

김치 발효소시지가 미생물학적 안정성에 미치는 효과

이 주 연

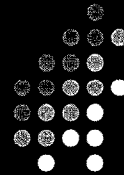
(한국식품개발연구원)



김치 발효소시지가 미생물학적 안정성에 미치는 효과

한국축산식품학회
2004년도 제 33차 춘계 학술발표

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Fermented sausages (Raw sausages)



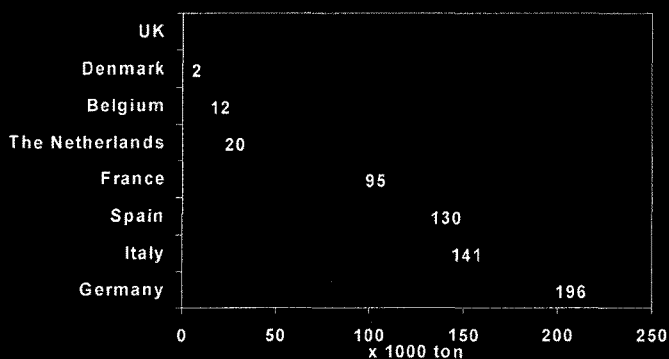
- Fermented raw sausages consist of raw, finely chopped meat and fat, which are mixed with salt, spices and few additives.
- It is then fermented and dried at the appropriate temperature and air humidity, fermented and dried for a sufficient length of time.

Classification of fermented sausages



Category	Ripening times	Final water activity (a_w)	Weight loss during drying	Application of smoke	Examples
Dry, mould-ripened	>4 weeks	<0.9	>30%	No	Genuine Italian salami, French 'saucisson sec'
Dry, mould-ripened	>4 weeks	<0.9	>30%	Yes	Genuine Hungarian salami
Dry, no mould growth	>4 weeks	<0.9	>30%	Yes or no	German 'Dauerwurst'
Semi-dry, mould-ripened	>4 weeks	0.90-0.95	<20%	No	Various French and Spanish raw sausages
Semi-dry, no mould growth	>4 weeks (usually 10-20days)	0.90-0.95	<20%	Yes (with exceptions)	Most fermented sausages in Germany, The Netherland, USA, etc.
Undried, spreadable	<2 weeks	0.94-0.96	<10%	Yes or no	German 'Streichmettwurst'; Spanish 'sobrasada'

Production of fermented sausages in EU



Source: Reading University, from various national statistics in 1988

Production and consumption of fermented sausages in Germany

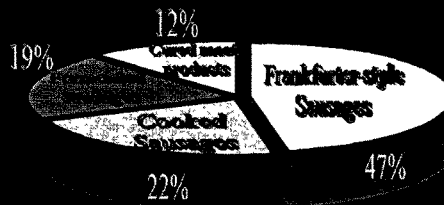


- Processed meat market in Germany:
1.05 million tonnes (1989; 288 factories employing 20 or more)
- Sausage Variation: Over 1500

{ Rohwurst
Bruehwurst
Kochwurst

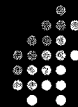


The world's leading
Sausage Country



Source: AID, 1996

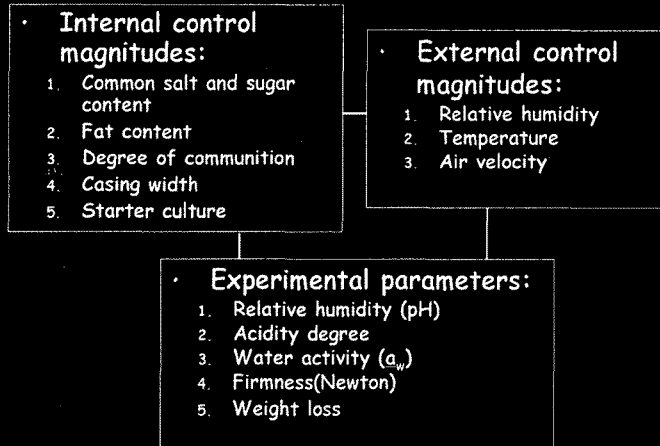
Safety of fermented sausages



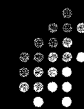
- Background:

Most fermented sausages are not heated during manufacture or before consumption

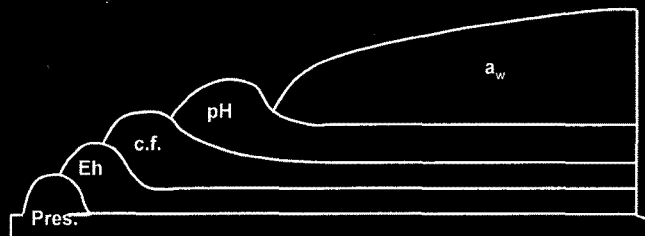
Safety of fermented sausages



Safety of fermented sausages

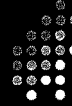


- **Hurdle effect (Leistner, 1987):**



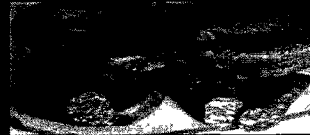
Pres.=preservation; Eh=redox potential; c.f.=competitive flora;
pH=acidity; a_w =water activity

Safety of fermented sausages



• Check Points:

- Preservation:
 - o Risk of nitrite
 - o Exhaust of nitrite
- High E_h value of outside
- Increase of pH



• Solutions:

- Smoking
- Air-drying
- Starter culture
- New ingredients (!!!)

Safety of fermented sausages



• Starter Culture:

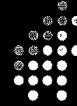
Lactic acid bacteria (LAB):

- PH-lowering effect
- Production of organic acids
- Production of anti-microbial substances, e.g. bacteriocins
- Bacteriocins („protective culture“)

• Beneficial properties of LAB:

- Hygienic safety, texture, color, and flavor of sausages

Starter culture of fermented sausages



• Requirements for Lactic acid Starter Culture:

- Health aspects: free from any microbial or chemical impurities
- Technological performance:
 - o Acidification activities
 - o Phage-resistant to perform optimally during fermentation
 - o No chemical or microbial components
 - o Phenotypically and genetically stable

Starter culture of fermented sausages



• Desirable characteristics of LAB in starter cultures:

- Salt tolerance (at least in the presence of 6 % NaCl);
- Growth in the temperature range of 15 - 40°C;
- Nitrite tolerance (at least 100 mg/kg of NaNO₂);
- Homofermentative;
- Not proteolytic

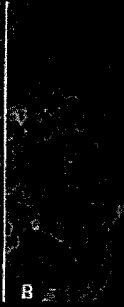
• LAB in today's starter cultures:

- LAB originating from plant material: e.g. *L. plantarum*, *P. pentosaceus*
- LAB originating from meat: e.g. *L. plantarum*, *L. sake*, *L. curvatus*

Topology of the bacteria in fermented sausage



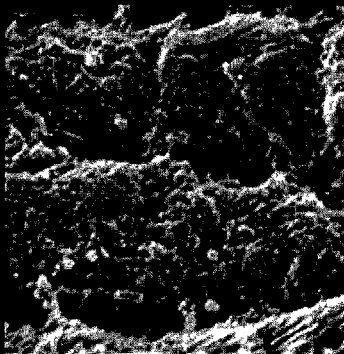
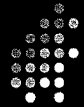
A



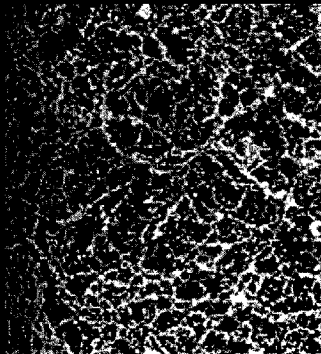
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Katsaras and Leistner, 1998

