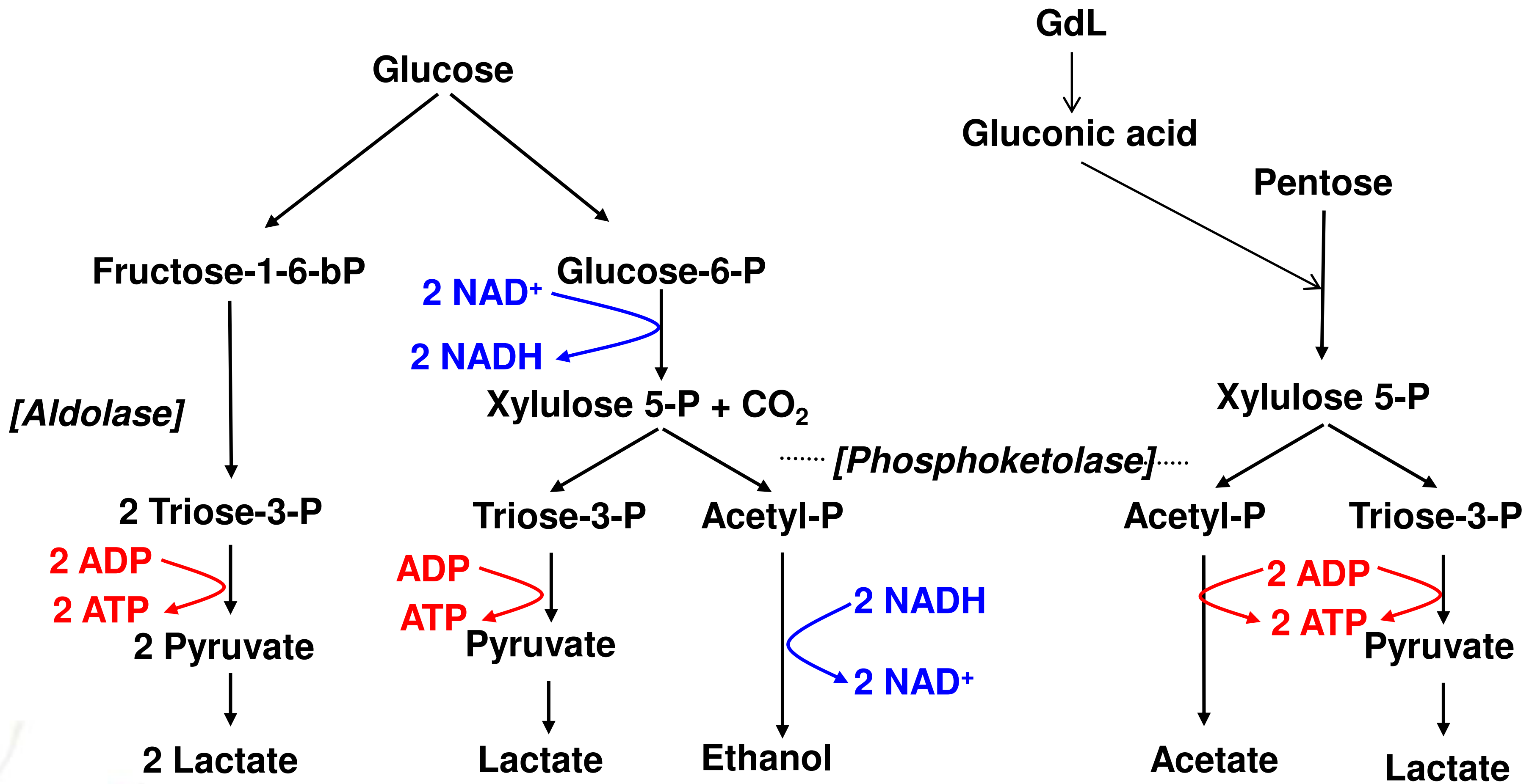
Fermentation type



homofermentative lactic acid fermentation

Heterofermentative lactic acid fermentation

L(+) : D(-) lactic acid 50 : 50
right-handed / left-handed
physiological / non-physiological

Gluconate metabolizing:
L. sakei strains: positive or (+)
L. curvatus strains: negative

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- Relationship to oxygen
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Salt tolerance and/or nitrite tolerance

- Water activity is the ratio of the water vapor pressure (*p*) above the food and the water vapor pressure above pure water (p_0):

$$a_W = p / p_0$$

	Water content	[salt]	a_W
Pork meat S II	73%		
Back bacon S VIII	8%		
Formula with 30% fat and 3% salt	53%	5.2%	0.97
Drying of 15%		7.1%	0.95
Drying of 30%		11.3%	0.92

Raw sausage $a_W < 0{,}91$ – Termination of bacterial growth and metabolism !
Enzymes are still active – proteolysis / lipolysis are further running !

Nitrite tolerance of our starter organism meet the salt tolerance.

