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Food safety of traditional alkaline fermented products

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Importance of fermentation

Fermentation: a process where a food substrate is transformed by microorganisms to obtain a better final product: nutrition, hygiene, stability, odor, color, taste, digestibility, safety.....

Fermentation: one of the oldest known and economical methods of preserving food.

In African countries: method to make food storable and by this way attempt to have sufficient food in the lean seasons .

Importance of fermentation

Today, food are fermented for many other reasons also:

- ❖ Improvement of sensory characteristics by development of diverse flavor and aroma compounds ,
- ❖ Increase of nutritive value through breakdown of proteins, carbohydrates and lipids to essential amino acids, easily digested sugars and essential fatty acids. Furthermore brings about synthesis of some vitamins.
- ❖ Improvement of safety (absence of toxins and partial and/ or complete elimination of antinutritional factors)
- ❖ Decrease in cooking time and thereby fuel requirements.

Alkaline traditional fermented foods

- ❖ Food obtained from a fermentation of a substrate in such a way that an pH increase(8-9) is observed
- ❖ Traditional: non controlled, with rudimentary equipment
- ❖ Raw material : Vegetable rich in protein
- ❖ Main microorganisms : *Bacillus* species especially *B. subtilis*
- ❖ Main biochemical change: degradation of proteins with sharp increase of quantity and quality of amino acid in the final product
- ❖ Further degradation of amino acid leads to a release of ammonia responsible of the pH increase(final pH about 8 to 9).
- ❖ Degradation of lipids and carbohydrates also occurs
- ❖ Usually used as food condiment but sometimes a part of main meal

Nutrition

- ❖ Proteins: up to 40%
- ❖ Essential amino acids : all including lysine
- ❖ Lipids
- ❖ Essential fatty acids: oleic and linoleic acids
- ❖ Carbohydrates
- ❖ Vitamins especially B but also A and C
- ❖ Minerals: Ca, Fe, Zn, Cu, Mg, P, Se, Na, Mn
- ❖ In many Africa countries Proteins/ calorie malnutrition is a major problem.
Fermented vegetable proteins therefore have great potential as key protein sources and as basic ingredients for food supplementation.

Example of African traditional alkaline fermented foods

Name	Location	Substrate	Associated microorganisms
Soumbala (Iru, Dawadawa, netetou, kinda)	West and central Africa	African locust beans	<i>B. subtilis</i> , <i>B. licheniformis</i> , <i>B. pumilus</i> , <i>B. megaterium</i> , <i>B. pumilus</i> , <i>B. sphaericus</i> , <i>B. cereus</i> , <i>Brevibacillus bortelensis</i> <i>Staphylococcus</i> spp., <i>Enterococcus</i> spp., <i>Pediococcus</i> spp. <i>Lactobacillus</i> spp., <i>Leuconostoc</i> spp.
Soy- dawadawa	West Africa	Soybeans	<i>Bacillus subtilis</i> , <i>B. licheniformis</i> , <i>B. pumilus</i> <i>Staphylococcus</i> spp, <i>Micrococcus</i> spp.
Ogiri	South- eastern Nigeria	Melon seeds	<i>B. subtilis</i> , <i>B. megaterium</i> , <i>B. firmus</i>
Ugba	Sierra Leone, eastern Nigeria	Oilbean seeds	<i>B. subtilis</i> <i>Staphylococcus</i> spp., <i>Micrococcus</i> spp.
Kawal	Sudan	<i>Cassia obtusifolia</i>	<i>B. subtilis</i> <i>Propionibacterium</i> spp.
Bikalga (Furundu, Mbuja)	West, East and central Africa	<i>Hibiscus sabdariffa</i>	<i>B. subtilis</i> , <i>B. licheniformis</i> <i>Enterococcus</i> spp., <i>Pediococcus</i> spp

Are traditional alkaline fermented foods safe?

Yes

Are traditional alkaline fermented foods risky?