Topology of the bacteria in fermented sausage



SEM Photograph, x 20000



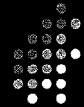
SEM Photograph, x 16400

Topology of the bacteria in fermented sausage



SEM Photograph, x 8700

Topology of the bacteria in fermented sausage



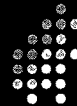
SEM Photograph, x 12600

Trends in the development of starter culture

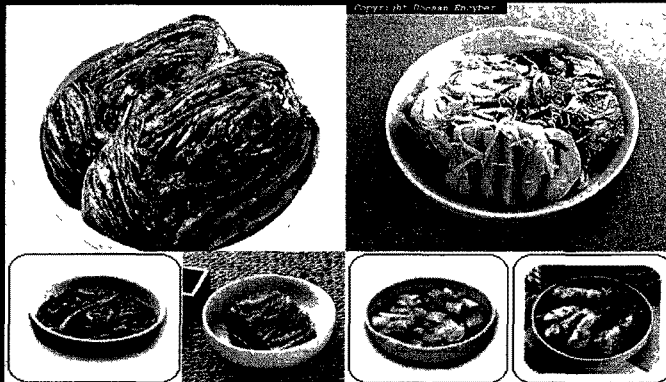


- Inhibition of the growth of pathogenic microorganisms (e.g. enterohaemorrhagic E. coli, EHEC)
- Formation of bacterial biogenic amines
- Development of genetic modified starter
- Finding bacteriocin producing bacteria
- Probiotics as starter culture

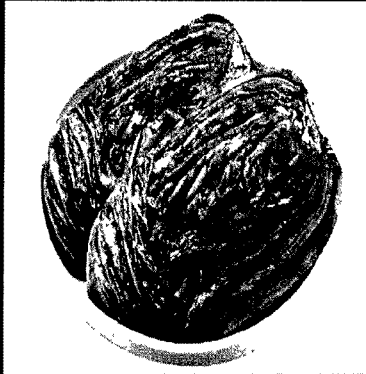
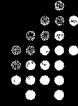
Kimchi



Korean, fermented vegetable product with different spice mixture



Baechu-kimchi



Typical ingredients:

- Chinese cabbage (80%)
- Radish (12%)
- Red pepper powder (1%)
- Garlic (0.5%)
- Ginger (0.4%)
- Leek or green onion (1.0%)
- Fermented fish-soup (1.1%)
- Sugar (1%)
- Salt (0.5%)
- Another ingredients (2.5%)

Kimchi LAB

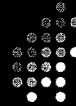


- *Lactobacillus* (*L. curvatus*, *L. plantarum*, *L. sake*)
 - Psychrophilic or psychrotrophic;
 - Fakultatively anaerobic;
 - Salt tolerance, acidity tolerance

Frequently used LAB as starter culture
in the production of fermented sausages

Potential of applying as lactic acid starter culture

Questions forming the investigation



- Are the LAB in kimchi able to adapt to the condition (the environment) of sausages and exhibit the desired abilities to be used as a substitute of starter culture?
- If so, what are the microbiological, physiological and chemical characteristics of the LAB isolated from kimchi in the sausage environment?
- What are influences of the addition of kimchi and kimchi-powder on the characteristics of fermented sausages considered from physical, biochemical and microbiological viewpoint compared to conventional sausages that are produced with commercial starter culture?

Isolation and identification of LAB from kimchi



• Methods

Kimchi fermented at 20°C for 5 days



Colonies on MRS agar plates



Selecting the colonies showing variations in appearance under the microscope



Classical identification and characterization

Isoation and identification of LAB from Kimchi



• Methods

Classical identification and characterization

By morphological criteria:

- Colony morphology
- Cell morphology
- Differentiation by GRAM

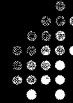
By physiological and biochemical criteria:

- Catalase production; Gas production
- Gas production;
- Growth at different temperatures;
- Growth at pH 3.9; Salt tolerance
- Ammonia production;
- Configuration of the lactic acid enantiomers
- Growth on acetate agar