Technologically relevant properties of starter organisms

- Relationship to oxygen
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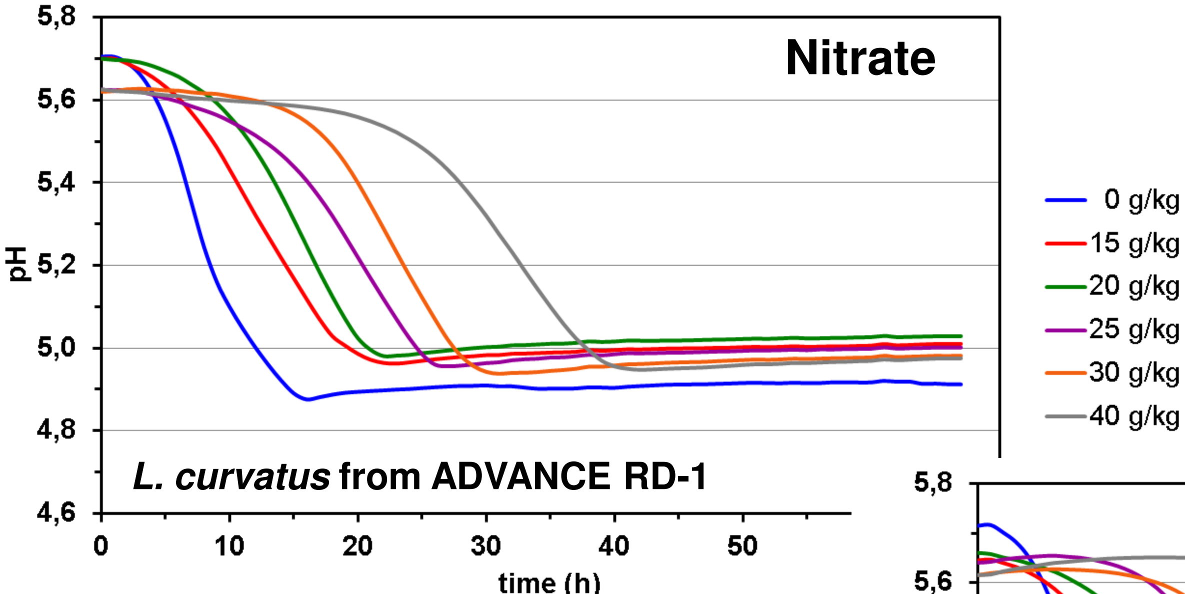
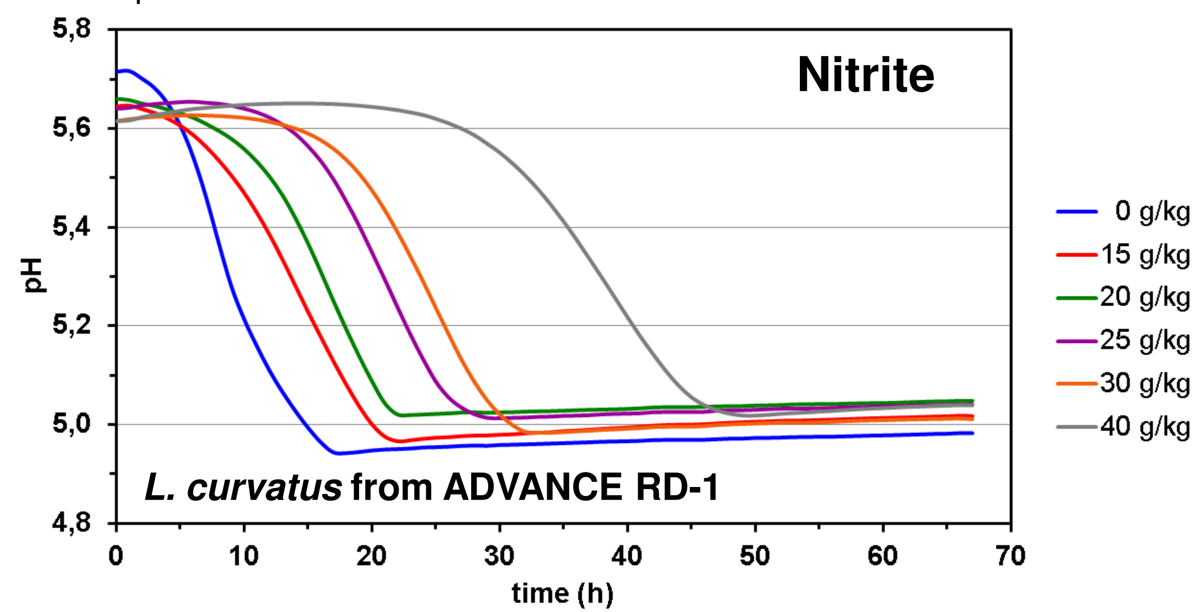
Effect of the salt concentration on the kinetics of pH during raw sausage fermentation

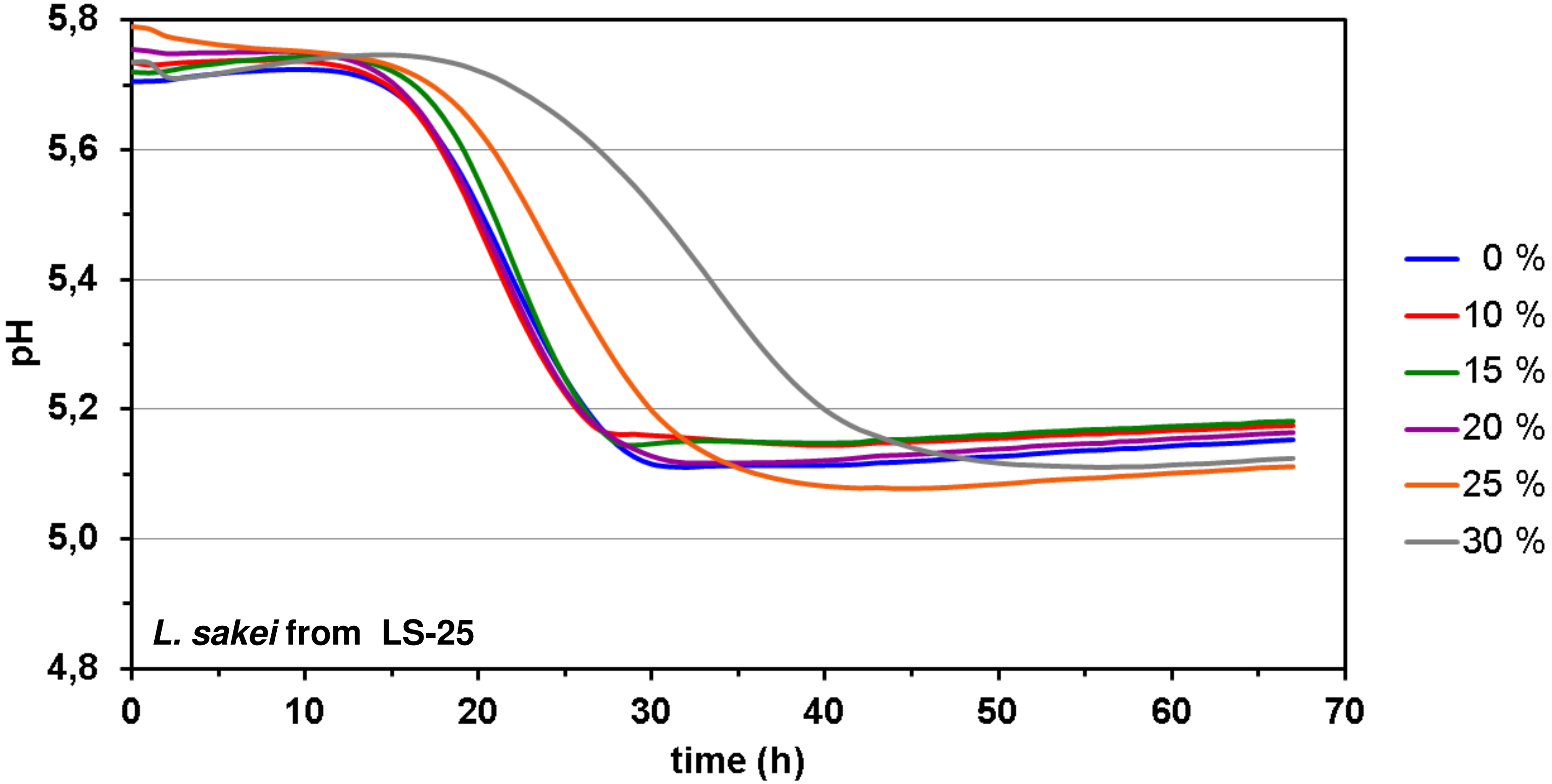
(0.4 % dextrose, 24°C)





Effect of the fat content on the kinetics of pH during raw sausage fermentation

(0.4% dextrose, nitrite curing salt, 24°C)



The fat content corresponds to the content of added fat. The absolute value is 8% higher.