Yes

Traditional alkaline fermented products as safe foods

- ❖ The high pH inhibits invasion by pathogenic and spoilage microorganisms.
- ❖ During fermentation inedible foods are made edible: elimination/decrease of antinutritional factors
- ❖ Alkaline fermentation increases digestibility due to extensive hydrolysis of protein and lipids respectively to peptides, essential amino acids and fatty acids.
- ❖ Degradation of non digestible oligosaccharides as stachyose and raffinose into simple sugars.
- ❖ Decreased toxicity: elimination/decrease of toxic components of raw materials: African locust beans contains high levels of toxic substances such as oxalic acid, phytic acid and hydrocyanic acid which are reduced during the fermentation.

Traditional alkaline fermented products as safe foods

During fermentation of cassava leaves for Ntoba Mbodi production, cyanogenic components are drastically reduced

- ❖ Most raw material undergoes a long cooking time (up to 40 h in some cases) prior to the fermentation step: eliminating of non spore forming pathogenic bacteria.
- ❖ Fermented products are most of the time cooked in various dishes: elimination of pathogenic contaminants after during drying and storage
- ❖ Some *Bacillus* isolates have strong antimicrobial activity, inhibiting and inactivating pathogenic Gram positive and Gram negative bacteria as well pathogenic mould (case study)
- ❖ Predominant *B. subtilis* do not produce enterotoxins(case study)
- ❖ *Bacillus* isolates do not harbor transferable antibiotic resistance genes

Case study: antimicrobial activity of *Bacillus subtilis* and *B.pumilus* isolated from Soumbala

Ouoba , L. I.I., Diawara, B., Jespersen, L and Jakobsen, M. (2007) Antimicrobial activity of *Bacillus subtilis* and *Bacillus pumilus* during the fermentation of African locust bean (*Parkia biglobosa*) for Soumbala production. *Journal of Applied Microbiology*, 102, 963-970.

Inhibition of indicator of pathogenic bacteria by *B. subtilis* and *B.pumilus* isolated from Soumbala

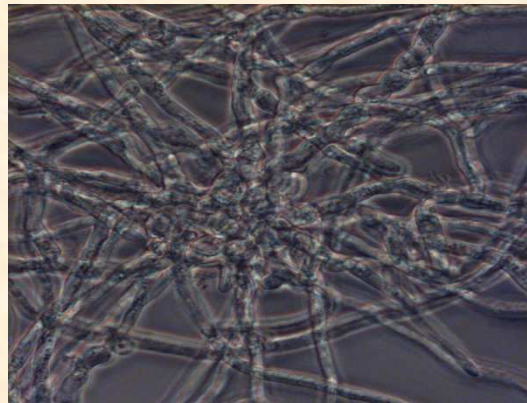
Indicator bacteria	Inhibition (24h)				
	<i>B. subtilis</i>			<i>B. pumilus</i>	
	B7	B9	B15	B6	B10
<i>Micrococcus luteus</i> A1	++	+	+	+	+
<i>Bacillus cereus</i> A4	+	-	+	-	-
<i>Bacillus cereus</i> A10	++	++	+	++	++
<i>Staphylococcus aureus</i> A11	-	-	+	+	+
<i>Staphylococcus aureus</i> A12	++(p)	-	+++	+	+
<i>Enterococcus faecium</i> A19	-	-	-	+	+
<i>Listeria monocytogenes</i> A18	++	+	+	+	+
<i>Escherichia coli</i> A13	++	-	+(p)	-	++(p)
<i>Salmonella enteritidis</i> A14	+	-	+	-	-
<i>Salmonella typhimurium</i> A15	++	-	+	-	+
<i>Shigella flexneri</i> A16	+	-	-	-	-
<i>Shigella dysenteriae</i> A17	++	-	+	-	++
<i>Yersinia enterocolitica</i> A21	++(p)	-	++	-	+

-: No inhibition, +: 1 ≤ inhibition < 3 mm, ++: 3 ≤ inhibition < 5 mm, +++: inhibition ≥ 5 mm,
p: partial i.e. the inhibition zone was not totally clear.