API 50 CH test

LAB from kimchi



	Group				
	I	II	III	IV	V
Distribution (%)	12.9 (4)*	9.7 (3)*	35.5 (11)*	25.8 (8)*	16.1 (5)*
Characterization	Leuconostoc-like bacteria	Streptobacteria	Heterofermentative lactobacilli	Streptobacteria	Streptobacteria
Identification by id. Program API	<i>Leuconostoc mes.mes./dent</i>	<i>L. curvatus</i>	<i>L. brevis</i>	<i>L. sake</i>	<i>L. plantarum</i>
Id. %	98.9-99.9	89.2-99.9	95.9-99.9	-	90.0-98.1

* Number of strains

Isolated LAB from kimchi in model-medium



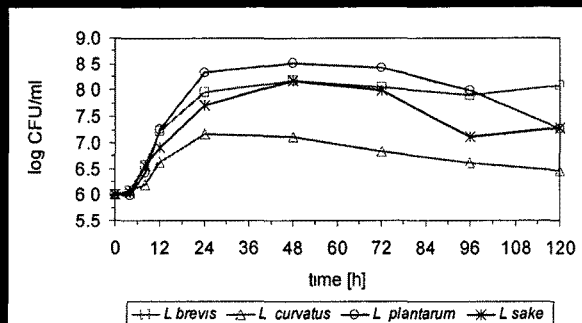
Materials and Methods

- Microorganisms:
L. brevis, *L. curvatus*, *L. plantarum*, *L. sake*
- Model-systems submerged model-medium
 solid-state model-medium
- Investigation parameters:
 - o Adaptation and growth;
 - o Souring properties;
 - o Nitrite tolerance;
 - o Competition capability

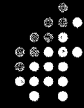
Isolated LAB from kimchi in model-medium