Technologically relevant properties of starter organisms

- Relationship to oxygen
- Type of fermentation
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- pH tolerance
- Temperature range
- Spectrum of fermentable sugars
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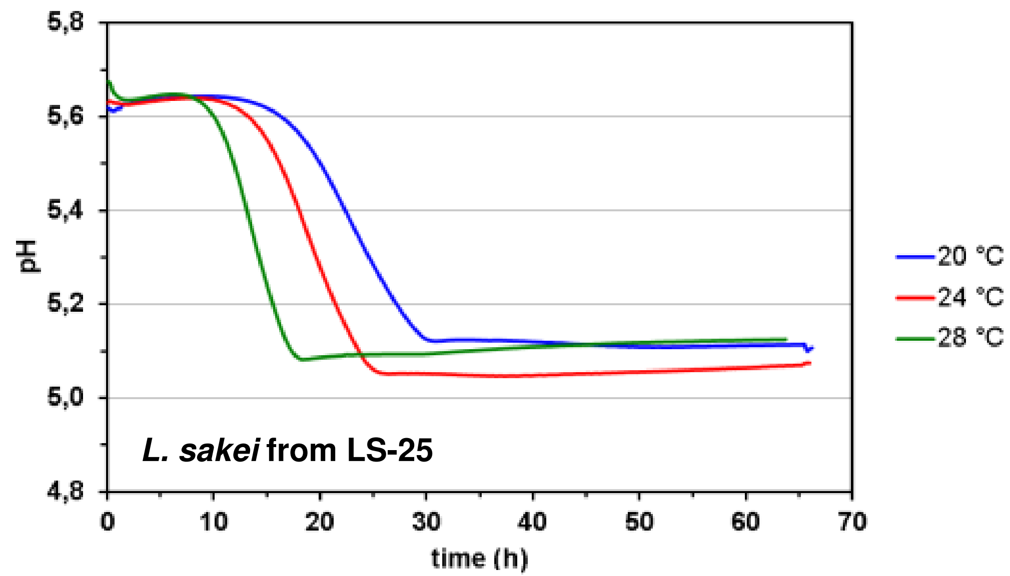
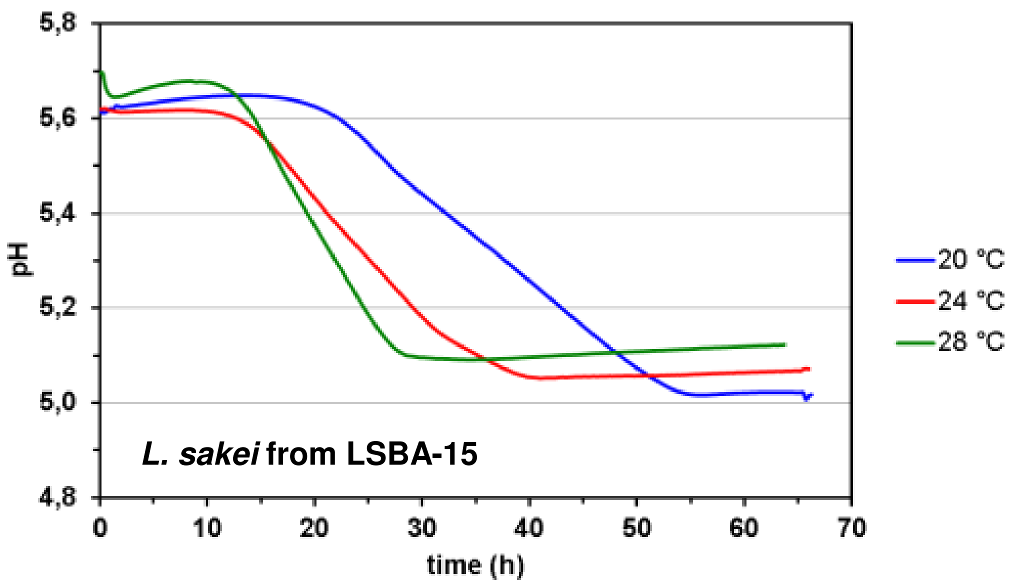
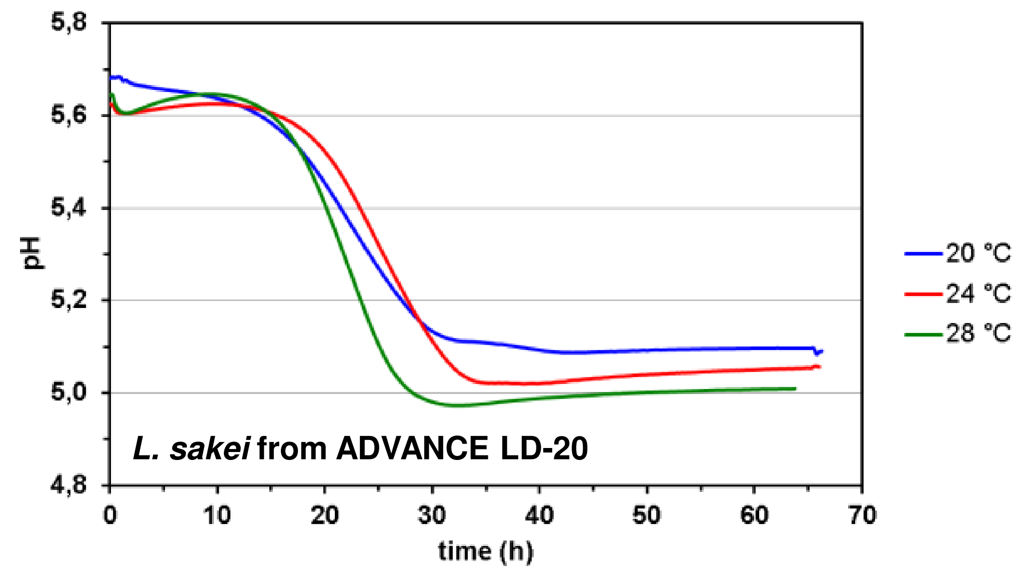
- Properties involved in the safety assessment

Effect of the temperature on the kinetics of pH during raw sausage fermentation (0.4% dextrose, nitrite curing salt)