Inactivation of indicator of pathogenic bacteria during the fermentatin of African locust bean for soubmala production

Indicators	Number of microorganisms (CFU)			
	0h	24h	48h	72h
<i>Micrococcus luteus</i> A1	$5.3 \times 10^5 \pm 1.3$	-	-	-
<i>Micrococcus luteus</i> A1a	$4.8 \times 10^5 \pm 1.4$	$1.0 \times 10^9 \pm 0.8$	$8 \times 10^{10} \pm 5.7$	$5.5 \times 10^{11} \pm 3.7$
<i>Bacillus cereus</i> A4	$2.5 \times 10^5 \pm 2.0$	-	-	-
<i>Bacillus cereus</i> A4a	$2.4 \times 10^5 \pm 1.7$	$4.5 \times 10^8 \pm 1.7$	$4.3 \times 10^9 \pm 1.8$	$1.5 \times 10^9 \pm 0.4$
<i>Bacillus cereus</i> A10	$3.1 \times 10^5 \pm 1.9$	-	-	-
<i>Bacillus cereus</i> A10a	$3.0 \times 10^5 \pm 1.6$	$9.3 \times 10^7 \pm 4.7$	$4.3 \times 10^9 \pm 2.9$	$1.0 \times 10^9 \pm 0.0$
<i>Escherichia coli</i> A13	$2.3 \times 10^5 \pm 0.9$	-	-	-
<i>Escherichia coli</i> A13a	$8.8 \times 10^6 \pm 5.9$	$5.6 \times 10^{10} \pm 4.4$	$5.1 \times 10^{11} \pm 3.9$	$4.0 \times 10^{12} \pm 1.6$
<i>Staphylococcus aureus</i> A11	$1.5 \times 10^7 \pm 1.5$	-	-	-
<i>Staphylococcus aureus</i> A11a	$1.5 \times 10^7 \pm 1.1$	$3.9 \times 10^6 \pm 2.8$	$3.2 \times 10^6 \pm 2.3$	$4.3 \times 10^6 \pm 3.0$
<i>Staphylococcus aureus</i> A12	$1.2 \times 10^6 \pm 4.2$	-	-	-
<i>Staphylococcus aureus</i> A12	$2.7 \times 10^6 \pm 0.9$	$1.0 \times 10^9 \pm 0.3$	$3.1 \times 10^9 \pm 0.1$	$1.1 \times 10^{10} \pm 0.0$

Inactivation of *Aspergillus ochraceus* by *B.subtilis* B7



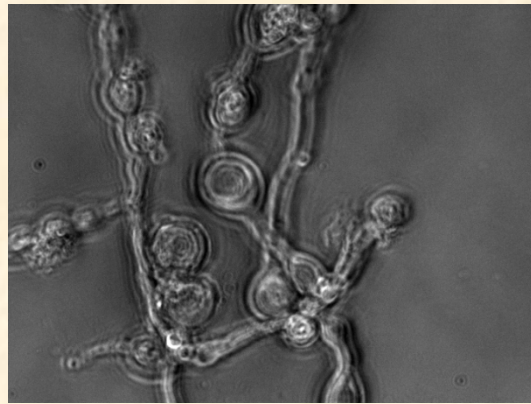
7 days growth of *A.ochraceus*:
normal mycellium

7 days growth with cells of B7



abnormal mycellium

2 days growth in supernatant of B7 culture



abnormal mycellium

7 days growth in supernatant of B7 culture



abnormal mycellium

Traditional alkaline fermented products as risky foods

- ❖ This problem is common to all types of traditional fermented foods
- ❖ Traditionally work in the families using rudimentary equipment



- ❖ Poor hygienic conditions: constant reinfection of the fermented seeds
- ❖ Producers not trained to apply at least GMP
- ❖ For economical reasons, raw material not let to cook and/or ferment for the required time
- ❖ Totally uncontrolled fermentation with variable microorganisms

Traditional alkaline fermented products as risky foods

- ❖ Presence of fermenting but pathogenic bacteria as *B. cereus*
- ❖ Presence of pathogenic bacteria (Enterobacteriaceae and fecal coliforms) especially during drying and storage
- ❖ **Consequence: variation of the nutritional and hygienic quality of the fermented product as well as their stability.**