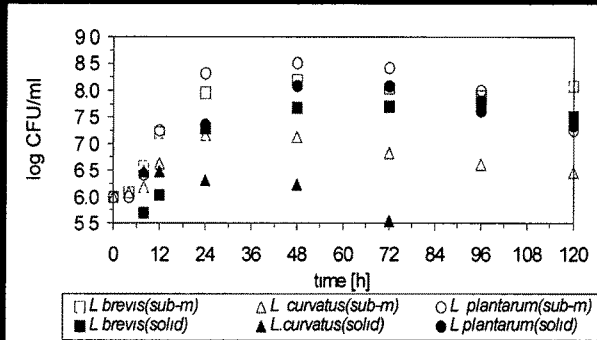
Adaptation and Growth



Isolated LAB from kimchi in model-medium



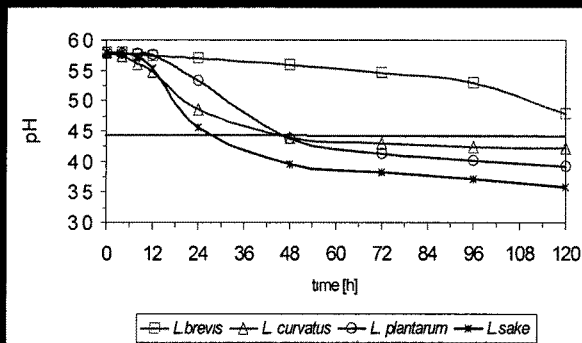
Influence of immobilized condition



Isolated LAB from kimchi in model-medium



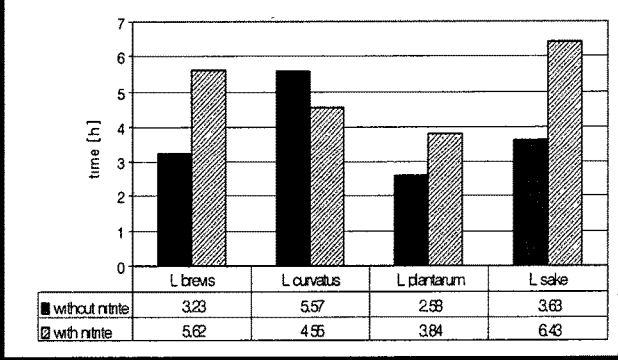
Souring properties



Isolated LAB from kimchi in model-medium



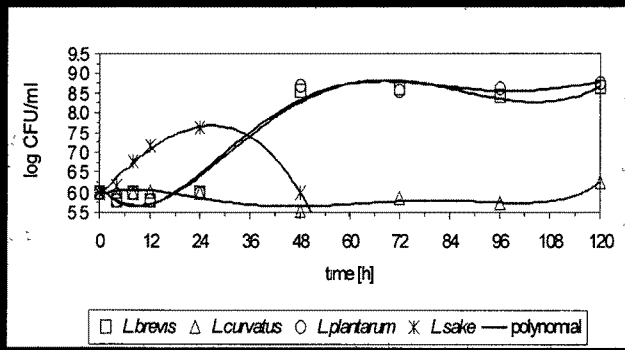
Influence of the added potassium nitrite on the generation time



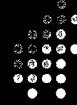
Isolated LAB from kimchi in model-medium



Competition capabilities

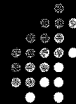


Kimchi and kimchi-powder in model-medium

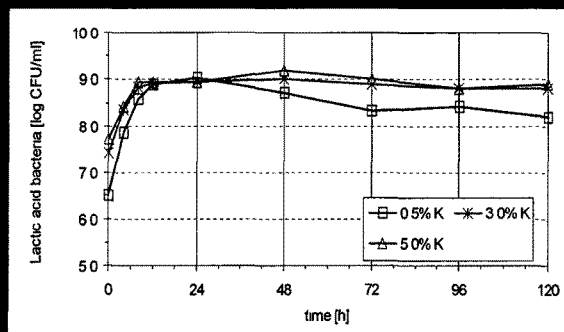


- **Materials and Methods**
 - Kimchi (0.1 - 1.5%)
 - Kimchi-powder (0.2, 0.5%)
 - Model-systems
 - Investigation parameters:
 - o Adaptation and growth
 - o Souring properties

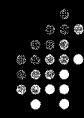
Kimchi in model-medium



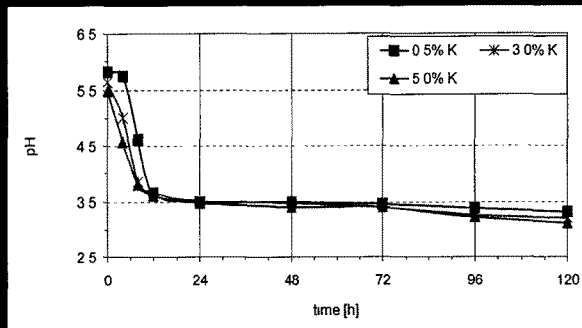
Growth of kimchi LAB in model-medium



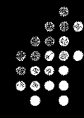
Kimchi in model-medium



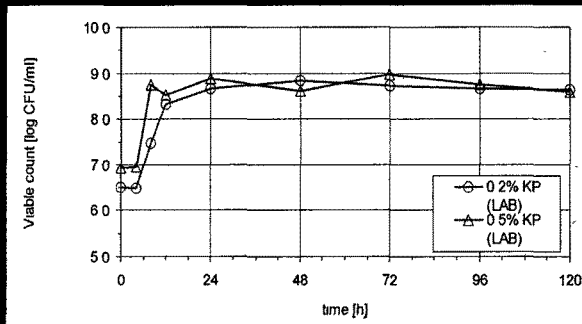
Changes in pH-value



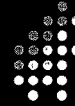
Kimchi-powder in model-medium



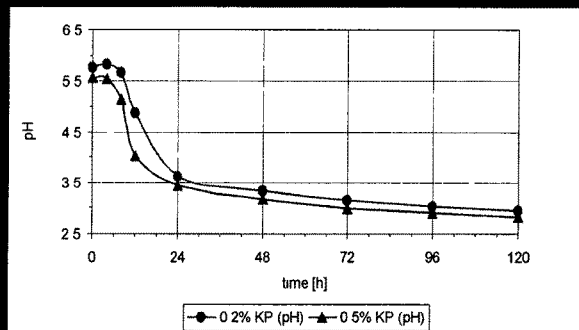
Growth of kimchi-powder LAB in model medium