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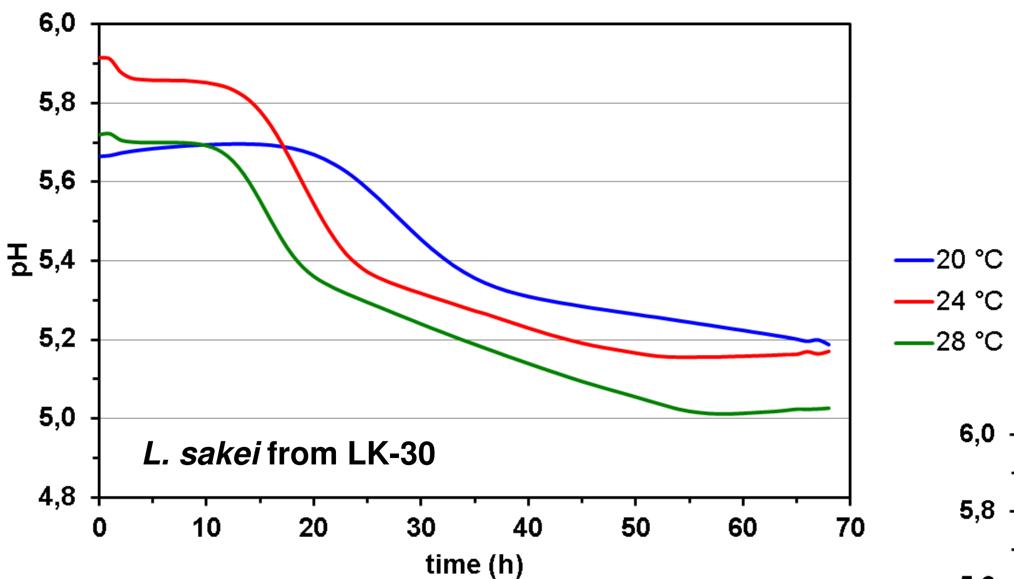
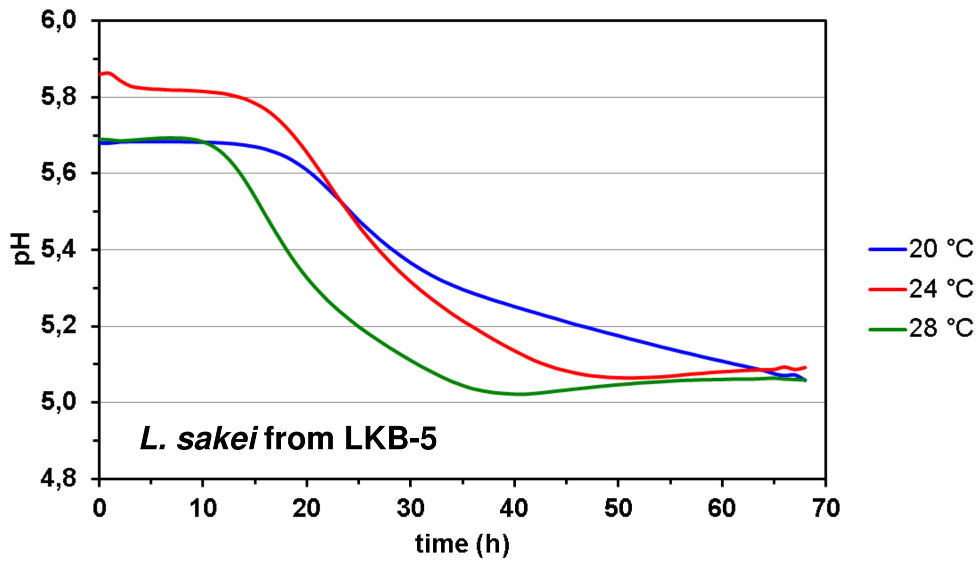
Effect of the temperature on the kinetics of pH during raw sausage fermentation (0.4% dextrose, nitrate)



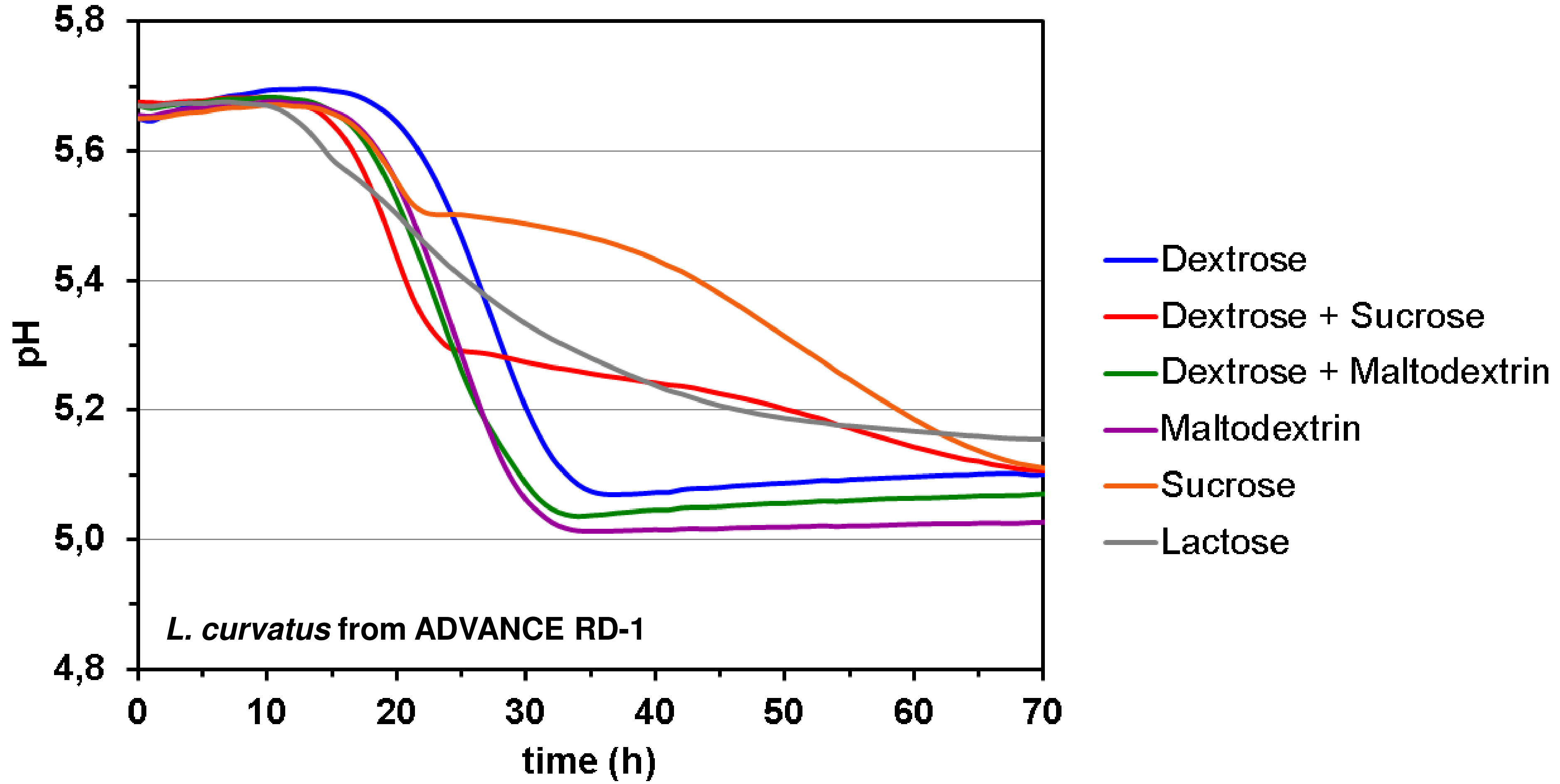


Technologically relevant properties of starter organisms

- Relationship to oxygen
- Type of fermentation
- Salt tolerance
- Nitrite tolerance
- pH tolerance
- Temperature range
- Spectrum of fermentable sugars
- Physiological enzyme activities
- Properties involved in the safety assessment