Case study: production of enterotoxins by *B.cereus* and other species of *Bacillus* from Soumbala and Bikalga

Ouoba L.I.I., Thorsen, L., Varnam, A.H. (2008) Enterotoxins and emetic toxins production by *Bacillus cereus* and other species of *Bacillus* isolated from Soumbala and Bikalga, African alkaline fermented food condiments. International Journal of Food Microbiology 124,224-230.
Poster session

Methods

- ❖ Haemolytic activity on blood agar
- ❖ Detection of genes encoding cytotoxin K (CytK), haemolysin BL (Hbl A, Hbl C, Hbl D), non-haemolytic enterotoxin (NheA, NheB, NheC) and EM1 specific of emetic toxin producers : PCR with single pair and multiplex
- ❖ Production of enterotoxins in broth and during the fermentation of African locust beans for soumbala production
- *B. cereus* Enterotoxin Reverse Passive Latex Agglutination test kit (BCET-RPLA, Oxoid): detection of haemolytic toxins
- *Bacillus* Diarrhoeal Enterotoxin Visual Immunoassay (BDEVIA, Tecra): detection of non- haemolytic toxins

Results

<i>Bacillus</i> isolates		Haemolysis on Blood agar	Broth		Fermented seeds		Toxin genes
			RPLA	BDEVIA	RPLA	BDEVIA	
<i>B. cereus</i>	B1	+	-	4 +	++	4+	Nhe(A, B, C)
	B2	+	-	4 +	++	4+	Nhe(A, B, C)
	B8	+	-	3 +	++	4+	Nhe(A, B, C), CytK
	B13	+	+++	3 +	+++	4+	Nhe(A,B,C), Hbl(A,C,D), CytK
	B17	+	+++	3 +	+++	4+	Nhe(A,B, C),Hbl (A,C,D) CytK
	B18	+	-	4 +	++	4+	Nhe(A, B, C)
	D2	+	±	4 +	+	4+	Nhe(A,B, C), HblC, CytK
	D3	±	±	5 +	++	4+	Nhe(A,B, C), HblC, CytK
	D7	-	±	5 +	++	4+	Nhe(A,B, C), Hbl(C, D),CytK
	<i>B. subtilis</i>	B7	+	-	-	-	-
A1		+	-	-	-	-	Hbl(A,D)
C3		+	-	-	-	-	Hbl D
<i>B. licheniformis</i>	E3	-	-	-	-	-	CytK, Hbl A

Can traditional alkaline fermented food be much safer?

Yes Yes Yes Yes

Optimisation of the process without unduly altering the traditional appearance and taste of the products.

- ❖ Training of producers to ferment foods in hygienic conditions: GMP
- ❖ Well characterisation of the fermented product: factors like moisture, quantity of substrate used, time, temperature and organoleptic characteristics must be known.
- ❖ Development better equipment:
 - ie improved fermentor: constructed with maximum emphasis on maintenance, sanitation and reliability for the process, and minimum capital investment and operating cost,
 - ie dehulling machine in the of long cooking step in the process: in case of African locust bean, cooking time is reduced from 24-40h to 6-8h

Optimisation of the process without unduly altering the traditional appearance and taste of the products.

- ❖ Development of starter cultures: microorganisms might be well identified and selected to conduct the fermentation and to produce a desirable product. (case study: next presentation)
- ❖ Development of small-scale industrial productions rather than highly technological approaches
- ❖ Quality assurance system and training:
 - well defined diagram of production,
 - Identification of all critical points of the process
 - Effective control of critical points
 - Training of producer to apply the quality assurance system
- ❖ Development of standard for commercialization

Example: optimization of the processing conditions during the production of Soumbala

- **GMP and HACCP:**
- **Dehulling machine(CNRST): decrease of the processing time and energy,**
- **Development of starter cultures: controlled fermentation with enhanced nutritional and hygienic quality as well as stability(case study, next presentation).**
- **Improved fermentation containers,**
- **Soumbala cube machine(CNRST),**
- **Protected dryer,**
- **Improved packages,**
- **Semi- industrial production sites.**