Kimchi-powder in model-medium



Changes in pH-value



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Kimchi LAB in model-medium



Summary

- *L. plantarum* :
 - Salt tolerance;
 - Good souring properties;
 - Good competition against other LAB;
 - It's growth rate was affected by immobilized condition and nitrite
- Good growth and souring properties of the totality of LAB that naturally occurred in kimchi under the new habitat of fermenting sausages
- Advantageous utility of LAB as an integral part of kimchi as compare to the use of pure LAB

Kimchi(-powder) sausages



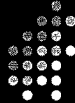
· Materials and Methods

- Composition of sausages

- o Meat mixture (lean pork:lean beef:bäck fat=1:1:1);
- o Nitrite curing salt (10 g / Kg);
- o Kimchi (KS; 5, 10, 15 %);
- o kimchi-powder (KPS; 2, 5 %);
- o Starter culture (control; LS 25; *L. sake + Staphylococcus carnosus*)

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Kimchi(-powder) sausages



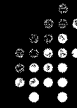
Conditions during fermentation and ripening

1-7. day: fermentation (24 °C – 15 °C, rH 90 – 80 %)

8-28. day: ripening (15 °C, rH 70 – 75 %)



Kimchi(-powder) sausages

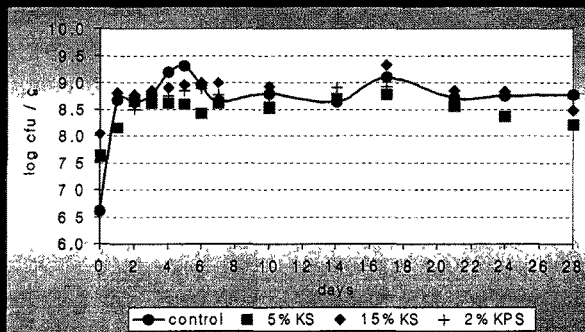


- Materials and Methods
 - Experimental parameters
 - o Microbiological changes
 - o pH value
 - o Water activity, weight loss