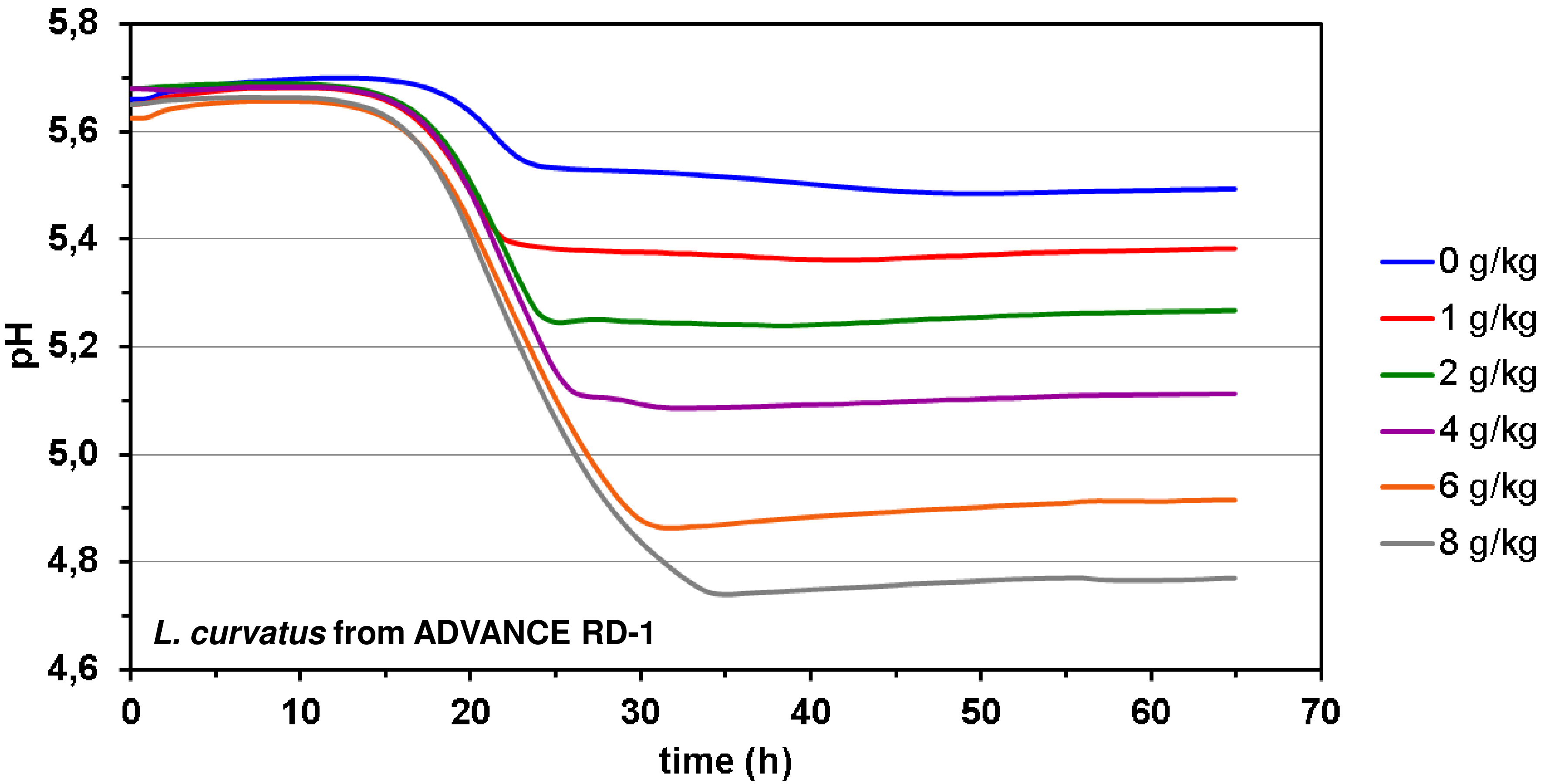
Effect of different sugars on the kinetics of pH during raw sausage fermentation

(0.4% dextrose, nitrite curing salt, 24°C)



Effect of the dextrose concentration on the kinetics of pH during raw sausage fermentation

(0.4% dextrose, nitrite curing salt, 24°C)



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- Relationship to oxygen
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Nitric oxide formation during curing

pH drop

Lactic acid bacteria → Lactic acid

GdL + H_2O → Gluconic acid

Redox system

$2\ HNO_2 + C_6H_8O_6 \longrightarrow 2\ NO + 2\ H_2O + C_6H_6O_6$

Ascorbic acid → Dehydro ascorbic acid

Bacterial reduction pH >5,5

$$3\ HNO_2 \rightleftarrows 2\ NO + HNO_3 + H_2O$$

Acid catalysed decay pH <5,5

Enzymatic redox systems of meat

Cystein cystin system: $2\ NO_2^- + 2\ R\text{-}SH + 2\ H_3O^+ \longrightarrow 2\ NO + R\text{-}S\text{-}S\text{-}R + 2\ H_2O$

Ferro cytochrome C system: $NO_2^- + \text{Cyt-c (red)} \longrightarrow \text{NO-Cyt-c (ox)}$

Flavor formation in meat fermentation

Taste

Meat enzymes | **Starter culture**

Meat proteins
Cathepsin D
Calpain
↓
Peptides
↓
Amino acids

Carbohydrate
↓
Lactate
Acetate
Ethanol
Acetoin
Diacetyl

Aroma

Meat enzymes | **Staphylococci**

Fat
Lipase
↓
Free fatty acids

Fatty acids
Oxidative degradation
↓
Alkane
Alkene
Alcohols
Aldehydes
Ketones
Furanes

Amino acids
Leu
Ile
Val
↓
Alcohols
Aldehydes
Ketone
Acids

- Mainly peptidases of *Staphylococcus* spec.
- Exo-peptidases of *Lactobacillus* spec.

- Key aroma compounds of fermented sausages:
  3-Methylbutanal (Leu)
  2-Methylbutanal (Ile)
  2-Methylpropanal (Val)

Technologically relevant properties of starter organisms

– Relationship to oxygen
– Type of fermentation
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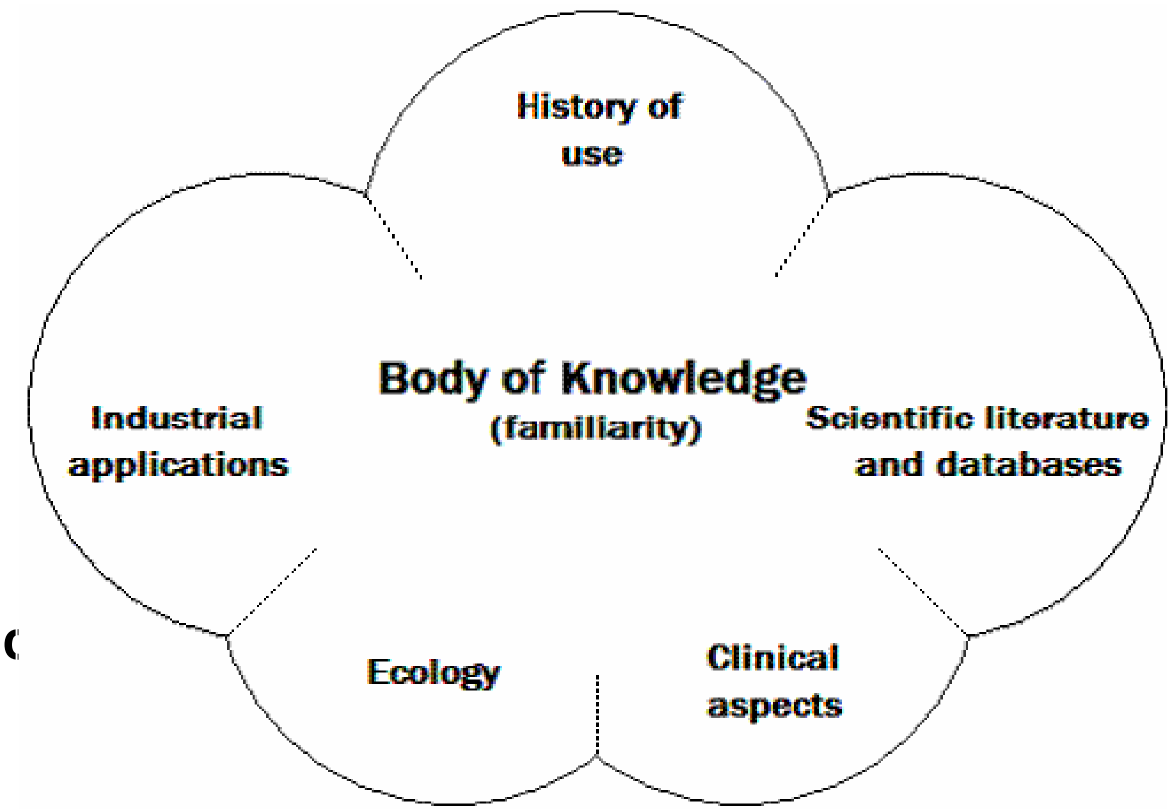
The Qualified Presumption of Safety (QPS) system

Four pillars

- Identity
- Body of knowledge
- Safety concerns
  - possible pathogenicity
  - acquired antibiotic resistanc
- End use



Source:
European Commission (2003). On a generic approach to the safety assessment of micro-organisms used in feed/food and feed/food production.
EFSA (2005). Opinion of the Scientific Committee on a request from EFSA related to A generic approach to the safety assessment by EFSA of microorganisms used in food/feed and the production of food/feed additives.

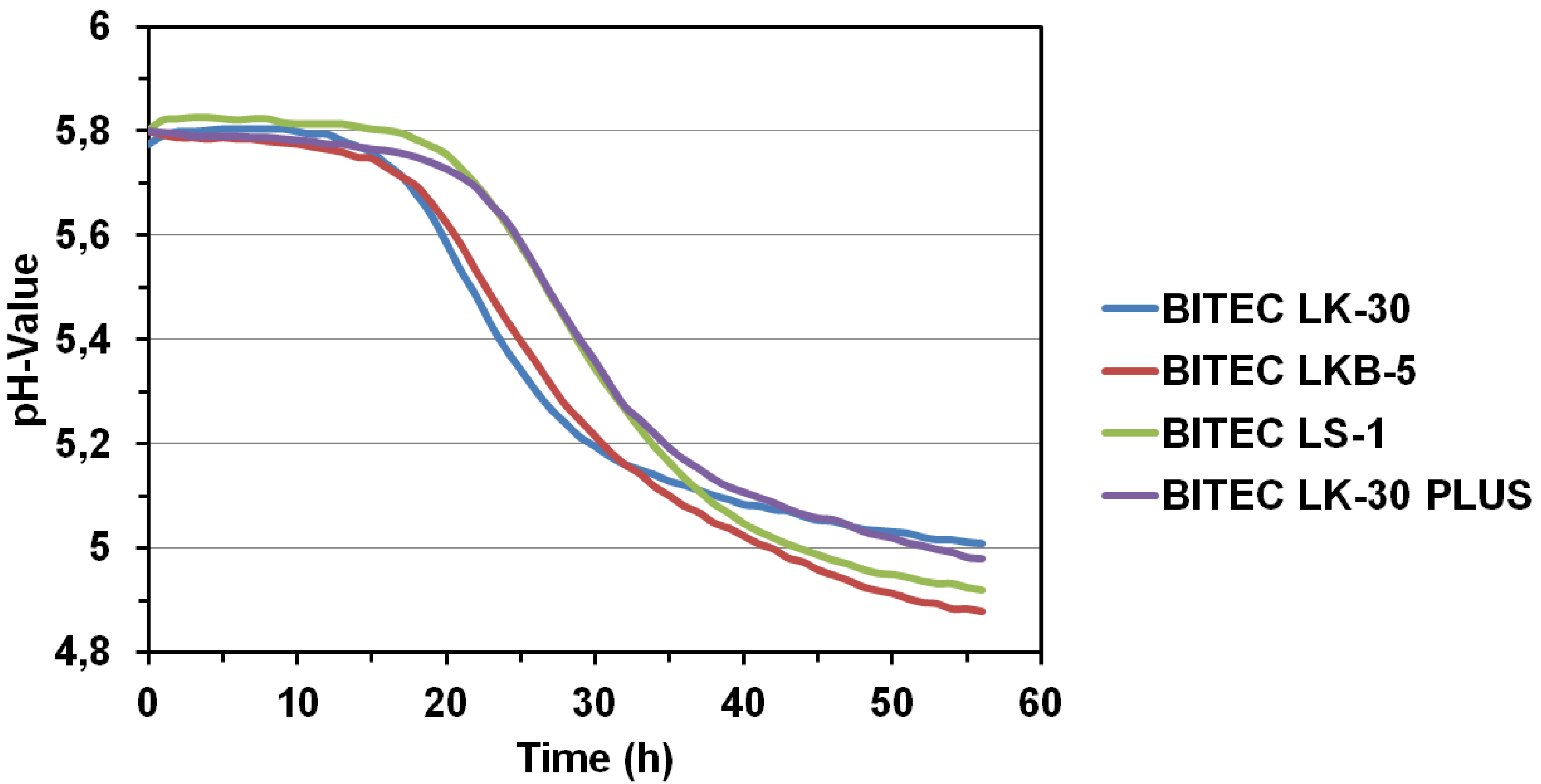
Species in FRUTAROM's meat starter cultures

Organism	Function	Effect
Lactic acid bacteria *Lactobacillus sakei* *Lactobacillus curvatus* *Lactobacillus paracasei* *Pediococcus acidilactici* *Pediococcus pentosaceus*	Acidification Possibly, formation of bacteriocins	Preservation Contribution to the formation of flavor, texture and red color
Catalase-positive cocci *Staphylococcus carnosus subsp. utilis* *Kocuria salsicia*	Nitrate reduction Proteolysis and lipolysis Cleavage of H_2O_2 (catalase) Reduction of redox potential	Formation and/or stabilization of flavor and red color
Molds *Penicillium nalgiovense* *Penicillium candidum*	Proteolysis and Lipolysis Growth on the surface	Formation of flavor Prevention of growth of undesired organisms Protection against water loss, oxygen and light