Kimchi(-powder) sausages



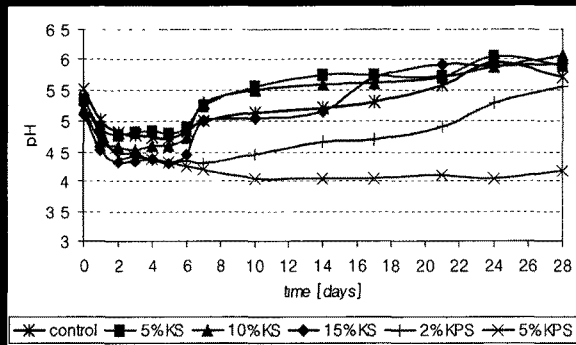
Growth of LAB



Kimchi(-powder) sausages



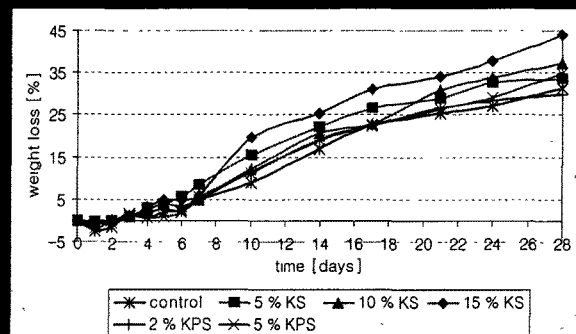
pH



Kimchi(-powder) sausages



Weight loss



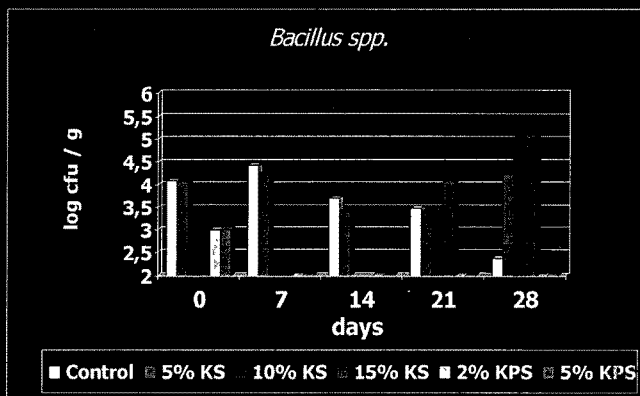
Kimchi(-powder) sausages



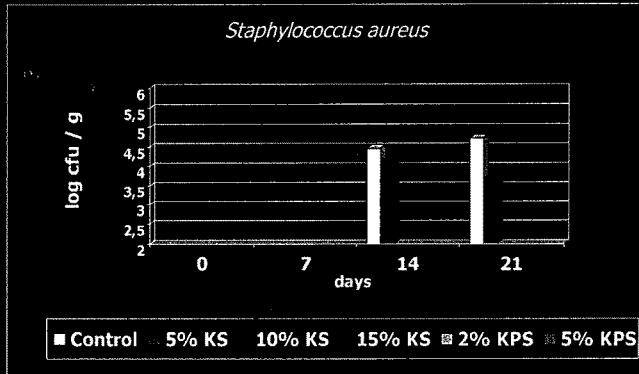
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Day	Control	5 % KS	10% KS	15% KS	2% KPS	5% KPS
0	0.95	0.94	0.94	0.95	0.95	0.93
7	0.95	0.94	0.94	0.95	0.95	0.93
14	0.94	0.93	0.93	0.94	0.94	0.92
21	0.94	0.92	0.92	0.94	0.93	0.92
28	0.93	0.92	0.91	0.93	0.93	0.89

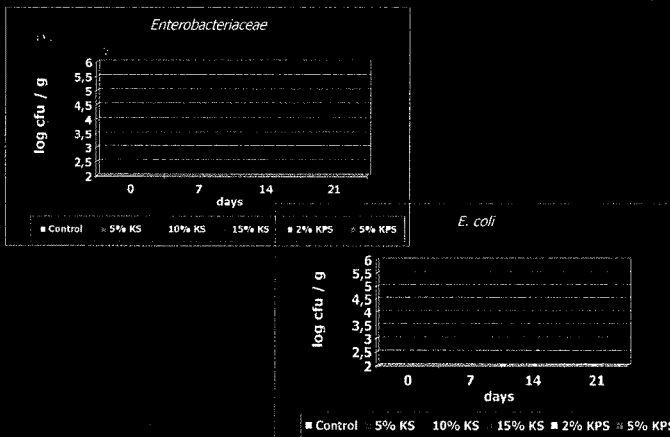
Microbial stability of Kimchi(-powder) sausages



Microbial stability of Kimchi(-powder) sausages



Microbial stability of kimchi(-powder) sausages



Kimchi(-powder) sausages



Summary

- The LAB as an integrated part of kimchi were well adapted to the new habitat of fermenting sausage and exhibited good souring properties that are comparable to those of commercial starter cultures.
- With the added kimchi (5-15%) and kimchi-powder (2-5%), the necessary microbial stability of real fermented sausages was achieved.
- In particular, kimchi-powder contributed to improving the safety of the fermented sausages as compared to the conventional one treated with starter culture.

Conclusion



- ☞ Potential Utility of LAB in kimchi as well as in kimchi-powder as a substitute of lactic starter culture in the production of fermented sausages
- ☞ A Good microbial stability achieved by the addition of kimchi and kimchi-powder