Starter cultures for slow raw sausage fermentation

Culture	Species	Characteristics
LK-30	*L. sakei, S. carnosus, K. salsicia*	Harmonic pH-drop, highly competitive
LK-30 plus	*L. sakei, L. paracasei, S. carnosus, K. salsicia*	Milder than LK-30
LKB-5	*L. sakei, S. carnosus, K. salsicia*	Harmonizes and assures acidification process
LS-1	*L. curvatus, S. carnosus, K. salsicia*	Evenly pH-drop, highly competitive

Kinetics of pH of starter cultures for slow fermentation

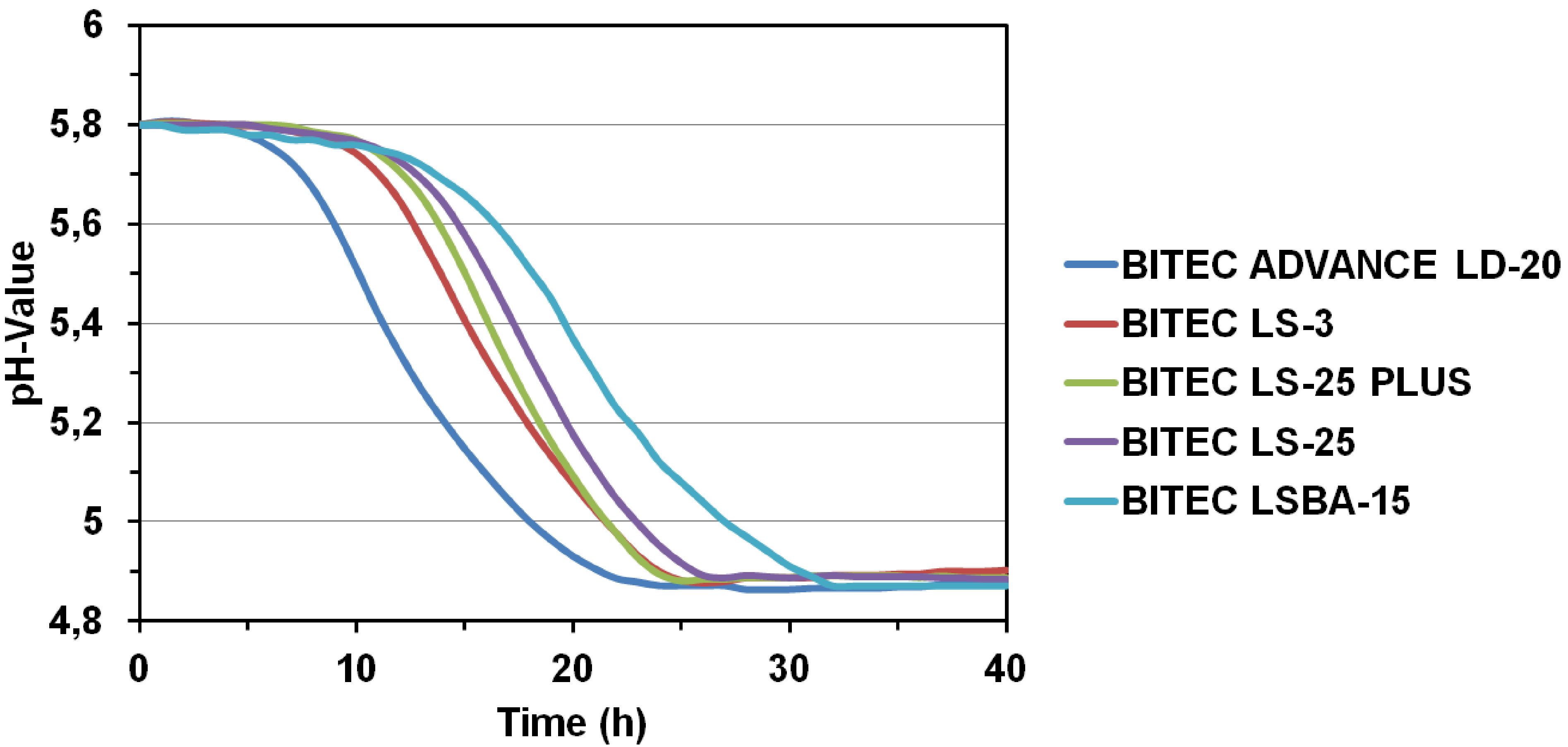


0.4 % dextrose and 2.8 % nitrite curing salt.
Temperature: 21°C

Starter cultures for fast raw sausage fermentation

Culture	Species	Characteristics
LS-25	*L. sakei, S. carnosus*	fast pH drop, highly competitive
LS-25 plus	*L. sakei, L. paracasei, S. carnosus*	milder than LS-25
LS-3	*L. curvatus, S. carnosus*	fast pH drop, highly competitive
CONDI rasant	*P. pentosaceus, S. carnosus*	Fast pH drop, suitable for high temperatures
LSBA-15	*L. sakei, S. carnosus, K. salsicia*	Bacteriocin producer
ADVANCE LD-20	*L. sakei, S. carnosus*	Very fast pH drop, Extra mild Highly competitive

Kinetics of pH of starter cultures for fast fermentation



0.4 % dextrose and 2.8 % nitrite curing salt.
Temperature: 24°C

BITEC ADVANCE LD-20 (launch: 2012)

Composition

Multi-strain mixed culture → ***Lactobacillus sakei***
Staphylococcus carnosus

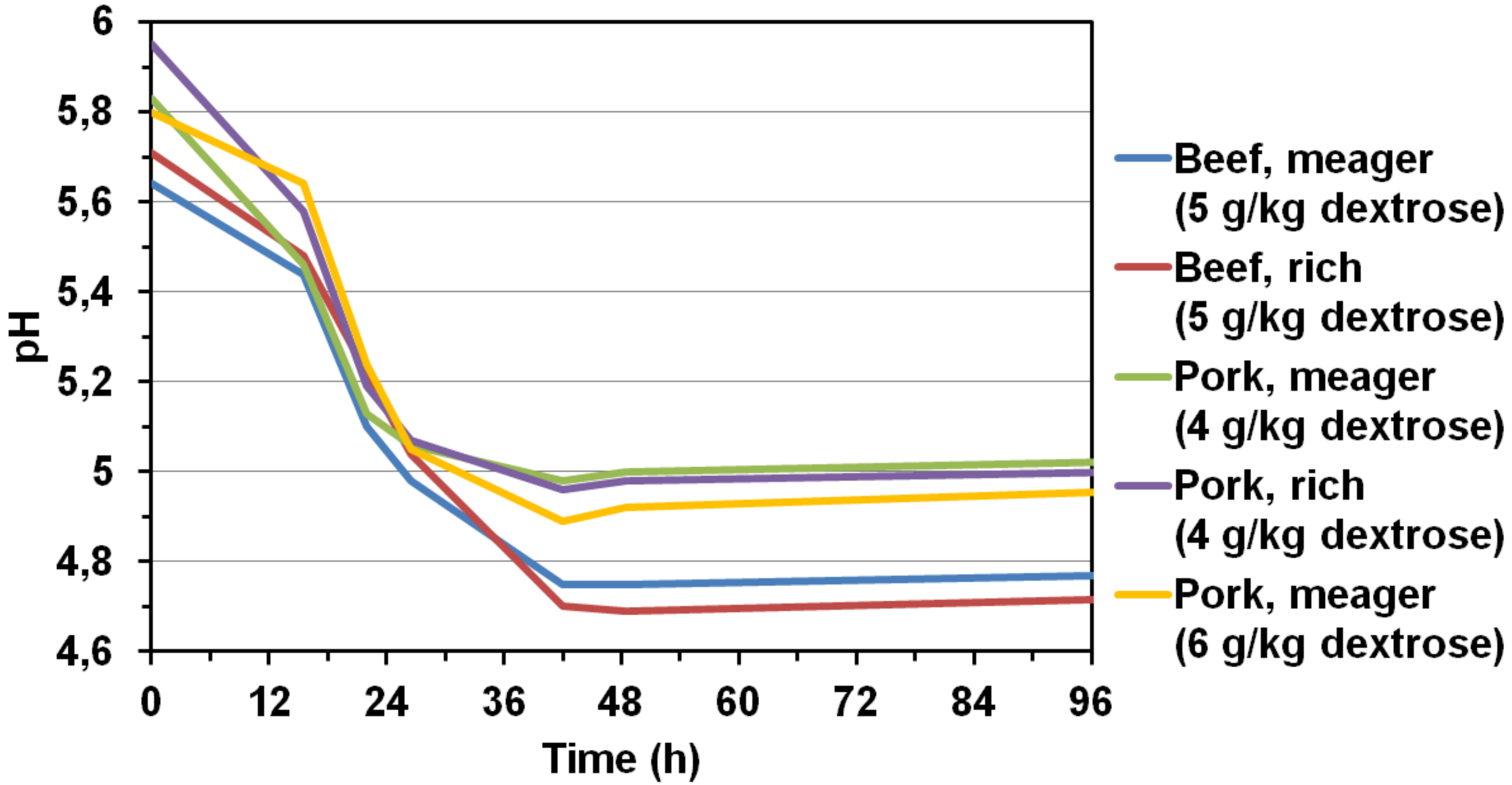
Properties

- **Mild acid taste**
- **Fast acidification**
- **Good development and stabilisation of the color**
- **Highly competitive**

Application

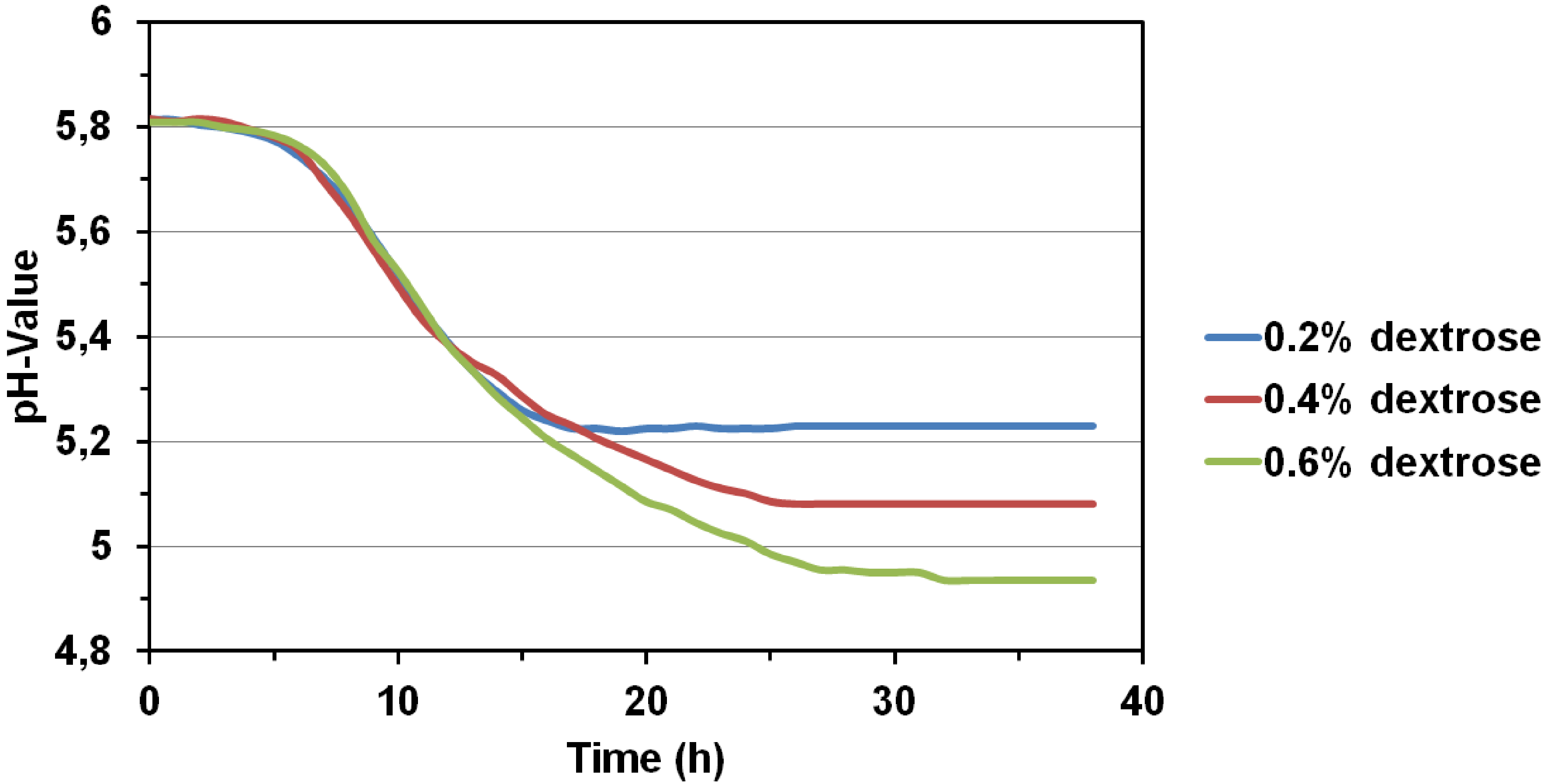
- **Sliceable, spreadable, and fresh fermented sausages**
- **Fermentation with nitrite curing salt**
- **Fermentation nitrate and salt**

Examples of pH drops in different fermenting sausages produced with ADVANCE LD-20



Kinetics of pH of BITEC ADVANCE LD-20

(pork, 24 °C, nitrite curing salt)



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Thank you!

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